

## MICROBIAL CONSORTIA FOR TREATING VAGINAL CONDITIONS

### RELATED APPLICATIONS

This application claims the benefit under 35 U.S.C. §119(e) to U.S. Provisional Application No. 63/599,555, filed November 15, 2023, entitled “Vaginal Bacterial Compositions”, the contents of which are incorporated herein by reference.

### REFERENCE TO AN ELECTRONIC SEQUENCE LISTING

The contents of the electronic sequence listing (F089570001WO00-SEQ-GJM.xml; Size: 42,018 bytes; and Date of Creation: November 15, 2024) is herein incorporated by reference in its entirety.

### BACKGROUND OF THE INVENTION

[0001] The vagina is a fibromuscular tubular tract leading from the uterus to the exterior of the body in females. A healthy vagina is colonized by a mutually symbiotic flora of microorganisms, in particular of the genus *Lactobacillus*, that protects its host from vaginal infections. The acidity of a healthy vagina of a woman of child-bearing age (pH about 3.5-4.5) is thought to be due to the degradation of secreted glycogen/glucose to lactic acid by *lactobacilli*. Acidic conditions are unfavorable for the growth of many pathogenic microorganisms and pathobionts, including bacteria, protozoa, and viruses. However, an imbalance in the vaginal microbiota may result in overgrowth of pathogenic microorganisms and pathobionts, resulting in dysbiosis, inflammation, and/or infections.

[0002] *Lactobacillus*-containing products (comprising, *e.g.*, *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, or *Lactobacillus gasseri*) for intravaginal or oral use have been available for many years. These products include vaginal suppositories containing lyophilized *Lactobacillus acidophilus* or other *Lactobacillus* species (*e.g.*, *Lactobacillus rhamnosus*, or *Lactobacillus gasseri*) of human origin as well as various nutritional supplements.

[0003] A need exists for the identification of specific bacteria and bacterial consortia capable of colonizing the human female genitourinary tract and a better understanding of the

*in vivo* environment of the genitourinary tract and overall health profile of subjects receiving such isolates.

### SUMMARY OF THE INVENTION

[0004] Provided herein are compositions of microbial consortia comprising two or more bacterial isolates capable of engraftment of the human female genitourinary tract, *e.g.*, of a female subject that exhibits, *e.g.*, a dysbiosis of the genitourinary tract.

[0005] A microbial consortium of the present disclosure is designed, in some embodiments, to include specific *Lactobacillus* isolates (*e.g.*, *Lactobacillus crispatus* and/or *Lactobacillus jensenii* isolates) having a diverse set of functional and genomic characteristics. For example, a microbial consortium of the disclosure may include two or more *Lactobacillus* isolates, wherein each isolate has different functional characteristics (*e.g.*, different growth rates, different acidification rates, different carbohydrate utilization profiles, different rates of engraftment). Additionally, the two or more *Lactobacillus* isolates may further comprise different genomic characteristics. This difference in genomic characteristics can allow for the microbial consortium comprising the two or more *Lactobacillus* isolates to encompass a preselected portion of a species-level pangenome (*e.g.*, all of the genes of a species-level pangenome that are linked to engraftment) even though any single *Lactobacillus* isolate might not encompass the preselected portion by itself.

[0006] A microbial consortium having a diverse set of functional and genomic characteristics as described herein can engraft in the female genitourinary tract of a diverse set of subjects, each of whom may have different vaginal environments (*e.g.*, different resident microbial communities, *e.g.*, different resident dysbiotic microbial communities). Subjects differ with respect to age, racial and national background, hormonal status (*e.g.*, different stages of menstrual cycle, menopausal), presence of comorbidities, lifestyle, diet and geographic factors, among others. Each of these differences between subjects can affect the resident microbial community (*e.g.*, including the composition of dysbiotic microbial communities and bacterial species) and the local environment (*e.g.*, pH, levels of H<sub>2</sub>O<sub>2</sub>, levels of glycogen, levels of estrogen, etc.) in the genitourinary tract. The inventors of the present disclosure have identified that a microbial consortium having a diverse set of functional and genomic characteristics as described herein is able to be highly adaptable to such different



environments present in a diverse set of subjects and to successfully engraft within the genitourinary tract of these diverse subjects. In some embodiments, the microbial consortia of the present disclosure can successfully engraft without the use of antibiotics such as metronidazole (*e.g.*, as a pre-treatment step prior to administration of the microbial consortia).

**[0001]** Some aspects of the present disclosure provide a microbial consortium comprising a defined population of isolated lactobacilli, the defined population comprising:

- (a) a *Lactobacillus jensenii* isolate obtained from a bacterial sample deposited as DSM Deposit Number 34770;
- (b) a *Lactobacillus crispatus* isolate obtained from a bacterial sample deposited as DSM Deposit Number 34772;
- (c) a *Lactobacillus crispatus* isolate obtained from a bacterial sample deposited as DSM Deposit Number 34771, and
- (d) a *Lactobacillus crispatus* isolate obtained from a bacterial sample deposited as DSM Deposit Number 34773.

**[0002]** Some aspects of the disclosure provide a microbial consortium comprising a defined population of isolated lactobacilli, the defined population comprising:

- (a) a *Lactobacillus jensenii* isolate having a genome that, when aligned with the genome of a bacterial sample deposited as DSM 34770, has a percent identity greater than or equal to a threshold value;
- (b) a *Lactobacillus crispatus* isolate having a genome that, when aligned with the genome of a bacterial sample deposited as DSM 34771, has a percent identity greater than or equal to a threshold value;
- (c) a *Lactobacillus crispatus* isolate having a genome that, when aligned with the genome of a bacterial sample deposited as DSM 34772, has a percent identity greater than or equal to a threshold value; and
- (d) a *Lactobacillus crispatus* isolate having a genome that, when aligned with the genome of a bacterial sample deposited as DSM 34773, has a percent identity greater than or equal to a threshold value.

**[0003]** Some aspects of the disclosure provide a method of increasing the relative abundance of lactobacilli in the female genitourinary tract of a subject comprising

administering any one of the microbial consortia described herein to the genitourinary tract of the subject.

- [0004] Some aspects of the disclosure provide a composition comprising *Lactobacillus jensenii* isolate obtained from a bacterial sample deposited as DSM Deposit Number 34770.
- [0005] Some aspects of the disclosure provide a composition comprising *Lactobacillus crispatus* isolate obtained from a bacterial sample deposited as DSM Deposit Number 34772.
- [0006] Some aspects of the disclosure provide a composition comprising *Lactobacillus crispatus* isolate obtained from a bacterial sample deposited as DSM Deposit Number 34771.
- [0007] Some aspects of the disclosure provide a composition comprising *Lactobacillus crispatus* isolate obtained from a bacterial sample deposited as DSM Deposit Number 34773.
- [0008] Some aspects of the disclosure provide a composition comprising two or more *Lactobacillus* bacteria selected from the group consisting of:
- (a) a *Lactobacillus jensenii* isolate obtained from a bacterial sample deposited as DSM Deposit Number 34770;
  - (b) a *Lactobacillus crispatus* isolate obtained from a bacterial sample deposited as DSM Deposit Number 34772;
  - (c) a *Lactobacillus crispatus* isolate obtained from a bacterial sample deposited as DSM Deposit Number 34771, and
  - (d) a *Lactobacillus crispatus* isolate obtained from a bacterial sample deposited as DSM Deposit Number 34773.
- [0009] Some aspects of the disclosure provide a composition comprising *Lactobacillus jensenii* isolate having a genome that, when aligned with the genome of a bacterial sample deposited as DSM 34770, has a percent identity greater than or equal to a threshold value.
- [0010] Some aspects of the disclosure provide a composition comprising *Lactobacillus crispatus* isolate having a genome that, when aligned with the genome of a bacterial sample deposited as DSM 34771, has a percent identity greater than or equal to a threshold value.

[0011] Some aspects of the disclosure provide a composition comprising *Lactobacillus crispatus* isolate having a genome that, when aligned with the genome of a bacterial sample deposited as DSM 34772, has a percent identity greater than or equal to a threshold value.

[0012] Some aspects of the disclosure provide a composition comprising *Lactobacillus crispatus* isolate having a genome that, when aligned with the genome of a bacterial sample deposited as DSM 34773, has a percent identity greater than or equal to a threshold value.

[0013] Some aspects of the disclosure provide a composition comprising two or more *Lactobacillus* bacteria selected from the group consisting of:

- (a) *Lactobacillus jensenii* isolate having a genome that, when aligned with the genome of a bacterial sample deposited as DSM 34770, has a percent identity greater than or equal to a threshold value;
- (b) *Lactobacillus crispatus* isolate having a genome that, when aligned with the genome of a bacterial sample deposited as DSM 34771, has a percent identity greater than or equal to a threshold value;
- (c) *Lactobacillus crispatus* isolate having a genome that, when aligned with the genome of a bacterial sample deposited as DSM 34772, has a percent identity greater than or equal to a threshold value; and
- (d) *Lactobacillus crispatus* isolate having a genome that, when aligned with the genome of a bacterial sample deposited as DSM 34773, has a percent identity greater than or equal to a threshold value.

[0014] In one embodiment, the disclosure provides a microbial consortium, comprising (i) a *Lactobacillus jensenii* isolate and (ii) at least two phenotypically distinct *Lactobacillus crispatus* isolates.

[0015] In another embodiment the disclosure provides a microbial consortium, comprising (i) a *Lactobacillus jensenii* isolate and (ii) at least three phenotypically distinct *Lactobacillus crispatus* isolates.

[0016] In yet another embodiment, the disclosure provides the disclosure provides a microbial consortium, comprising (i) a *Lactobacillus jensenii* isolate and (ii) at least two phenotypically distinct *Lactobacillus crispatus* isolates, wherein the at least two phenotypically distinct *Lactobacillus crispatus* isolates are each characterized by a different carbon substrate utilization profile. In various embodiments, the at least two phenotypically

distinct *Lactobacillus crispatus* isolates is characterized as utilizing both mannitol and glucose. In certain embodiments, a first phenotypically distinct *Lactobacillus crispatus* isolate is characterized as utilizing mannitol to a lesser degree than a second phenotypically distinct *Lactobacillus crispatus* isolate.

**[0017]** The disclosure provides in another embodiment a microbial consortium, comprising (i) a *Lactobacillus jensenii* isolate and (ii) at least three phenotypically distinct *Lactobacillus crispatus* isolates, wherein the at least two phenotypically distinct *Lactobacillus crispatus* isolates are each characterized by a different carbon substrate utilization profile. In various embodiments, the at least three phenotypically distinct *Lactobacillus crispatus* isolates is characterized as utilizing both mannitol and glucose. In certain embodiments, a first phenotypically distinct *Lactobacillus crispatus* isolate is characterized as utilizing mannitol to a lesser degree than a second phenotypically distinct *Lactobacillus crispatus* isolate.

**[0018]** In other embodiments, the disclosure provides a microbial consortium comprising (i) a *Lactobacillus jensenii* isolate and (ii) at least two phenotypically distinct *Lactobacillus crispatus* isolates, wherein the at least two phenotypically distinct *Lactobacillus crispatus* isolates have different growth and/or acidification rates during logarithmic growth phase on media comprising mannitol or glucose as carbon substrates. In certain embodiments, the growth rate is determined according to the steps of Table 7. In other embodiments, the acidification rate is determined according to the steps of Table 8.

**[0019]** In various embodiments, each isolate of a microbial consortium has engraftment potential in recipient subjects. The engraftment potential can be assessed according to the various methods, including a method of detecting engraftment potential in a recipient subject of a microbial consortium, said method comprising detecting the presence of at least one bacterial isolate in the recipient at least 24 hours after administration of the microbial consortium to the recipient, e.g., to the vaginal lumen. In various embodiments for measuring engraftment, the microbial consortium comprises (i) a *Lactobacillus jensenii* isolate and (ii) at least two phenotypically distinct *Lactobacillus crispatus* isolates. In other embodiments, the microbial consortium comprises (i) a *Lactobacillus jensenii* isolate and (ii) at least three phenotypically distinct *Lactobacillus crispatus* isolates. In certain embodiments, measuring engraftment can include detecting at least two or three bacterial isolates after at least 24 hours after administration of the microbial consortium to the recipient. The detecting step in other embodiments can also be after at least 48 hours, 72

hours, 7 day, 4 weeks, 8 weeks, and 16 weeks after administration of the microbial consortium to the recipient.

[0020] In various embodiments, the detecting step may comprise nucleic acid sequencing of the nucleic acid molecule, a genome or a genome region of at least one bacterial isolate. The sequencing can be whole genome sequencing.

[0021] When detecting engraftment, in certain embodiment, the detection may comprise measuring a relative abundance of the least one isolate of at least 1%, 5%, 10%, 15%, or 20% of a sample taken from the vaginal lumen after administration of the microbial consortium.

[0022] In various embodiments, the relative abundance of each isolate in the microbial consortium is ratiometrically equal.

[0023] In other embodiments, the percentage relative abundance of *Lactobacillus* in the consortium that are *Lactobacillus jensenii* is in the range of 15%-60% and the remaining balance of *Lactobacillus* are *Lactobacillus crispatus*. In still other embodiments, the percentage of *Lactobacillus* in the consortium that are *Lactobacillus jensenii* is in the range of 25% to 40% and the remaining balance of *Lactobacillus* are *Lactobacillus crispatus*. In other embodiments, the percentage of *Lactobacillus* in the consortium that are *Lactobacillus jensenii* is in the range of 20% to 30% and the remaining balance of *Lactobacillus* are *Lactobacillus crispatus*.

[0024] In various embodiments, the *Lactobacillus* isolates originated from vaginal secretions obtained from at least 2 female donors, or from at least 3 female donors. In various embodiments, the vaginal secretions comprise microbes from a eubiotic vaginal microbiome.

[0025] In other embodiments of the microbial consortia, the *Lactobacillus jensenii* isolate originates from a eubiotic vaginal microbiome comprising *Lactobacillus jensenii* and *Lactobacillus crispatus*, wherein a first of the *Lactobacillus crispatus* isolates originates from a eubiotic vaginal microbiome comprising *Lactobacillus jensenii* and *Lactobacillus crispatus*, and wherein a second of the *Lactobacillus crispatus* isolates originates from a eubiotic vaginal microbiome comprising *Lactobacillus crispatus* and lacking detectable *Lactobacillus jensenii*.

[0026] In embodiments, at least two of the at least three phenotypically distinct *Lactobacillus crispatus* isolates have different growth rates and/or acidification rates on one or more carbon substrates during logarithmic growth phase. In various embodiments the

growth rate is determined according to the steps of Table 7 and the acidification rate is determined according to the steps of Table 8.

**[0027]** In various embodiments, at least two of the lactobacilli of the microbial consortia have different carbon utilization profiles. The carbon utilization profile can be selected from the group consisting of: a glycogen utilization profile; a glucose utilization profile; a fructose utilization profile; a mannose utilization profile; a maltodextrin utilization profile; and a mannitol utilization profile. In certain embodiments, the carbon utilization profile is a glycogen utilization profile. In certain other embodiments, the carbon utilization profile is a mannitol utilization profile.

**[0028]** In other embodiments, at least two of the lactobacilli of the microbial consortia have different growth rates, acidification rates, lactic acid production rates, and/or carbon utilization profiles. The carbon utilization profile can be selected from the group consisting of: a glycogen utilization profile; a glucose utilization profile; a fructose utilization profile; a mannose utilization profile; a maltodextrin utilization profile; and a mannitol utilization profile. In certain embodiments, the carbon utilization profile is a glycogen utilization profile. In certain other embodiments, the carbon utilization profile is a mannitol utilization profile. In certain embodiments, the microbial consortium has a growth rate that improves upon the lowest performing individual isolate of the consortium when grown in modified MRS medium, modified MRS + maltodextrin, modified MRS + fructose, modified MRS + mannose, or modified MRS + mannitol. In other embodiments, the microbial consortium has an acidification rate that improves upon the lowest performing individual isolate of the microbial consortium when grown in modified MRS medium, modified MRS + maltodextrin, or modified MRS + glycogen.

**[0029]** In various embodiments, the total bacterial content in the microbial consortium is in the range of  $10^6$  to  $10^{11}$  CFUs. In other embodiments, the total bacterial content in the consortium is in the range of  $10^7$  to  $10^9$  CFUs. In still other embodiments, the total content of at least one lactobacillus isolate present in the microbial consortium is in the range of  $2.0 \times 10^6$  -  $3.0 \times 10^6$  to  $2.0 \times 10^{11}$  -  $3.0 \times 10^{11}$  CFUs. In yet other embodiments, the total content of at least one lactobacillus isolate present in the consortium is in the range of  $2.0 \times 10^7$  -  $3.0 \times 10^7$  to  $2.0 \times 10^9$  -  $3.0 \times 10^9$  CFUs. The at least one lactobacillus isolate can be *Lactobacillus jensenii*.

**[0030]** In certain embodiments, the consortium has a growth rate greater than a two-member consortium comprising the *Lactobacillus jensenii* isolate and any one of the at least

two *Lactobacillus crispatus* isolates. The consortium also may have an acidification rate greater than a two-member consortium comprising the *Lactobacillus jensenii* isolate and any one of the at least two *Lactobacillus crispatus* isolates.

[0031] In other aspects, the disclosure provides a composition comprising a microbial consortium described herein, and a pharmaceutically acceptable buffer, diluent, or excipient. The composition may be in a dosage form, e.g., a suppository, a tablet, a capsule, a film, a gel, or a cream. In one embodiment, the dosage form is a capsule.

[0032] The composition in various embodiments may comprise a total bacterial content in the microbial consortium in the range of  $10^6$  to  $10^{11}$  CFUs. In other embodiments, the total bacterial content in the consortium is in the range of  $10^7$  to  $10^9$  CFUs. In still other embodiments, the total content of at least one lactobacillus isolate present in the microbial consortium is in the range of  $2.0 \times 10^6$ - $3.0 \times 10^6$  to  $2.0 \times 10^{11}$  -  $3.0 \times 10^{11}$  CFUs. In yet other embodiments, the total content of at least one lactobacillus isolate present in the consortium is in the range of  $2.0 \times 10^7$ - $3.0 \times 10^7$  to  $2.0 \times 10^9$ - $3.0 \times 10^9$  CFUs. The at least one lactobacillus isolate can be *Lactobacillus jensenii*.

[0033] In another aspect, the disclosure relates to a kit comprising a microbial consortium of described herein or a composition comprising a microbial consortium described herein, and further comprising a device or applicator suitable for delivery of the microbial consortium or the composition to the vaginal lumen, and optionally instructions for use. In embodiments, kit may include an antiseptic wash and/or cleanse solution, which can be included in a device in some embodiments, such as, but not limited to a syringe or a pre-soaked gauze. In embodiments, the antiseptic wash and/or cleanse solution can comprise chlorhexidine.

[0034] In another aspect, the disclosure provides a method comprising administering the microbial consortium to the urogenital tract of a subject, e.g., a subject having a dysbiotic vaginal microbiome. The microbial consortium or composition can be administered as a capsule. In embodiments, the method results in populating the urogenital tract of the subject with lactobacilli and/or increasing the relative abundance of lactobacilli in the female urogenital tract of the subject, each of which may be assessed by detecting the presence of at least one bacterial isolate of the microbial consortium in the recipient at least 24 hours after administration of said microbial consortium to the recipient. In other embodiments, the detecting step can be performed after at least 48 hours, 72 hours, 7 day, 4 weeks, 8 weeks, and 16 weeks after administration of the microbial consortium to the recipient. In other

embodiments, the detecting step can comprise nucleic acid sequencing of a nucleic acid molecule, a genome or a genome region of at least one bacterial isolate. The sequencing may be whole genome sequencing. In some embodiments, the subject does not exhibit vaginal clinical symptoms, such as vaginal discharge, vaginal itching, vaginal irritation, and/or vaginal malodor. In other embodiments, the subject does not exhibit bacterial vaginosis (BV) clinical symptoms as diagnosed by Amsel criteria or Nugent score.

**[0035]** In another aspect, the disclosure provides a method comprising administering the microbial consortium to the urogenital tract of a subject, e.g., a subject having a dysbiotic vaginal microbiome, which is converted to a eubiotic vaginal microbiome after administering the microbial consortium. In embodiments, the eubiotic vaginal microbiome is stable for at least 1 month, at least 2 months, at least 3 months, at least 4 months, at least 5 months, at least 6 months, or at least up to 1 year after administering the microbial consortium. In still other embodiments the eubiotic vaginal microbiome is stable for at least 1 menstrual cycle, at least 2 menstrual cycles, at least 3 menstrual cycles, at least 4 menstrual cycles, at least 5 menstrual cycles, at least 6 menstrual cycles, and at least 7 menstrual cycles, at least 8 menstrual cycles, at least 9 menstrual cycles, at least 10 menstrual cycles, at least 11 menstrual cycles, and at least 12 menstrual cycles, after administering the microbial consortium.

**[0036]** In other aspects, the disclosure provides a method of preparing a microbial consortium, the method comprising: (i) obtaining a *Lactobacillus jensenii* isolate; (ii) obtaining at least two phenotypically distinct *Lactobacillus crispatus* isolates; and (iii) combining the *Lactobacillus jensenii* isolate and the at least two phenotypically distinct *Lactobacillus crispatus* isolates in a mixture to form the microbial consortium.

**[0037]** In some embodiments, the method of preparing a microbial consortium comprises (i) obtaining a *Lactobacillus jensenii* isolate; (ii) obtaining at least three phenotypically distinct *Lactobacillus crispatus* isolates; and (iii) combining the *Lactobacillus jensenii* isolate and the at least three phenotypically distinct *Lactobacillus crispatus* isolates in a mixture to form the microbial consortium. In some embodiments, the microbial consortia formed is a 3-isolate microbial consortium comprising a *Lactobacillus jensenii* isolate and two phenotypically distinct *Lactobacillus crispatus* isolates. In other embodiments, the microbial consortia formed is a 4-isolate microbial consortium comprising a *Lactobacillus jensenii* isolate and three phenotypically distinct *Lactobacillus crispatus* isolates.



[0038] In embodiments, the *Lactobacillus jensenii* isolate and each of the *Lactobacillus crispatus* isolates of the prepared consortia are ratiometrically equally represented in the mixture. The consortia in various embodiments may comprise a total bacterial content in the range of  $10^6$  to  $10^{11}$  CFUs. In other embodiments, the total bacterial content in the consortium is in the range of  $10^7$  to  $10^9$  CFUs. In still other embodiments, the total content of at least one lactobacillus isolate present in the microbial consortium is in the range of  $2.0 \times 10^6$ - $3.0 \times 10^6$  to  $2.0 \times 10^{11}$ - $3.0 \times 10^{11}$  CFUs. In yet other embodiments, the total content of at least one lactobacillus isolate present in the consortium is in the range of  $2.0 \times 10^7$ - $3.0 \times 10^7$  to  $2.0 \times 10^9$ - $3.0 \times 10^9$  CFUs. The at least one lactobacillus isolate can be *Lactobacillus jensenii*.

[0039] In various embodiments, the *Lactobacillus jensenii* isolate is derived from a first eubiotic vaginal donor as a *Lactobacillus jensenii* isolate that engrafts in a recipient subject following a vaginal microbiome transfer of an SCVMP; and wherein at least one *Lactobacillus crispatus* isolate is derived from a second eubiotic vaginal donor as a *Lactobacillus crispatus* isolate that engrafts in a recipient subject following a vaginal microbiome transfer of an SCVMP.

[0040] In certain embodiments, the *Lactobacillus jensenii* isolate comprises one or more carbohydrate utilization genes comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 9-10, or a nucleotide sequence having at least 98%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9%, or up to 100% of a nucleotide sequence selected from the group consisting of SEQ ID NOs: 9-10.

[0041] In other embodiment, a first of the *Lactobacillus crispatus* isolates comprises one or more carbohydrate utilization genes comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 11-14, or a nucleotide sequence having at least 98%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9%, or up to 100% of a nucleotide sequence selected from the group consisting of SEQ ID NOs: 11-14.

[0042] In another embodiment, a second of the *Lactobacillus crispatus* isolates comprises one or more carbohydrate utilization genes comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 15-18, or a nucleotide sequence having at least 98%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9%, or up to 100% of a nucleotide sequence selected from the group consisting of SEQ ID NOs: 15-18.

[0043] In still another embodiment, a third of the *Lactobacillus crispatus* isolates comprises one or more carbohydrate utilization genes comprising a nucleotide sequence

selected from the group consisting of SEQ ID NOs: 19-22, or a nucleotide sequence having at least 98%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9%, or up to 100% of a nucleotide sequence selected from the group consisting of SEQ ID NOs: 19-22.

**[0044]** In another aspect, the disclosure provides a method of treating a subject having vaginal dysbiosis, comprising administering a therapeutically effective amount of the microbial consortium described herein, a composition described herein, or kit described herein, thereby treating the vaginal dysbiosis. In embodiments, the microbial consortium is a 3-isolate microbial consortium comprising a *Lactobacillus jensenii* isolate and two phenotypically distinct *Lactobacillus crispatus* isolates. In other embodiments, the microbial consortium is a 4-isolate microbial consortium a *Lactobacillus jensenii* isolate and three phenotypically distinct *Lactobacillus crispatus* isolates.

**[0045]** In still another aspect, the disclosure provides a method of treating a subject having bacterial vaginosis (BV), comprising administering a therapeutically effective amount of the consortium described herein, a composition described herein, or kit described herein, thereby treating the bacterial vaginosis (BV). In embodiments, the microbial consortium is a 3-isolate microbial consortium comprising a *Lactobacillus jensenii* isolate and two phenotypically distinct *Lactobacillus crispatus* isolates. In other embodiments, the microbial consortium is a 4-isolate microbial consortium a *Lactobacillus jensenii* isolate and three phenotypically distinct *Lactobacillus crispatus* isolates.

**[0046]** In another aspect, the disclosure provides a method for manufacturing a microbial consortium described herein comprising independently culturing each isolate of the microbial consortium in a bioreactor, harvesting the isolates, and combining the isolates in ratiometrically equal amounts to result in the microbial consortium. The bioreactor can be a 10L, 50L, 500L, 1000L, or up to 3000L bioreactor. The culturing step can result in at least 1g, 25g, 50g, 500g, 1kg, 10kg, or 50kg of lyophilized/dry weight of each isolate.

**[0047]** In some embodiments, the present disclosure provides a microbial consortium comprising a defined population of isolated *lactobacilli* configured to promote engraftment in a female genitourinary tract of a subject, the defined population comprising: a *Lactobacillus jensenii* isolate; a *Lactobacillus crispatus* isolate that thrives in the presence of *Lactobacillus jensenii*; and at least one additional *Lactobacillus crispatus* isolate that thrives independent of the presence of *Lactobacillus jensenii*.

[0048] In some embodiments, the *Lactobacillus crispatus* isolate that thrives independent of the presence of *Lactobacillus jensenii* is capable of engrafting the female genitourinary tract of the subject for at least a pre-determined period of time.

[0049] In some embodiments, the defined population of isolated *lactobacilli* comprises: a relative abundance of 15% to 35% of the total viable cells are *Lactobacillus jensenii* bacteria; a relative abundance of 15% to 35% of the total viable cells are *Lactobacillus crispatus* bacteria that thrive in the presence of *Lactobacillus jensenii*; and a relative abundance of 30% to 70% of the total viable cells are additional *Lactobacillus crispatus* bacteria that thrive independent of the presence of *Lactobacillus jensenii*.

[0050] In some embodiments, the defined population of isolated *lactobacilli* comprises: a relative abundance of about 25% of the total viable cells are *Lactobacillus jensenii* bacteria; a relative abundance of about 25% of the total viable cells are *Lactobacillus crispatus* bacteria that thrive in the presence of *Lactobacillus jensenii*; and a relative abundance of about 50% of the total viable cells are additional *Lactobacillus crispatus* bacteria that thrive independent of the presence of *Lactobacillus jensenii*.

[0051] In some embodiments, wherein: the *Lactobacillus jensenii* isolate comprises a plurality of *Lactobacillus jensenii* bacteria; the *Lactobacillus crispatus* isolate that thrives in the presence of *Lactobacillus jensenii* comprises a plurality of *Lactobacillus crispatus* bacteria that thrives in the presence of *Lactobacillus jensenii*; and the at least one additional *Lactobacillus crispatus* isolate that thrives independent of the presence of *Lactobacillus jensenii* comprises a plurality of additional *Lactobacillus crispatus* bacteria that thrives independent of the presence of *Lactobacillus jensenii*.

[0052] Some aspects of the present disclosure provide a microbial consortium comprising a defined population of isolated *Lactobacilli* configured to promote engraftment in a female genitourinary tract of a subject, the defined population comprising:

a *Lactobacillus jensenii* isolate;

a *Lactobacillus crispatus* isolate that thrives in the presence of *Lactobacillus jensenii*; and

at least two additional *Lactobacillus crispatus* isolate, wherein the at least two additional *Lactobacillus crispatus* isolates have different growth rates, and wherein the at least two additional *Lactobacillus crispatus* isolates thrive independent of the presence of

*Lactobacillus jensenii*.

[0053] In some embodiments, wherein a first of the at least two additional *Lactobacillus crispatus* isolates has a growth rate of 0.27-0.29 hours<sup>-1</sup> and a second of the at least two

additional *Lactobacillus crispatus* isolates has a growth rate of 0.24-0.26 hours<sup>-1</sup> over a period of 8 hours in culture (starting pH of 4.7), wherein the growth rate is calculated using the following equation: natural logarithm (Ln) of (final OD600 divided by initial OD600) divided by (final time minus initial time); or a first of the at least two additional *Lactobacillus crispatus* isolates has a growth rate of 0.35-0.37 hours<sup>-1</sup> and a second of the at least two additional *Lactobacillus crispatus* isolates has a growth rate of 0.31-0.33 hours<sup>-1</sup> over a period of 8 hours in culture (starting pH of 5.2), wherein the growth rate is calculated using the following equation: natural logarithm (Ln) of (final OD600 divided by initial OD600) divided by (final time minus initial time).

[0054] In some embodiments, at least two of the *Lactobacillus crispatus* isolates differ in their growth rates, glycogen utilization rate, rate of producing lactic acid, acidification rate, and/or time to engraftment in the female genitourinary tract.

[0055] In some embodiments, the defined population of isolated lactobacilli comprises:  
a relative abundance of 15% to 35% of the total viable cells are *Lactobacillus jensenii* bacteria;  
a relative abundance of 15% to 35% of the total viable cells are *Lactobacillus crispatus* bacteria that thrive in the presence of *Lactobacillus jensenii*; and  
a relative abundance of 15% to 35% of the total viable cells are additional *Lactobacillus crispatus* bacteria.

[0056] In some embodiments, the defined population of isolated lactobacilli comprises:  
a relative abundance of about 25% of the total viable cells are *Lactobacillus jensenii* bacteria;  
a relative abundance of about 25% of the total viable cells are *Lactobacillus crispatus* bacteria that thrive in the presence of *Lactobacillus jensenii*; and  
a relative abundance of about 50% of the total viable cells are additional *Lactobacillus crispatus* bacteria.

[0057] In some embodiments, the *Lactobacillus jensenii* isolate comprises a plurality of *Lactobacillus jensenii* bacteria;  
the *Lactobacillus crispatus* isolate that thrives in the presence of *Lactobacillus jensenii* comprises a plurality of *Lactobacillus crispatus* bacteria that thrive in the presence of *Lactobacillus jensenii*; and  
each of the at least two additional *Lactobacillus crispatus* isolates comprises a plurality of *Lactobacillus crispatus* bacteria.

[0058] Some aspects of the present disclosure provide a microbial consortium comprising a defined population of isolated lactobacilli configured to promote engraftment in a female genitourinary tract of a subject, the defined population comprising:

[0059] a *Lactobacillus jensenii* isolate;

a *Lactobacillus crispatus* isolate that thrives in the presence of *Lactobacillus jensenii*; and

at least two additional *Lactobacillus crispatus* isolates, wherein the at least two additional *Lactobacillus crispatus* isolates have different acidification rates, and wherein the at least two additional *Lactobacillus crispatus* isolates thrive independent of the presence of *Lactobacillus jensenii*.

[0060] In some embodiments, a first of the at least two additional *Lactobacillus crispatus* isolates has an acidification rate of about  $-0.006 \text{ hours}^{-1}$  and a second of the at least two additional *Lactobacillus crispatus* isolates has an acidification rate of about  $-0.007 \text{ hours}^{-1}$  over a period of 24 hours in culture (starting pH of 4.7), wherein the acidification rate is calculated using the following equation: natural logarithm (Ln) of (final pH divided by initial pH) divided by (final time minus initial time); or

a first of the at least two additional *Lactobacillus crispatus* isolates has an acidification rate of  $-0.008$  to  $-0.006 \text{ hours}^{-1}$  and a second of the at least two additional *Lactobacillus crispatus* isolates has an acidification rate of  $-0.012$  to  $-0.010 \text{ hours}^{-1}$  over a period of 24 hours in culture (starting pH of 5.2), wherein the acidification rate is calculated using the following equation: natural logarithm (Ln) of (final pH divided by initial pH) divided by (final time minus initial time).

[0061] In some embodiments, at least two of the *Lactobacillus crispatus* isolates differ in their growth rates, capability of utilizing glycogen, rate of producing lactic acid, and/or time to engraftment in the female genitourinary tract.

[0062] In some embodiments, the defined population of isolated lactobacilli comprises:

a relative abundance of 15% to 35% of the total viable cells are *Lactobacillus jensenii* bacteria;

a relative abundance of 15% to 35% of the total viable cells are *Lactobacillus crispatus* bacteria that thrives in the presence of *Lactobacillus jensenii*;

a relative abundance of 30% to 70% of the total viable cells are the at least two additional *Lactobacillus crispatus* bacteria.

[0063] In some embodiments, the defined population of isolated lactobacilli comprises:

a relative abundance of about 25% of the total viable cells are *Lactobacillus jensenii* bacteria;

a relative abundance of about 25% of the total viable cells are *Lactobacillus crispatus* bacteria that thrives in the presence of *Lactobacillus jensenii*; and

a relative abundance of about 50% of the total viable cells are *Lactobacillus crispatus* bacteria.

[0064] In some embodiments, the *Lactobacillus jensenii* isolate comprises a plurality of *Lactobacillus jensenii* bacteria;

the *Lactobacillus crispatus* isolate that thrives in the presence of *Lactobacillus jensenii* comprises a plurality of *Lactobacillus crispatus* bacteria that thrive in the presence of *Lactobacillus jensenii*; and

each of the at least two additional *Lactobacillus crispatus* isolates comprises a plurality of *Lactobacillus crispatus* bacteria.

[0065] In some embodiments, the *Lactobacillus crispatus* isolate that thrives in the presence of *Lactobacillus jensenii* is capable of engrafting the female genitourinary tract of the subject for at least a pre-determined period of time in the presence of *Lactobacillus jensenii*.

[0066] In some embodiments, each of the lactobacilli are characterized by a genome having a distinct set of single nucleotide polymorphisms (SNPs), a distinct haplotype signature and/or a distinct set of single nucleotide variants (SNV).

[0067] In some embodiments, the distinct set of SNPs define a SNP profile.

[0068] In some embodiments, each *Lactobacillus* comprises a core genome, wherein the distinct set of SNVs is contained within the core genome.

[0069] In some embodiments, the genome of a *Lactobacilli* comprises a number of SNVs such that the core genome of the *Lactobacilli*, when aligned with a core genome of a reference *Lactobacilli*, has a percent identity greater than a threshold value, the threshold value defining a distinction between two different *Lactobacilli* isolates.

[0070] In some embodiments, the genome of each *Lactobacillus crispatus* isolate comprises a number of SNVs such that the genome of the *Lactobacillus crispatus* isolate, when aligned with a genome of a reference *Lactobacillus crispatus* isolate, has a percent identity greater than or equal to a threshold value, the threshold value defining a distinction between two different *Lactobacillus crispatus* isolates.

[0071] In some embodiments, the percent identity is greater than or equal to 99.81%.

- [0072] In some embodiments, the percent identity is greater than or equal to 99.90%.
- [0073] In some embodiments, the percent identity is greater than or equal to 99.96%.
- [0074] In some embodiments, at least two of the lactobacilli differ in their growth rates.
- [0075] In some embodiments, the *Lactobacillus jensenii* and at least one *Lactobacillus crispatus* differ in their growth rates.
- [0076] In some embodiments, at least two *Lactobacillus crispatus* differ in their growth rates.
- [0077] In some embodiments, the growth rates are growth rates in vitro (e.g., in culture) and/or in vivo.
- [0078] In some embodiments, the growth rate of the *Lactobacillus jensenii* is 0.32-0.36 hours<sup>-1</sup> over a period of 8 hours in culture (starting pH of 4.7), wherein the growth rate is calculated using the following equation: natural logarithm (Ln) of (final OD600 divided by initial OD600) divided by (final time minus initial time); or 0.41-0.43 hours<sup>-1</sup> over a period of 8 hours in culture (starting pH of 5.2), wherein the growth rate is calculated using the following equation: natural logarithm (Ln) of (final OD600 divided by initial OD600) divided by (final time minus initial time).
- [0079] In some embodiments, the growth rate of the *Lactobacillus crispatus* isolate that thrives in the presence of *Lactobacillus jensenii* is 0.35-0.41 hours<sup>-1</sup> over a period of 8 hours in culture (starting pH of 4.7), wherein the growth rate is calculated using the following equation: natural logarithm (Ln) of (final OD600 divided by initial OD600) divided by (final time minus initial time); or 0.39-0.41 hours<sup>-1</sup> over a period of 8 hours in culture (starting pH of 5.2), wherein the growth rate is calculated using the following equation: natural logarithm (Ln) of (final OD600 divided by initial OD600) divided by (final time minus initial time).
- [0080] In some embodiments, the growth rate of a first additional *Lactobacillus crispatus* isolate is 0.27-0.29 hours<sup>-1</sup> over a period of 8 hours in culture (starting pH of 4.7), wherein the growth rate is calculated using the following equation: natural logarithm (Ln) of (final OD600 divided by initial OD600) divided by (final time minus initial time); or 0.35-0.37 hours<sup>-1</sup> over a period of 8 hours in culture (starting pH of 5.2), wherein the growth rate is calculated using the following equation: natural logarithm (Ln) of (final OD600 divided by initial OD600) divided by (final time minus initial time).
- [0081] In some embodiments, the growth rate of a second additional *Lactobacillus crispatus* isolate is 0.24-0.26 hours<sup>-1</sup> over a period of 8 hours in culture (starting pH of 4.7), wherein the growth rate is calculated using the following equation: natural logarithm (Ln) of

(final OD600 divided by initial OD600) divided by (final time minus initial time); or 0.31-0.33 hours<sup>-1</sup> over a period of 8 hours in culture (starting pH of 5.2), wherein the growth rate is calculated using the following equation: natural logarithm (Ln) of (final OD600 divided by initial OD600) divided by (final time minus initial time).

**[0082]** In some embodiments, at least two of the lactobacilli differ in their acidification rates.

**[0083]** In some embodiments, the *Lactobacillus jensenii* and at least one *Lactobacillus crispatus* differ in their acidification rates.

**[0084]** In some embodiments, at least two *Lactobacillus crispatus* differ in their acidification rates. In some embodiments, the acidification rates are acidification rates in vitro (e.g., in culture) and/or in vivo.

**[0085]** In some embodiments, the acidification rate of the *Lactobacillus jensenii* is -0.005 to -0.003 over a period of 24 hours in culture (starting pH of 4.7), wherein the acidification rate is calculated using the following equation: natural logarithm (Ln) of (final pH divided by initial pH) divided by (final time minus initial time); or -0.009 to -0.007 over a period of 24 hours in culture (starting pH of 5.2), wherein the acidification rate is calculated using the following equation: natural logarithm (Ln) of (final pH divided by initial pH) divided by (final time minus initial time).

**[0086]** In some embodiments, the acidification rate of the *Lactobacillus crispatus* isolate that thrives in the presence of *Lactobacillus jensenii* is -0.009 to -0.007 over a period of 24 hours in culture (starting pH of 4.7), wherein the acidification rate is calculated using the following equation: natural logarithm (Ln) of (final pH divided by initial pH) divided by (final time minus initial time); or -0.012 to -0.010 over a period of 24 hours in culture (starting pH of 5.2), wherein the acidification rate is calculated using the following equation: natural logarithm (Ln) of (final pH divided by initial pH) divided by (final time minus initial time).

**[0087]** In some embodiments, the acidification rate of a first additional *Lactobacillus crispatus* isolate is -0.007 to -0.005 hours<sup>-1</sup> over a period of 24 hours in culture (starting pH of 4.7), wherein the acidification rate is calculated using the following equation: natural logarithm (Ln) of (final pH divided by initial pH) divided by (final time minus initial time); or -0.008 to -0.006 hours<sup>-1</sup> over a period of 24 hours in culture (starting pH of 5.2), wherein the acidification rate is calculated using the following equation: natural logarithm (Ln) of (final pH divided by initial pH) divided by (final time minus initial time).



[0088] In some embodiments, the acidification rate of a second additional *Lactobacillus crispatus* isolate is -0.008 to -0.006 hours<sup>-1</sup> over a period of 24 hours in culture (starting pH of 4.7), wherein the acidification rate is calculated using the following equation: natural logarithm (Ln) of (final pH divided by initial pH) divided by (final time minus initial time); or -0.012 to -0.010 hours<sup>-1</sup> over a period of 24 hours in culture (starting pH of 5.2), wherein the acidification rate is calculated using the following equation: natural logarithm (Ln) of (final pH divided by initial pH) divided by (final time minus initial time).

[0089] In some embodiments, at least two of the lactobacilli differ in their capability of utilizing glycogen. In some embodiments, the *Lactobacillus jensenii* and at least one *Lactobacillus crispatus* differ in their capability of utilizing glycogen. In some embodiments, at least two *Lactobacillus crispatus* differ in their capability of utilizing glycogen. In some embodiments, the capability of utilizing glycogen is a capability of utilizing glycogen in vitro (e.g., in culture) and/or in vivo.

[0090] In some embodiments, at least two of the lactobacilli differ in their rate of producing lactic acid. In some embodiments, the *Lactobacillus jensenii* and at least one *Lactobacillus crispatus* differ in their rate of producing lactic acid. In some embodiments, at least two *Lactobacillus crispatus* differ in their rate of producing lactic acid. In some embodiments, the rate of producing lactic acid is a rate of producing lactic acid in vitro (e.g., in culture) and/or in vivo.

[0091] In some embodiments, at least two of the lactobacilli differ in their time to engraftment in the female genitourinary tract. In some embodiments, the *Lactobacillus jensenii* and at least one *Lactobacillus crispatus* differ in their time to engraftment in the female genitourinary tract. In some embodiments, at least two *Lactobacillus crispatus* differ in their time to engraftment in the female genitourinary tract. In some embodiments, the time to engraftment in the female genitourinary tract is determined in vivo.

[0092] In some embodiments, at least one of the lactobacilli isolates is capable of engrafting the female genitourinary tract of the subject within 24 hours after administration to the female genitourinary tract of the subject. In some embodiments, at least one of the lactobacilli isolates is capable of persisting within the female genitourinary tract of the subject for at least 4 days after administration to the female genitourinary tract of the subject.

[0093] In some embodiments, 2, 3 or 4 of the lactobacilli isolates are capable of persisting within the female genitourinary tract of the subject for at least 4, 5, 6, or 7 days after administration to the female genitourinary tract of the subject, optionally wherein 2, 3 or

4 of the lactobacilli isolates are capable of persisting within the female genitourinary tract of the subject for at least 1 menstrual cycle after administration to the female genitourinary tract of the subject.

[0094] In some embodiments, the lactobacilli of the consortium:

are capable of competing with the resident microbiota within the female genitourinary tract of the subject, optionally, without at least partial removal the resident microbiota, e.g., by administration to the genitourinary tract of an antimicrobial agent, such as, e.g., an antibiotic; or are capable of engrafting in the presence of the resident microbiota.

[0095] In some embodiments, each of the lactobacilli of the consortium were obtained from a healthy (e.g., *Lactobacillus*-dominated) human donor. In some embodiments, the lactobacilli comprised in the defined population were obtained from at least two different healthy (e.g., *Lactobacillus*-dominated) human donors, optionally wherein the lactobacilli comprised in the defined population were obtained from three different healthy (e.g., *Lactobacillus*-dominated) human donors. In some embodiments, the *Lactobacillus jensenii* isolate and the *Lactobacillus crispatus* isolate that thrives in the presence of *Lactobacillus jensenii* were obtained from different healthy (e.g., lactobacillus-dominated) human donors. In some embodiments, the at least two additional *Lactobacillus crispatus* isolates were obtained from the same healthy (e.g., lactobacillus-dominated) human donor.

[0096] In some embodiments, the at least two *Lactobacillus crispatus* isolates thrive independent of the presence of *Lactobacillus jensenii* each of the lactobacilli of the consortium are individually propagated, optionally using a large-scale culture method.

[0097] Some aspects of the disclosure provide a microbial consortium comprising a defined population of isolated lactobacilli configured to promote engraftment in a female genitourinary tract of a subject, the defined population comprising:

a *Lactobacillus jensenii* isolate; and

two or three *Lactobacillus crispatus* isolates, wherein at least two of the two or three *Lactobacillus crispatus* isolates have different growth rates, acidification rates, and/or rates of lactic acid production.

[0098] Some aspects of the disclosure provide a pharmaceutical composition comprising any one of the microbial consortia described herein and a pharmaceutically acceptable buffer, diluent or excipients.

[0099] Some aspects of the disclosure provide a method of increasing the relative abundance of lactobacilli in the female genitourinary tract of a subject comprising

administering any one of the microbial consortia described herein to the genitourinary tract of the subject.

**[0100]** In some embodiments, the female genitourinary tract of the subject comprises a bacterial population having a relative abundance of *Lactobacillus* that is less than 90% (e.g., less than 80%, 70%, 60%, 50%, 40%, 30%, or 20%) of the total bacterial population prior to administration of the microbial consortium.

**[0101]** In some embodiments, the female genitourinary tract of the subject comprises a bacterial population having a relative abundance of *Lactobacillus* that is greater than 70% (e.g., greater than 80%, 85%, 90%, 95%, or 98%) of the total bacterial population following administration of the microbial consortium.

**[0102]** In some embodiments, the relative abundance of *Lactobacillus* in the bacterial population of the female genitourinary tract of the subject is increased by at least 5 percentage points (e.g., at least 10, 15, 20, 25, 30, 40, 50, or 60 percentage points) following administration of the microbial consortium.

**[0103]** In some embodiments, any one of the microbial consortia described herein is for use in therapy.

**[0104]** In some embodiments, any one of the microbial consortia described herein is for use in a method of treating dysbiosis of the female genitourinary tract of a subject, said method comprising administering the microbial consortium to the genitourinary tract of the subject, optionally wherein the subject is asymptomatic.

**[0105]** In some embodiments, the any one of the microbial consortia described herein is for use in a method of treating inflammation of the female genitourinary tract of a subject, said method comprising administering the microbial consortium to the genitourinary tract of the subject, optionally wherein the subject is asymptomatic.

**[0106]** Some aspects of the present disclosure provide a method of assembling a microbial consortium comprising two or more isolates belonging to one or more bacterial species, the method comprising:

- obtaining genetic sequencing data obtained from a purported sample of each of the two or more isolates;

- obtaining genetic sequencing data obtained from a reference sample of each of the isolates;

- using at least one computer processor to:

align at least a core portion of the genetic sequencing data from the purported sample to the genetic sequencing data from the reference sample;  
calculate, using the aligned genetic sequencing data, a percent identity value describing a percent of the aligned genetic sequencing data that is identical; and  
determine that the purported sample and the reference sample comprise the same set of bacteria by determining that the percent identity value is greater than or equal to a threshold value defining a distinction between different isolates of the same bacterial species; and  
assembling the microbial consortium using the purported sample.

**[0107]** In some embodiments, the method further comprises determining the threshold value by:

obtaining genetic sequencing data obtained from two or more samples of each of the two or more isolates;  
aligning, using at least one computer processor, at least a core portion of the obtained genetic sequencing data for pairs of the two or more isolates;  
calculating, using the at least one computer processor, a percent identity between each of the aligned pairs; and  
determining the threshold value by determining, using the at least one computer processor, a largest value of the calculated percent identities.

**[0108]** In some embodiments, the bacterial species comprises a species of *Lactobacilli*.

**[0109]** In some embodiments, the bacterial species comprises *Lactobacillus crispatus*.

**[0110]** In some embodiments, determining that the purported sample and the reference sample comprise the same set of bacteria comprises determining that the percent identity is greater than or equal to a threshold value of 99.81%. In some embodiments, determining that the purported sample and the reference sample comprise the same set of bacteria comprises determining that the percent identity is greater than or equal to a threshold value of 99.90%. In some embodiments, determining that the purported sample and the reference sample comprise the same set of bacteria comprises determining that the percent identity is greater than or equal to a threshold value of 99.96%.

**BRIEF DESCRIPTION OF THE DRAWINGS**

- [0111] **FIG. 1** provides a schematic overview of isolation of bacteria from donor vaginal microbiomes.
- [0112] **FIG. 2** provides a selection scheme for identification of individual lactobacilli for inclusion in an exemplary microbial consortium.
- [0113] **FIGs. 3A-C** provide a graph showing that genomic assemblies can be useful in distinguishing one *Lactobacillus crispatus* from another on the basis of percent identity across a segment of bacterial genomes.
- [0114] **FIGs. 4A-B** show the relative abundance of *L. crispatus* bacteria in samples taken from the vaginal tract over multiple visits from a single donor
- [0115] **FIGs. 5A-D** show the relative abundance of *L. crispatus* bacteria in samples taken from example recipient subjects following administration of the donor-obtained material from FIG. 4.
- [0116] **FIG. 6** shows a schematic overview of selection based on pangenome analysis.
- [0117] **FIGs. 7A-7B** show graphs of growth (**FIG. 7A**) and pH (**FIG. 7B**) over time for tested isolates.
- [0118] **FIGs. 8A-8D** show growth rate comparisons between single bacteria and the exemplary 4-member consortium (MC-1).
- [0119] **FIGs. 9A-9D** show acidification change (pH change) comparisons between single bacteria and the exemplary 4-member consortium (MC-1).
- [0120] **FIGs. 10A-10H** show D-lactic acid and L-lactic acid production comparisons between single bacteria and the exemplary 4-member consortium (MC-1).
- [0121] **FIGs. 11A-11B** compares the percentage of D-/L-lactic acid produced across time in single bacteria and the exemplary 4-member consortium (MC-1).
- [0122] **FIGs. 12A-12D** show the abundance of *L. crispatus* and *L. jensenii* within the exemplary 4-member (MC-1) consortium grown on MRS agar plates and relative abundance by species using qPCR over time.
- [0123] **FIG. 13** is a diagram depicting a flowchart of an illustrative process 1300 for assembling a microbial consortium, according to some embodiments of the technology described herein.
- [0124] **FIG. 14** depicts an illustrative implementation of a computer system that may be used in connection with some embodiments of the technology described herein.

[0125] FIG. 15 shows a schematic of the isolates contained in the exemplary 4-member consortium (MC-1).

[0126] FIGs. 16A-16L show the growth and growth rate of individual isolates and the exemplary 4-member consortium (MC-1) grown in MRS medium modified to include 20mg/mL maltodextrin (FIGs. 16A-B), glycogen (FIGs. 16C-D), glucose (FIGs. 16E-F), fructose (FIGs. 16G-H), mannose (FIGs. 16I-J), or mannitol (FIGs. 16K-L).

[0127] FIGs. 17A-17L show the acidification of individual isolates and the exemplary 4-member (MC-1) consortium grown in MRS medium modified to include 20mg/mL maltodextrin (FIGs. 17A-B), glycogen (FIGs. 17C-D), glucose (FIGs. 17E-F), fructose (FIGs. 17G-H), mannose (FIGs. 17I-J), or mannitol (FIGs. 17K-L).

[0128] FIGs. 18A-18F show the relative abundance (measured by qPCR) of the individual isolates of the 4-member consortium (MC-1) over time when grown in MRS medium modified to include 20mg/mL maltodextrin (FIGs. 18A), glycogen (FIGs. 18B), glucose (FIGs. 18C), fructose (FIGs. 18D), mannose (FIGs. 18E), or mannitol (FIGs. 18F).

[0129] FIGs. 19A-19D show comparisons of the exemplary 4-member consortium (MC-1) and competitor strains in growth (FIG. 19A-B) and acidification (FIG. 19C-D).

[0130] FIGs. 20A-20C shows the growth comparison of individual isolates and the exemplary 4-member consortium (MC-1) (FIG. 20A) and the growth comparison of 2-member consortia and the exemplary 4-member consortium (FIG. 20B). A comparison of growth rates is shown after 8h for all individual isolates, 2-member and 4-member consortia (FIG. 20C).

[0131] FIGs. 21A-21C show the acidification comparison of individual isolates and the exemplary 4-member consortium (MC-1) (FIG. 21A) and the acidification comparison of 2-member consortia and the exemplary 4-member consortium (FIG. 21B). A comparison of acidification rates is shown (FIG. 21C) after 8h for all individual isolates, 2-member and 4-member consortia.

[0132] FIGs. 22A-22B show the growth comparison of 3-member consortia and the exemplary 4-member consortium (MC-1) (FIG. 22A) and comparison of growth rates after 8h for 3-member and 4-member consortia (MC-1) (FIG. 22B).

[0133] FIGs. 23A-23B show the acidification comparison of 3-member consortia and the exemplary 4-member consortium (MC-1) (FIG. 23A) and comparison of acidification rates after 8h for 3-member and 4-member consortia (MC-1) (FIG. 23B).

[0134] FIGs. 24A-24C show the relative abundance in percentages of each isolate in the 2-member consortia measured on plates (CFUs/ml based).

[0135] FIGs. 25A-25B show the relative abundance in percentages of each isolate in the 3-member consortia measured by qPCR (CFUs/ml based).

#### DETAILED DESCRIPTION

[0136] A healthy vaginal microbiome is primarily composed of bacterial species from the genus *Lactobacillus* (also referred to as “*Lactobacillus*-dominated”), which provides protection against harmful pathogens and helps maintain a healthy or “eubiotic” vaginal environment. For purposes herein, a “eubiotic” vaginal environment or microbiome excludes a dysbiotic vaginal environment or microbiome. An important function of *Lactobacillus* in the vaginal microbiome is the maintenance of a healthy acidified state in the range of about pH 3.5-4.5 resulting from the metabolic activity of *Lactobacillus* on secreted glycogen/glucose in the vagina to produce lactic acid. Acidic conditions are unfavorable for the growth of many pathogenic microorganisms and pathobionts, including bacteria, protozoa, and viruses. When vaginal dysbiosis (i.e., “dysbiotic” vaginal environment) occurs, characterized by a decline in *Lactobacillus* and an increase in microbial diversity (including in some cases vaginal pathogens), it can elevate the risk of various genital tract conditions such as bacterial vaginosis, inflammation, aerobic vaginitis, vulvovaginal candidiasis, sexually transmitted infections, and complications during pregnancy, including preterm birth. If clinical symptoms are present, such as, e.g., vaginal discharge, vaginal itching, vaginal irritation, and/or vaginal malodor. Antibiotics and antifungals are frequently the first-choice treatments for treating these conditions, however, such treatments can become ineffective and cause conditions to recur.

[0137] Recently, live biotherapeutic products have emerged as an alternative category of therapeutic agents with a wide range of applications, including for potentially addressing vaginal microbiome issues. The United States Food and Drug Administration (FDA) defines live biotherapeutic products as biological products that contain living organisms, such as bacteria, and are intended to prevent, treat, or cure human diseases or conditions, excluding vaccines. Live biotherapeutic products particularly containing *Lactobacillus* species (e.g., *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, or *Lactobacillus gasseri*) for intravaginal or oral use have been available for many years. These products include vaginal

suppositories containing lyophilized *Lactobacillus acidophilus* or other *Lactobacillus* species (e.g., *Lactobacillus rhamnosus*, or *Lactobacillus gasseri*) of human origin as well as various nutritional supplements. However, more effective live biotherapeutic products for vaginal health and for resolving vaginal dysbiosis are much needed. In particular, a need exists for the development of effective consortia of bacterial isolates that may be administered as a live biotherapeutic product for the treatment and resolution of vaginal dysbiosis, vaginal infections, and for improving the overall health profile of subjects receiving such isolates.

**[0138]** Provided herein are microbial consortia comprising a defined population comprising two or more different bacteria capable of engraftment of the human female genitourinary tract, e.g., of a female subject that exhibits, e.g., a dysbiosis of the genitourinary tract. The microbial consortia of the disclosure, in some embodiments, comprise a defined population of isolated lactobacilli configured to promote engraftment in a female genitourinary tract of a subject, the defined population comprising: a *Lactobacillus jensenii* isolate; a *Lactobacillus crispatus* isolate that thrives in the presence of *Lactobacillus jensenii*; and at least one additional *Lactobacillus crispatus* isolate that thrives independent of the presence of *Lactobacillus jensenii* (e.g., two different *Lactobacillus crispatus* isolate, e.g., two different *Lactobacillus crispatus* isolate having different acidification rates). The genitourinary tract includes the reproductive tract, and it is thought that a link exists between the vaginal and endometrial spaces (e.g., that they represent interconnected biogeographical niches) and the exchange of, e.g., microorganisms and immune molecules between the niches (Chen C et al., The microbiota continuum along the female reproductive tract and its relation to uterine-related diseases, Nature Communications vol 8, 875 (2017)).

**[0139]** Preparations of lactic acid-producing bacteria (e.g., including *Lactobacilli*) known in the art generally are not sufficiently capable of colonizing and engrafting *in vivo*, e.g., in the human female genitourinary tract of a diverse set of female subjects, e.g., female subjects exhibiting dysbiosis or infection of the vaginal microbial niche. Bacterial intervention strategies in the art that have attempted to revert dysbiosis in the genitourinary tract by changing the microbial environment to *Lactobacillus* dominance have focused on the selection of individual strains of *Lactobacillus* that are formulated into a probiotic supplement or live biotherapeutic product (LBP) that is then orally or locally administered, sometimes following an antibiotic treatment. However, these strategies lack convincing data showing the reversion of dysbiosis or infection in part due to poor engraftment potential of the strains (Vieira-Baptista et al 2022; van de Wijgert and Verwijs 2020; Thanaboonyawat et



al 2023). The probiotic supplements have often consisted of non-vaginal *Lactobacillus* species such as *L. rhamnosus*, which are not suitable for durable vaginal engraftment, and most supplements use a single species or strain approach. With respect to LBPs, the only extensively studied vaginal LBP is *L. crispatus* CTV-05. In a recent double-blind randomized clinical study, the LBP containing *L. crispatus* CTV-05 did not impact reproductive outcomes in women with abnormal vaginal microbiota undergoing assisted reproductive procedures (Haahr et al. Human Reproduction, Volume 38, Issue Supplement\_1, June 2023), emphasizing that a single-strain product is not effective in affecting the resident microbial communities of genitourinary tract of a diverse set of female subjects.

**[0140]** Four main issues have hampered the discovery of lactic acid-producing bacteria (*e.g.*, including *Lactobacilli*) capable of colonizing and engrafting *in vivo*: (1) the source of the bacteria, (2) the process of identifying and isolating suitable bacteria, (3) the route of administration of the bacteria, and (4) the combination of the plurality of bacteria, *e.g.*, the specific combination of bacterial isolates.

**[0141]** The inventors realized that in order to identify lactic acid-producing bacteria (*e.g.*, including *Lactobacilli*) that are capable of colonizing and engrafting the female reproductive tract *in vivo* the following conditions are favorable:

**[0142]** First, the preparations of lactic acid-producing bacteria (*e.g.*, including *Lactobacilli*) are sourced from a human female donor exhibiting healthy (non-dysbiotic) vaginal microbiota; *i.e.*, the lactic acid-producing bacteria that will be administered to a human female subject are derived from a microbial niche (the donor female's urogenital tract microbiota) that is adapted to survive and thrive in this environment; the lactic acid-producing bacteria are not sourced from food or other animal sources. Provided herein are methods that include bacterial preparations derived from healthy human female donors.

**[0143]** Second, bacteria or bacterial isolates capable of colonizing and engrafting *in vivo* in human subjects are identified *after* the donor preparation (comprising the lactic acid-producing bacteria) has been administered to a female recipient, *e.g.*, after a predetermined period of time, not prior to the administration to a subject which is typically described in the art. Generally, the art describes methods that include first taking a (vaginal) sample from a healthy individual, then isolating and propagating individual isolates *in vitro* (in culture), followed by *in vitro* testing to assess the ability to colonize (*e.g.*, adherence testing to vaginal epithelia cells (VEC) or HeLa cells, and to produce anti-microbial agents, *e.g.*, hydrogen peroxide) *in vitro*, and finally administering an isolate(s) to a recipient. This has the

disadvantage that any *in vitro* testing comprises a reductionist simulation of the *in vivo* environment and results have been mixed as to the extent of predictability of success, using such *in vitro* methods.

**[0144]** Provided herein are microbial consortia comprising defined populations of isolated lactobacilli that were generated using methods that include engrafting bacterial preparations derived from healthy human female donors in human females (*e.g.*, females who exhibit a dysbiosis), followed by taking a (vaginal) sample from the female subject after a predetermined amount of time, then determining which species and isolates were successful in colonizing and engrafting in the female subject's urogenital tract *in vivo*, *e.g.*, by comparing the constituents (bacterial taxa and isolates) of the donor female with those of the female subject (*e.g.*, by nucleic acid sequencing), and then upon obtaining the identification of isolates that are successful in colonizing and engrafting the microbial niche *in vivo*, return to the healthy donor or a stored sample from the donor, from which the original material was prepared (or optionally the recipient's sample) to isolate those specific isolates, propagate and formulate into bacterial preparations and/or (pharmaceutical) compositions. These methods thus remove the need for cumbersome and labor-intensive *in vitro* testing procedures and the uncertainty inherent in the current methods of having to predict from *in vitro* assays the ability to colonize an environment *in vivo*, thereby increasing the likelihood of identifying relevant, highly adapted, and successful isolates capable of colonizing and engrafting *in vivo*. The forward approach of first administering a donor sample comprising a microbial consortium and subsequently identifying the specific species and isolates that were capable of engrafting and improving the dysbiosis in the recipient's vaginal tract, not only provides a higher chance of identifying therapeutically effective isolates that are capable of improving dysbiosis, but also allows for identification of isolate consortia, *e.g.*, preferred combinations of bacterial isolates, which represent a sub-population of the donor sample, which provide for the therapeutic effect.

**[0145]** A healthy vaginal flora is characterized by an acidic environment inhabited predominantly by lactic acid bacteria, primarily, species of *Lactobacillus* (residing in the vaginal microbial niche). The microbial composition in healthy women can differ, though it is typically dominated by *Lactobacillus* species such as *L. crispatus*, *L. iners*, *L. gasseri*, *L. jensenii*, and mixtures thereof. A healthy vagina of a women of child-bearing age is estimated to be dominated by  $10^6$ - $10^9$  colony forming units of lactic acid-producing bacteria (*e.g.*, *Lactobacillus*) per gram of fluid. The species distribution differs between women of different

geographical background, race (*e.g.*, Asian, white women, black, Hispanic), age, lifestyle and the like. The composition of the vaginal flora is also influenced by which specific isolates the woman has inherited from her mother and/or which isolates have migrated from her digestive tract to the urogenital tract. Healthy, fertile women present with a pH of about 3 to 5.5 (more specifically between pH 3.5 and 4.5) in the vagina, primarily as a result of lactic acid production. Vaginal pH undergoes physiological changes from birth to menopause. The increase of vaginal pH is detrimental for the survival of *Lactobacillus* bacteria, but not for other microorganisms. The vaginal lactobacilli are believed to have a protective effect against vaginal colonization by pathogenic microorganisms (*e.g.*, yeast (*Candida albicans*), *Trichomonas vaginalis*, *Neisseria gonorrhoeae*, and *Chlamydia trachomatis*, and viruses, *e.g.*, HIV, HSV-2, and various anaerobes) and prevent the vaginal establishment of, for instance, bacteria that are present in the colon, such as *Gardnerella vaginalis*, *Mobiluncus*, *Bacteroides*, *Prevotella* and *Escherichia coli*.

**[0146]** Several factors may contribute to the disturbance of the vaginal flora. Factors may include, a) use of antibiotics to kill pathogenic bacteria which can lead to significantly reduced levels of lactobacilli in the vagina; b) hormonal changes, in particular changes in estrogen levels, which are observed in several phases of a woman's life (*e.g.*, puberty, pregnancy, childbearing age, pre- and post-menopause); estrogen levels are thought to be associated with *Lactobacillus* levels (dominance) in the vagina; c) sexual intercourse, which can be associated with pH increases (semen generally is alkaline) that may disturb the vaginal flora, because bacteria other than lactobacilli may start to flourish once the vaginal pH increases; d) use of medications, *e.g.*, chemotherapeutics or antimycotics; e) use of birth control products; f) during menstruation; g) insufficient hygiene (*e.g.*, promoting undesirable spread of the microorganisms from rectum to the urogenital area); h) general health status, such as having comorbidities, *e.g.*, being diabetic; and (i) use of excessive hygienic measures in particular the use of vaginal douches.

**[0147]** Disturbance of the vaginal flora may lead to vaginal dysbiosis and vaginal disorders, *e.g.*, candidiasis and bacterial vaginosis, which are two common vaginal disorders that affect women worldwide. Bacterial vaginosis is believed to be the result of displaced vaginal lactic acid-producing bacteria which are replaced by a range of unwanted species such as *Gardnerella vaginalis*, *Bacteroides*, *Mobiluncus*, *Prevotella*, and *Mycoplasma hominis*. Vaginal infections are most often associated with one or more of: *Escherichia*, *Enterococcus*, *Pseudomonas*, *Proteus*, *Klebsiella*, *Streptococcus*, *Staphylococcus*,

*Gardnerella, Ureaplasma, Bacteroides, Peptococcus, Neisseria, Serratia, Sneathia, Megaspheara, Corynebacterium, Clostridium, and Candida.*

**[0148]** Vaginal lactobacilli predominance is thought to play an important role in resistance to infection via production of lactic acid and acidification of the vagina and by production of other antimicrobial products, such as, *e.g.*, hydrogen peroxide. The presence of lactobacilli in the vagina has been linked to decreased frequencies of bacterial vaginosis, yeast vaginitis and sexually transmitted pathogens, including *Neisseria gonorrhea*, *Chlamydia trachomatis*, and *Trichomonas vaginalis* and viruses, *e.g.*, HIV and HSV. *Lactobacillus* dominance varies among ethnic groups (they are thought to be very predominant in Asian and white women but less so in black and Hispanic women, though they still often represent the majority).

**[0149]** Studies have shown that *Lactobacillus*-depleted communities can be transient, lasting just a few days, while in other instances the depleted communities persist for many weeks. Some women with *Lactobacillus*-depleted communities remain asymptomatic, *e.g.*, with respect to vaginal health conditions, and healthy. However, such women may be at higher risk for infections and STDs.

**[0150]** The female urogenital (also known as genitourinary) tract consists of interconnected biogeographical niches, such as the vaginal niche and the endometrial niche. Bacteria from the vaginal microbial community can migrate through the cervix to remote sites of the urogenital tract. Dysbiosis in the vaginal microbial community can result in a dysbiosis at remote sites. Dysbiosis at these sites has been associated with a range of diseases and conditions, including urinary tract infection (UTI), pelvic inflammatory disease (PID), and bacterial vaginosis (BV). In some embodiments, administration of a microbial consortium comprising individual and unique lactobacilli (*e.g.*, *L. jensenii* and/or *L. crispatus* bacteria) described herein to the vagina includes resolving dysbiosis in remote sites of the genitourinary tract.

#### **Lactobacillus jensenii**

**[0151]** *Lactobacillus jensenii* is a Gram-positive, rod-shaped, aerotolerant species of *Lactobacillus* and is a naturally-occurring component of the vaginal microflora. *In vitro* studies have demonstrated that *L. jensenii* inhibit the release of pro-inflammatory mediators from vaginal epithelial cells, promoting anti-microbial defense while not inducing immune mediated inflammation (Witkin SS and Linhares IM, Why do lactobacilli dominate the

human vaginal microbiota? BJOG 2017; 124:606-611). *L. jensenii* contains two D-lactate dehydrogenase genes and one L-lactate dehydrogenase gene for the production of lactic acid.

**[0152]** In some embodiments, the *Lactobacillus jensenii* isolate is obtained from a bacterial sample deposited as DSM Deposit Number 34770 (“DSM 34770”) with DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Inhoffenstr. 7B, D-38124 Braunschweig, on October 4, 2023. The aforementioned deposit was made pursuant to the terms of the Budapest treaty on the international recognition of the deposit of microorganisms for the purposes of patent procedures.

**[0153]** In some embodiments, the *Lactobacillus jensenii* isolate is derived or propagated from a bacterial sample deposited as DSM 34770. In some embodiments, the genome of the *Lactobacillus jensenii* isolate, when aligned with the genome of a bacterial sample deposited as DSM 34770, has a percent identity greater than or equal to a threshold value (*e.g.*, a threshold value of 99.81%, 99.90%, or 99.96%).

**[0154]** In some embodiments, *L. jensenii* (*e.g.*, *L. jensenii* isolates) can be obtained from a donor (*e.g.*, a human donor) from vaginal secretions. In some embodiments, *L. jensenii* (*e.g.*, *L. jensenii* isolates) can be obtained using a method as described in International Patent Publication WO 2022/185121, published on September 9, 2022, the content of which is incorporated herein by reference. For example, vaginal fluid can be collected from a donor in a menstrual cup or using a vaginal swab and plated on a selective culture medium (*e.g.*, diffuse media, such as broth, and/or solid media, such as agar) to allow individual bacterial isolates to colonize and propagate. Non-limiting examples of selective culture media include De Man–Rogosa–Sharpe (MRS) media, Rogosa, M17, Elliker, and Thioglycolate. Individual colonies (*e.g.*, bacterial isolates) can then be selected and propagated by inoculation into large-scale cultures. A large-scale culture method, as used herein, refers to a method of multiplying individual bacterial isolates by letting them reproduce in at least 1 L of a selective diffuse medium (*e.g.*, broth). In some embodiments, a large-scale culture is about 1 L, about 2 L, about 3 L, about 4 L, about 5 L, about 6 L, about 7 L, about 8 L, about 9 L, about 10 L, about 15 L, about 20 L, about 25 L, about 30 L, about 35 L, about 40 L, about 45 L, about 50 L, about 100 L, about 500 L, about 1,000 L, about 2,000 L, about 3,000 L, about 4,000 L, or about 5,000 L or more of a selective diffuse medium (*e.g.*, broth). In some embodiments, a large-scale culture is between 1 L – 50L, between 1 L- 45 L, between 5 L – 45 L, between 5 L – 40 L, between 10 L – 40 L, between 10 L – 35 L, between 15 L – 35 L, between 15 L – 30 L, between 20 L – 30 L, between 20 L – 25 L, between 1 L – 5 L, between

1 L – 10 L, between 5 L – 10 L, between 5 L – 15 L, between 10 L – 15 L, between 10 L – 20 L, between 15 L – 20 L, between 15 L – 25 L, between 20 L – 25 L, between 20 L – 30 L, between 25 L – 30 L, between 25 L – 35 L, between 30 L – 35 L, between 30 L – 40 L, between 35 L – 40 L, between 35 L – 45 L, between 40 L – 45 L, between 40 L – 50 L, between 45 L – 50 L, between 50 L – 250 L, between 100 L – 5,000 L, between 100 L – 2,000 L, between 100 L – 1,000 L, between 500 L – 5,000 L, between 500 L – 3,000 L, between 500 L – 1,500 L, or between 1,000 L – 10,000 L of a selective diffuse medium (*e.g.*, broth).

**[0155]** In some embodiments, a microbial consortium of the present disclosure can include a *L. jensenii* isolate. In some embodiments, a growth rate is an *in vitro* growth rate that is calculated after 8 hours of bacterial growth in culture. In some embodiments, a growth rate is determined for a particular bacteria or consortia after an inoculation of MRS broth at 37 °C with 5 million CFU/mL. Growth rates may be determined according to the following equation: Growth rate equals natural logarithm (Ln) of (final OD600 divided by initial OD600) divided by (final time minus initial time). In some embodiments, an *L. jensenii* included in a microbial consortium of the present disclosure has a growth rate of about 0.2 hours<sup>-1</sup>, about 0.25 hours<sup>-1</sup>, about 0.3 hours<sup>-1</sup>, about 0.35 hours<sup>-1</sup>, about 0.4 hours<sup>-1</sup>, about 0.45 hours<sup>-1</sup>, or about 0.5 hours<sup>-1</sup> when grown in a selective diffuse medium (*e.g.*, MRS) over a period of 8 hours in culture (starting pH of 4.7), wherein the growth rate is calculated using the following equation: natural logarithm (Ln) of (final OD600 divided by initial OD600) divided by (final time minus initial time). In some embodiments, an *L. jensenii* included in a microbial consortium of the present disclosure has a growth rate of between 0.2 – 0.25 hours<sup>-1</sup>, between 0.2 – 0.3 hours<sup>-1</sup>, between 0.2 – 0.35 hours<sup>-1</sup>, between 0.2 – 0.4 hours<sup>-1</sup>, between 0.2 – 0.45 hours<sup>-1</sup>, between 0.2 – 0.5 hours<sup>-1</sup>, between 0.25 – 0.3 hours<sup>-1</sup>, between 0.25 – 0.35 hours<sup>-1</sup>, between 0.25 – 0.4 hours<sup>-1</sup>, between 0.25 – 0.45 hours<sup>-1</sup>, between 0.25 – 0.5 hours<sup>-1</sup>, between 0.3 – 0.35 hours<sup>-1</sup>, between 0.3 – 0.4 hours<sup>-1</sup>, between 0.3 – 0.45 hours<sup>-1</sup>, between 0.3 – 0.5 hours<sup>-1</sup>, between 0.35 – 0.4 hours<sup>-1</sup>, between 0.35 – 0.45 hours<sup>-1</sup>, between 0.35 – 0.5 hours<sup>-1</sup>, between 0.4 – 0.45 hours<sup>-1</sup>, between 0.4 – 0.5 hours<sup>-1</sup>, or between 0.45 – 0.5 hours<sup>-1</sup> when grown in a selective diffuse medium (*e.g.*, MRS) over a period of 8 hours in culture (starting pH of 4.7), wherein the growth rate is calculated using the following equation: natural logarithm (Ln) of (final OD600 divided by initial OD600) divided by (final time minus initial time). In some embodiments, an *L. jensenii* included in a microbial consortium of the present disclosure has a growth rate of about 0.35 hours<sup>-1</sup> (*e.g.*, 0.34 hours<sup>-1</sup>) when

grown in a selective diffuse medium (*e.g.*, MRS) over a period of 8 hours in culture (starting pH of 4.7), wherein the growth rate is calculated using the following equation: natural logarithm (Ln) of (final OD600 divided by initial OD600) divided by (final time minus initial time).

**[0156]** In some embodiments, an *L. jensenii* included in a microbial consortium of the present disclosure has a growth rate after 8 hours of growth in culture of about 0.2 hours<sup>-1</sup>, about 0.25 hours<sup>-1</sup>, about 0.3 hours<sup>-1</sup>, about 0.35 hours<sup>-1</sup>, about 0.4 hours<sup>-1</sup>, about 0.45 hours<sup>-1</sup>, or about 0.5 hours<sup>-1</sup> when grown in a selective diffuse medium (*e.g.*, MRS) over a period of 8 hours in culture (starting pH of 5.2), wherein the growth rate is calculated using the following equation: natural logarithm (Ln) of (final OD600 divided by initial OD600) divided by (final time minus initial time). In some embodiments, an *L. jensenii* included in a microbial consortium of the present disclosure has a growth rate after 8 hours of growth in culture of between 0.2 – 0.25 hours<sup>-1</sup>, between 0.2 – 0.3 hours<sup>-1</sup>, between 0.2 – 0.35 hours<sup>-1</sup>, between 0.2 – 0.4 hours<sup>-1</sup>, between 0.2 – 0.45 hours<sup>-1</sup>, between 0.2 – 0.5 hours<sup>-1</sup>, between 0.25 – 0.3 hours<sup>-1</sup>, between 0.25 – 0.35 hours<sup>-1</sup>, between 0.25 – 0.4 hours<sup>-1</sup>, between 0.25 – 0.45 hours<sup>-1</sup>, between 0.25 – 0.5 hours<sup>-1</sup>, between 0.3 – 0.35 hours<sup>-1</sup>, between 0.3 – 0.4 hours<sup>-1</sup>, between 0.3 – 0.45 hours<sup>-1</sup>, between 0.3 – 0.5 hours<sup>-1</sup>, between 0.35 – 0.4 hours<sup>-1</sup>, between 0.35 – 0.45 hours<sup>-1</sup>, between 0.35 – 0.5 hours<sup>-1</sup>, between 0.4 – 0.45 hours<sup>-1</sup>, between 0.4 – 0.5 hours<sup>-1</sup>, or between 0.45 – 0.5 hours<sup>-1</sup> when grown in a selective diffuse medium (*e.g.*, MRS) over a period of 8 hours in culture (starting pH of 5.2), wherein the growth rate is calculated using the following equation: natural logarithm (Ln) of (final OD600 divided by initial OD600) divided by (final time minus initial time). In some embodiments, an *L. jensenii* included in a microbial consortium of the present disclosure has a growth rate after 8 hours of growth in culture of about 0.4 hours<sup>-1</sup> (*e.g.*, 0.42 hours<sup>-1</sup>) when grown in a selective diffuse medium (*e.g.*, MRS) over a period of 8 hours in culture (starting pH of 5.2), wherein the growth rate is calculated using the following equation: natural logarithm (Ln) of (final OD600 divided by initial OD600) divided by (final time minus initial time).

**[0157]** In some embodiments, an acidification rate is an *in vitro* acidification rate that is calculated after 24 hours of bacterial growth in culture. In some embodiments, an acidification rate is determined for a particular bacteria or consortia after an inoculation of MRS broth at 37 °C with 5 million CFU/mL. Acidification rates may be determined according to the following equation: Acidification rate equals natural logarithm (Ln) of (final pH divided by initial pH) divided by (final time minus initial time). In some embodiments, an

*L. jensenii* included in a microbial consortium of the present disclosure has an acidification rate of about  $-0.003 \text{ hours}^{-1}$ , about  $-0.004 \text{ hours}^{-1}$ , about  $-0.006 \text{ hours}^{-1}$ , about  $-0.007 \text{ hours}^{-1}$ , about  $-0.008 \text{ hours}^{-1}$ , about  $-0.009 \text{ hours}^{-1}$ , about  $-0.010 \text{ hours}^{-1}$ , about  $-0.011 \text{ hours}^{-1}$ , or about  $-0.12 \text{ hours}^{-1}$  when grown in a selective diffuse medium (*e.g.*, MRS) over a period of 24 hours in culture (starting pH of 4.7), wherein the acidification rate is calculated using the following equation: natural logarithm (Ln) of (final pH divided by initial pH) divided by (final time minus initial time).. In some embodiments, an *L. jensenii* included in a microbial consortium of the present disclosure has an acidification rate between  $-0.003$  to  $-0.005 \text{ hours}^{-1}$ , between  $-0.003$  to  $-0.006 \text{ hours}^{-1}$ , between  $-0.003$  to  $-0.007 \text{ hours}^{-1}$ , between  $-0.003$  to  $-0.008 \text{ hours}^{-1}$ , between  $-0.003$  to  $-0.009 \text{ hours}^{-1}$ , between  $-0.003$  to  $-0.010 \text{ hours}^{-1}$ , between  $-0.003$  to  $-0.011 \text{ hours}^{-1}$ , between  $-0.003$  to  $-0.012 \text{ hours}^{-1}$ , between  $-0.004$  to  $-0.006 \text{ hours}^{-1}$ , between  $-0.004$  to  $-0.007 \text{ hours}^{-1}$ , between  $-0.004$  to  $-0.008 \text{ hours}^{-1}$ , between  $-0.004$  to  $-0.009 \text{ hours}^{-1}$ , between  $-0.004$  to  $-0.010 \text{ hours}^{-1}$ , between  $-0.004$  to  $-0.011 \text{ hours}^{-1}$ , between  $-0.004$  to  $-0.012 \text{ hours}^{-1}$ , between  $-0.005$  to  $-0.007 \text{ hours}^{-1}$ , between  $-0.005$  to  $-0.008 \text{ hours}^{-1}$ , between  $-0.005$  to  $-0.009 \text{ hours}^{-1}$ , between  $-0.005$  to  $-0.010 \text{ hours}^{-1}$ , between  $-0.005$  to  $-0.011 \text{ hours}^{-1}$ , between  $-0.001$  to  $-0.012 \text{ hours}^{-1}$ , between  $-0.006$  to  $-0.008 \text{ hours}^{-1}$ , between  $-0.006$  to  $-0.009 \text{ hours}^{-1}$ , between  $-0.006$  to  $-0.010 \text{ hours}^{-1}$ , between  $-0.006$  to  $-0.011 \text{ hours}^{-1}$ , between  $-0.006$  to  $-0.12 \text{ hours}^{-1}$ , between  $-0.007$  to  $-0.009 \text{ hours}^{-1}$ , between  $-0.007$  to  $-0.010 \text{ hours}^{-1}$ , between  $-0.007$  to  $-0.011 \text{ hours}^{-1}$ , between  $-0.007$  to  $-0.012 \text{ hours}^{-1}$ , between  $-0.008$  to  $-0.010 \text{ hours}^{-1}$ , between  $-0.008$  to  $-0.011 \text{ hours}^{-1}$ , between  $-0.008$  to  $-0.012 \text{ hours}^{-1}$ , between  $-0.009$  to  $-0.011 \text{ hours}^{-1}$ , between  $-0.009$  to  $-0.012 \text{ hours}^{-1}$ , or between  $-0.010$  to  $-0.012 \text{ hours}^{-1}$  when grown in a selective diffuse medium (*e.g.*, MRS) over a period of 24 hours in culture (starting pH of 4.7), wherein the acidification rate is calculated using the following equation: natural logarithm (Ln) of (final pH divided by initial pH) divided by (final time minus initial time). In some embodiments, an *L. jensenii* included in a microbial consortium of the present disclosure has an acidification rate of about  $-0.004 \text{ hours}^{-1}$  when grown in a selective diffuse medium (*e.g.*, MRS) over a period of 24 hours in culture (starting pH of 4.7), wherein the acidification rate is calculated using the following equation: natural logarithm (Ln) of (final pH divided by initial pH) divided by (final time minus initial time)..

**[0158]** In some embodiments, an *L. jensenii* included in a microbial consortium of the present disclosure has an acidification rate of about  $-0.003 \text{ hours}^{-1}$ , about  $-0.004 \text{ hours}^{-1}$ , about  $-0.006 \text{ hours}^{-1}$ , about  $-0.007 \text{ hours}^{-1}$ , about  $-0.008 \text{ hours}^{-1}$ , about  $-0.009 \text{ hours}^{-1}$ , about  $-0.010 \text{ hours}^{-1}$ , about  $-0.011 \text{ hours}^{-1}$ , or about  $-0.12 \text{ hours}^{-1}$  when grown in a selective diffuse



medium (*e.g.*, MRS) over a period of 24 hours in culture (starting pH of 5.2), wherein the acidification rate is calculated using the following equation: natural logarithm (Ln) of (final pH divided by initial pH) divided by (final time minus initial time). In some embodiments, an *L. jensenii* included in a microbial consortium of the present disclosure has an acidification rate between -0.003 to -0.005 hours<sup>-1</sup>, between -0.003 to -0.006 hours<sup>-1</sup>, between -0.003 to -0.007 hours<sup>-1</sup>, between -0.003 to -0.008 hours<sup>-1</sup>, between -0.003 to -0.009 hours<sup>-1</sup>, between -0.003 to -0.010 hours<sup>-1</sup>, between -0.003 to -0.011 hours<sup>-1</sup>, between -0.003 to -0.012 hours<sup>-1</sup>, between -0.004 to -0.006 hours<sup>-1</sup>, between -0.004 to -0.007 hours<sup>-1</sup>, between -0.004 to -0.008 hours<sup>-1</sup>, between -0.004 to -0.009 hours<sup>-1</sup>, between -0.004 to -0.010 hours<sup>-1</sup>, between -0.004 to -0.011 hours<sup>-1</sup>, between -0.004 to -0.012 hours<sup>-1</sup>, between -0.005 to -0.007 hours<sup>-1</sup>, between -0.005 to -0.008 hours<sup>-1</sup>, between -0.005 to -0.009 hours<sup>-1</sup>, between -0.005 to -0.010 hours<sup>-1</sup>, between -0.005 to -0.011 hours<sup>-1</sup>, between -0.001 to -0.012 hours<sup>-1</sup>, between -0.006 to -0.008 hours<sup>-1</sup>, between -0.006 to -0.009 hours<sup>-1</sup>, between -0.006 to -0.010 hours<sup>-1</sup>, between -0.006 to -0.011 hours<sup>-1</sup>, between -0.006 to -0.12 hours<sup>-1</sup>, between -0.007 to -0.009 hours<sup>-1</sup>, between -0.007 to -0.010 hours<sup>-1</sup>, between -0.007 to -0.011 hours<sup>-1</sup>, between -0.007 to -0.012 hours<sup>-1</sup>, between -0.008 to -0.010 hours<sup>-1</sup>, between -0.008 to -0.011 hours<sup>-1</sup>, between -0.008 to -0.012 hours<sup>-1</sup>, between -0.009 to -0.011 hours<sup>-1</sup>, between -0.009 to -0.012 hours<sup>-1</sup>, or between -0.010 to -0.012 hours<sup>-1</sup> when grown in a selective diffuse medium (*e.g.*, MRS) over a period of 24 hours in culture (starting pH of 5.2), wherein the acidification rate is calculated using the following equation: natural logarithm (Ln) of (final pH divided by initial pH) divided by (final time minus initial time). In some embodiments, an *L. jensenii* included in a microbial consortium of the present disclosure has an acidification rate of about -0.008 hours<sup>-1</sup> when grown in a selective diffuse medium (*e.g.*, MRS) over a period of 24 hours in culture (starting pH of 5.2), wherein the acidification rate is calculated using the following equation: natural logarithm (Ln) of (final pH divided by initial pH) divided by (final time minus initial time).

**[0159]** In some embodiments, an *L. jensenii* of the present disclosure thrives. The term “thrives”, as used herein, refers to a bacteria (*e.g.*, an *L. jensenii* isolate) that grows or propagates at a higher rate relative to a control bacteria when grown under the same conditions. A control bacteria can be, for example, a different isolate of the same species.

**[0160]** In some embodiments, an *L. jensenii* isolate is said to “thrive” if the *L. jensenii* isolate can grow and propagate *in vitro* or *in vivo*. In some embodiments, an *L. jensenii* isolate is said to “thrive” if the *L. jensenii* isolate has a growth rate that is at least 1%, at least 2%, at least 3%, at least 4%, at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, at

least 10%, at least 15%, at least 20%, or at least 25% higher than the growth rate of a control *L. jensenii* (under the same conditions). In some embodiments, an *L. jensenii* isolate is said to “thrive” if the *L. jensenii* isolate has a growth rate of between 1% - 5%, between 1% - 10%, between 5% - 10%, between 5% - 15%, between 10% - 15%, between 10% - 20%, between 15% - 20%, between 15% - 25%, between 20% - 25%, between 1% - 25%, between 1% - 20%, between 5% - 25%, between 5% - 20%, or between 5% - 15% higher than the growth rate of a control *L. jensenii* (under the same conditions).

#### **Lactobacillus crispatus**

[0161] *Lactobacillus crispatus* is a Gram-positive, rod-shaped, aerotolerant species of *Lactobacillus* and is a naturally-occurring component of the vaginal microflora. A vaginal microbiota dominated by *L. crispatus* is associated with a healthy vagina, as *L. crispatus* has been shown to provide a protective effect against sexually transmitted infections (STIs), bacterial vaginosis (BV), and vulvovaginal candidiasis (VVC) due to its ability to produce lactic acid and bacteriocin (Chee WJY et al., Vaginal microbiota and the potential of *Lactobacillus* derivatives in maintaining vaginal health, Microbial Cell Factories vol 19, 203 (2020)). *L. crispatus* contains two L-lactate dehydrogenase genes and one D-lactate dehydrogenase gene for the production of lactic acid.

[0162] In some embodiments, a *Lactobacillus crispatus* isolate of the present disclosure is obtained from a bacterial sample deposited as DSM Deposit Number 34771 (“DSM 34771”). In some embodiments, a *Lactobacillus crispatus* isolate of the present disclosure is obtained from a bacterial sample deposited as DSM Deposit Number 34772 (“DSM 34772”). In some embodiments, a *Lactobacillus crispatus* isolate of the present disclosure is obtained from a bacterial sample deposited as DSM Deposit Number 34773 (“DSM 34773”). DSM 34771, DSM 34772, and DSM 34773 were deposited with DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Inhoffenstr. 7B, D-38124 Braunschweig, on October 4, 2023. The aforementioned deposits were made pursuant to the terms of the Budapest treaty on the international recognition of the deposit of microorganisms for the purposes of patent procedures.

[0163] In some embodiments, *L. crispatus* (e.g., *L. crispatus* isolates) can be obtained from a donor (e.g., a human donor) from vaginal secretions. In some embodiments, *L. crispatus* (e.g., *L. crispatus* isolates) can be obtained using a method as described in International Patent Publication WO 2022/185121, published on September 9, 2022, the

content of which is incorporated herein by reference. For example, vaginal fluid can be collected from a donor in a menstrual cup or using a vaginal swab and plated on a selective culture medium (*e.g.*, diffuse media, such as broth, and/or solid media, such as agar) to allow individual bacterial isolates to colonize and propagate. Non-limiting examples of selective culture media include De Man–Rogosa–Sharpe (MRS), Rogosa, M17, Elliker, and Thioglycolate. Individual colonies can then be selected and propagated by inoculation into large-scale cultures. A large-scale culture method, as used herein, refers to a method of multiplying individual bacterial isolates by letting them reproduce in at least 1 L of a selective diffuse medium (*e.g.*, broth). In some embodiments, the large-scale culture is about 1 L, about 2 L, about 3 L, about 4 L, about 5 L, about 6 L, about 7 L, about 8 L, about 9 L, about 10 L, about 15 L, about 20 L, about 25 L, about 30 L, about 35 L, about 40 L, about 45 L, about 50 L, about 100 L, about 500 L, about 1,000 L, about 2,000 L, about 3,000 L, about 4,000 L, or about 5,000 L, or more of a selective diffuse medium (*e.g.*, broth). In some embodiments, the large-scale culture is between 1 L – 50L, between 1 L- 45 L, between 5 L – 45 L, between 5 L – 40 L, between 10 L – 40 L, between 10 L – 35 L, between 15 L – 35 L, between 15 L – 30 L, between 20 L – 30 L, between 20 L – 25 L, between 1 L – 5 L, between 1 L – 10 L, between 5 L – 10 L, between 5 L – 15 L, between 10 L – 15 L, between 10 L – 20 L, between 15 L – 20 L, between 15 L – 25 L, between 20 L – 25 L, between 20 L – 30 L, between 25 L – 30 L, between 25 L – 35 L, between 30 L – 35 L, between 30 L – 40 L, between 35 L – 40 L, between 35 L – 45 L, between 40 L – 45 L, between 40 L – 50 L, or between 45 L – 50 L, between 50 L – 250 L, between 100 L – 5,000 L, between 100 L – 2,000 L, between 100 L – 1,000 L, between 500 L – 5,000 L, between 500 L – 3,000 L, between 500 L – 1,500 L, or between 1,000 L – 10,000 L of a selective diffuse medium (*e.g.*, broth).

**[0164]** In some embodiments, a microbial consortium of the present disclosure can include one or more different *L. crispatus* isolates. In some embodiments, a microbial consortium of the present disclosure can include 1, 2, 3, 4, 5, or more different *L. crispatus* isolates.

**[0165]** In some embodiments, a growth rate is an *in vitro* growth rate that is calculated after 8 hours of bacterial growth in culture. In some embodiments, a growth rate is determined for a particular bacteria or consortia after an inoculation of MRS broth at 37 °C with 5 million CFU/mL. Growth rates may be determined according to the following

equation: Growth rate equals natural logarithm (Ln) of (final OD600 divided by initial OD600) divided by (final time minus initial time).

**[0166]** In some embodiments, an *L. crispatus* included in a microbial consortium of the present disclosure has a growth rate of about 0.2 hours<sup>-1</sup>, about 0.25 hours<sup>-1</sup>, about 0.3 hours<sup>-1</sup>, about 0.35 hours<sup>-1</sup>, about 0.4 hours<sup>-1</sup>, about 0.45 hours<sup>-1</sup>, or about 0.5 hours<sup>-1</sup> when grown in a selective diffuse medium (*e.g.*, MRS) at a starting pH of 4.7. In some embodiments, an *L. crispatus* included in a microbial consortium of the present disclosure has a growth rate of between 0.2 – 0.25 hours<sup>-1</sup>, between 0.2 – 0.3 hours<sup>-1</sup>, between 0.2 – 0.35 hours<sup>-1</sup>, between 0.2 – 0.4 hours<sup>-1</sup>, between 0.2 – 0.45 hours<sup>-1</sup>, between 0.2 – 0.5 hours<sup>-1</sup>, between 0.25 – 0.3 hours<sup>-1</sup>, between 0.25 – 0.35 hours<sup>-1</sup>, between 0.25 – 0.4 hours<sup>-1</sup>, between 0.25 – 0.45 hours<sup>-1</sup>, between 0.25 – 0.5 hours<sup>-1</sup>, between 0.3 – 0.35 hours<sup>-1</sup>, between 0.3 – 0.4 hours<sup>-1</sup>, between 0.3 – 0.45 hours<sup>-1</sup>, between 0.3 – 0.5 hours<sup>-1</sup>, between 0.35 – 0.4 hours<sup>-1</sup>, between 0.35 – 0.45 hours<sup>-1</sup>, between 0.35 – 0.5 hours<sup>-1</sup>, between 0.4 – 0.45 hours<sup>-1</sup>, between 0.4 – 0.5 hours<sup>-1</sup>, or between 0.45 – 0.5 hours<sup>-1</sup> when grown in a selective diffuse medium (*e.g.*, MRS) at a starting pH of 4.7. In some embodiments, an *L. crispatus* included in a microbial consortium of the present disclosure has a growth rate of about 0.3 hours<sup>-1</sup> (*e.g.*, 0.28 hours<sup>-1</sup>) when grown in a selective diffuse medium (*e.g.*, MRS) at a starting pH of 4.7. In some embodiments, an *L. crispatus* included in a microbial consortium of the present disclosure has a growth rate of about 0.4 hours<sup>-1</sup> (*e.g.*, 0.38 hours<sup>-1</sup>) when grown in a selective diffuse medium (*e.g.*, MRS) at a starting pH of 4.7. In some embodiments, an *L. crispatus* included in a microbial consortium of the present disclosure has a growth rate of about 0.25 hours<sup>-1</sup> when grown in a selective diffuse medium (*e.g.*, MRS) at a starting pH of 4.7.

**[0167]** In some embodiments, an *L. crispatus* included in a microbial consortium of the present disclosure has a growth rate of about 0.2 hours<sup>-1</sup>, about 0.25 hours<sup>-1</sup>, about 0.3 hours<sup>-1</sup>, about 0.35 hours<sup>-1</sup>, about 0.4 hours<sup>-1</sup>, about 0.45 hours<sup>-1</sup>, or about 0.5 hours<sup>-1</sup> when grown in a selective diffuse medium (*e.g.*, MRS) at a starting pH of 5.2. In some embodiments, an *L. crispatus* included in a microbial consortium of the present disclosure has a growth rate of between 0.2 – 0.25 hours<sup>-1</sup>, between 0.2 – 0.3 hours<sup>-1</sup>, between 0.2 – 0.35 hours<sup>-1</sup>, between 0.2 – 0.4 hours<sup>-1</sup>, between 0.2 – 0.45 hours<sup>-1</sup>, between 0.2 – 0.5 hours<sup>-1</sup>, between 0.25 – 0.3 hours<sup>-1</sup>, between 0.25 – 0.35 hours<sup>-1</sup>, between 0.25 – 0.4 hours<sup>-1</sup>, between 0.25 – 0.45 hours<sup>-1</sup>, between 0.25 – 0.5 hours<sup>-1</sup>, between 0.3 – 0.35 hours<sup>-1</sup>, between 0.3 – 0.4 hours<sup>-1</sup>, between 0.3 – 0.45 hours<sup>-1</sup>, between 0.3 – 0.5 hours<sup>-1</sup>, between 0.35 – 0.4 hours<sup>-1</sup>, between 0.35 – 0.45 hours<sup>-1</sup>, between 0.35 – 0.5 hours<sup>-1</sup>, between 0.4 – 0.45 hours<sup>-1</sup>, between 0.4 – 0.5 hours<sup>-1</sup>, or

between 0.45 – 0.5 hours<sup>-1</sup> when grown in a selective diffuse medium (*e.g.*, MRS) at a starting pH of 5.2. In some embodiments, an *L. crispatus* of the present disclosure has a growth rate of about 0.35 (*e.g.*, 0.36) when grown in a selective diffuse medium (*e.g.*, MRS) at a starting pH of 5.2. In some embodiments, an *L. crispatus* of the present disclosure has a growth rate of about 0.4 hours<sup>-1</sup> when grown in a selective diffuse medium (*e.g.*, MRS) at a starting pH of 5.2. In some embodiments, an *L. crispatus* of the present disclosure has a growth rate of about 0.3 hours<sup>-1</sup> (*e.g.*, 0.32 hours<sup>-1</sup>) when grown in a selective diffuse medium (*e.g.*, MRS) at a starting pH of 5.2.

**[0168]** In some embodiments, an acidification rate is an *in vitro* acidification rate that is calculated after 24 hours of bacterial growth in culture. In some embodiments, an acidification rate is determined for a particular bacteria or consortia after an inoculation of MRS broth at 37 °C with 5 million CFU/mL. Acidification rates may be determined according to the following equation: Acidification rate equals natural logarithm (Ln) of (final pH divided by initial pH) divided by (final time minus initial time).

**[0169]** In some embodiments, an *L. crispatus* of the present disclosure has an acidification rate of about -0.003 hours<sup>-1</sup>, about -0.004 hours<sup>-1</sup>, about -0.006 hours<sup>-1</sup>, about -0.007 hours<sup>-1</sup>, about -0.008 hours<sup>-1</sup>, about -0.009 hours<sup>-1</sup>, about -0.010 hours<sup>-1</sup>, about -0.011 hours<sup>-1</sup>, or about -0.12 hours<sup>-1</sup> when grown in a selective diffuse medium (*e.g.*, MRS) over a period of 24 hours in culture (starting pH of 4.7), wherein the acidification rate is calculated using the following equation: natural logarithm (Ln) of (final pH divided by initial pH) divided by (final time minus initial time). In some embodiments, an *L. crispatus* included in a microbial consortium of the present disclosure has an acidification rate between -0.003 to -0.005 hours<sup>-1</sup>, between -0.003 to -0.006 hours<sup>-1</sup>, between -0.003 to -0.007 hours<sup>-1</sup>, between -0.003 to -0.008 hours<sup>-1</sup>, between -0.003 to -0.009 hours<sup>-1</sup>, between -0.003 to -0.010 hours<sup>-1</sup>, between -0.003 to -0.011 hours<sup>-1</sup>, between -0.003 to -0.012 hours<sup>-1</sup>, between -0.004 to -0.006 hours<sup>-1</sup>, between -0.004 to -0.007 hours<sup>-1</sup>, between -0.004 to -0.008 hours<sup>-1</sup>, between -0.004 to -0.009 hours<sup>-1</sup>, between -0.004 to -0.010 hours<sup>-1</sup>, between -0.004 to -0.011 hours<sup>-1</sup>, between -0.004 to -0.012 hours<sup>-1</sup>, between -0.005 to -0.007 hours<sup>-1</sup>, between -0.005 to -0.008 hours<sup>-1</sup>, between -0.005 to -0.009 hours<sup>-1</sup>, between -0.005 to -0.010 hours<sup>-1</sup>, between -0.005 to -0.011 hours<sup>-1</sup>, between -0.001 to -0.012 hours<sup>-1</sup>, between -0.006 to -0.008 hours<sup>-1</sup>, between -0.006 to -0.009 hours<sup>-1</sup>, between -0.006 to -0.010 hours<sup>-1</sup>, between -0.006 to -0.011 hours<sup>-1</sup>, between -0.006 to -0.12 hours<sup>-1</sup>, between -0.007 to -0.009 hours<sup>-1</sup>, between -0.007 to -0.010 hours<sup>-1</sup>, between -0.007 to -0.011 hours<sup>-1</sup>, between -0.007 to -0.012 hours<sup>-1</sup>, between -0.008 to -0.010

hours<sup>-1</sup>, between -0.008 to -0.011 hours<sup>-1</sup>, between -0.008 to -0.012 hours<sup>-1</sup>, between -0.009 to -0.011 hours<sup>-1</sup>, between -0.009 to -0.012 hours<sup>-1</sup>, or between -0.010 to -0.012 hours<sup>-1</sup> when grown in a selective diffuse medium (*e.g.*, MRS) over a period of 24 hours in culture (starting pH of 4.7), wherein the acidification rate is calculated using the following equation: natural logarithm (Ln) of (final pH divided by initial pH) divided by (final time minus initial time). In some embodiments, an *L. crispatus* included in a microbial consortium of the present disclosure has an acidification rate of about -0.006 hours<sup>-1</sup> when grown in a selective diffuse medium (*e.g.*, MRS) over a period of 24 hours in culture (starting pH of 4.7), wherein the acidification rate is calculated using the following equation: natural logarithm (Ln) of (final pH divided by initial pH) divided by (final time minus initial time). In some embodiments, an *L. crispatus* included in a microbial consortium of the present disclosure has an acidification rate of about -0.007 hours<sup>-1</sup> when grown in a selective diffuse medium (*e.g.*, MRS) over a period of 24 hours in culture (starting pH of 4.7), wherein the acidification rate is calculated using the following equation: natural logarithm (Ln) of (final pH divided by initial pH) divided by (final time minus initial time). In some embodiments, an *L. crispatus* included in a microbial consortium of the present disclosure has an acidification rate of about -0.008 hours<sup>-1</sup> when grown in a selective diffuse medium (*e.g.*, MRS) over a period of 24 hours in culture (starting pH of 4.7), wherein the acidification rate is calculated using the following equation: natural logarithm (Ln) of (final pH divided by initial pH) divided by (final time minus initial time). In some embodiments, an *L. crispatus* included in a microbial consortium of the present disclosure has an acidification rate of between -0.006 to -0.008 hours<sup>-1</sup> when grown in a selective diffuse medium (*e.g.*, MRS) over a period of 24 hours in culture (starting pH of 4.7), wherein the acidification rate is calculated using the following equation: natural logarithm (Ln) of (final pH divided by initial pH) divided by (final time minus initial time).

**[0170]** In some embodiments, an *L. crispatus* included in a microbial consortium of the present disclosure has an acidification rate of about -0.003 hours<sup>-1</sup>, about -0.004 hours<sup>-1</sup>, about -0.006 hours<sup>-1</sup>, about -0.007 hours<sup>-1</sup>, about -0.008 hours<sup>-1</sup>, about -0.009 hours<sup>-1</sup>, about -0.010 hours<sup>-1</sup>, about -0.011 hours<sup>-1</sup>, or about -0.12 hours<sup>-1</sup> when grown in a selective diffuse medium (*e.g.*, MRS) over a period of 24 hours in culture (starting pH of 5.2), wherein the acidification rate is calculated using the following equation: natural logarithm (Ln) of (final pH divided by initial pH) divided by (final time minus initial time). In some embodiments, an *L. crispatus* included in a microbial consortium of the present disclosure has an acidification rate between -0.003 to -0.005 hours<sup>-1</sup>, between -0.003 to -0.006 hours<sup>-1</sup>, between -0.003 to -

0.007 hours<sup>-1</sup>, between -0.003 to -0.008 hours<sup>-1</sup>, between -0.003 to -0.009 hours<sup>-1</sup>, between -0.003 to -0.010 hours<sup>-1</sup>, between -0.003 to -0.011 hours<sup>-1</sup>, between -0.003 to -0.012 hours<sup>-1</sup>, between -0.004 to -0.006 hours<sup>-1</sup>, between -0.004 to -0.007 hours<sup>-1</sup>, between -0.004 to -0.008 hours<sup>-1</sup>, between -0.004 to -0.009 hours<sup>-1</sup>, between -0.004 to -0.010 hours<sup>-1</sup>, between -0.004 to -0.011 hours<sup>-1</sup>, between -0.004 to -0.012 hours<sup>-1</sup>, between -0.005 to -0.007 hours<sup>-1</sup>, between -0.005 to -0.008 hours<sup>-1</sup>, between -0.005 to -0.009 hours<sup>-1</sup>, between -0.005 to -0.010 hours<sup>-1</sup>, between -0.005 to -0.011 hours<sup>-1</sup>, between -0.001 to -0.012 hours<sup>-1</sup>, between -0.006 to -0.008 hours<sup>-1</sup>, between -0.006 to -0.009 hours<sup>-1</sup>, between -0.006 to -0.010 hours<sup>-1</sup>, between -0.006 to -0.011 hours<sup>-1</sup>, between -0.006 to -0.012 hours<sup>-1</sup>, between -0.007 to -0.009 hours<sup>-1</sup>, between -0.007 to -0.010 hours<sup>-1</sup>, between -0.007 to -0.011 hours<sup>-1</sup>, between -0.007 to -0.012 hours<sup>-1</sup>, between -0.008 to -0.010 hours<sup>-1</sup>, between -0.008 to -0.011 hours<sup>-1</sup>, between -0.008 to -0.012 hours<sup>-1</sup>, between -0.009 to -0.011 hours<sup>-1</sup>, between -0.009 to -0.012 hours<sup>-1</sup>, or between -0.010 to -0.012 hours<sup>-1</sup> when grown in a selective diffuse medium (*e.g.*, MRS) over a period of 24 hours in culture (starting pH of 5.2), wherein the acidification rate is calculated using the following equation: natural logarithm (Ln) of (final pH divided by initial pH) divided by (final time minus initial time). In some embodiments, an *L. crispatus* included in a microbial consortium of the present disclosure has an acidification rate of about -0.007 hours<sup>-1</sup> when grown in a selective diffuse medium (*e.g.*, MRS) over a period of 24 hours in culture (starting pH of 5.2), wherein the acidification rate is calculated using the following equation: natural logarithm (Ln) of (final pH divided by initial pH) divided by (final time minus initial time). In some embodiments, an *L. crispatus* included in a microbial consortium of the present disclosure has an acidification rate of about -0.0011 hours<sup>-1</sup> when grown in a selective diffuse medium (*e.g.*, MRS) over a period of 24 hours in culture (starting pH of 5.2), wherein the acidification rate is calculated using the following equation: natural logarithm (Ln) of (final pH divided by initial pH) divided by (final time minus initial time).

**[0171]** In some embodiments, an *L. crispatus* of the present disclosure thrives in the presence of *L. jensenii* bacteria. The term “**thrives**”, as used herein, refers to a bacteria (*e.g.*, an *L. crispatus* isolate) that grows or propagates at a higher rate relative to a control bacteria when grown under the same conditions. A control bacteria can be, for example, a different isolate of the same species.

**[0172]** In some embodiments, an *L. crispatus* isolate is said to “thrive” if the *L. crispatus* isolate can grow and propagate *in vitro* or *in vivo*. In some embodiments, an *L. crispatus* isolate is said to “thrive” if the *L. crispatus* isolate has a growth rate that is at least 1%, at

least 2%, at least 3%, at least 4%, at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, at least 10%, at least 15%, at least 20%, or at least 25% higher than the growth rate of a control *L. crispatus* (under the same conditions). In some embodiments, an *L. crispatus* isolate is said to “thrive” if the *L. crispatus* isolate has a growth rate of between 1% - 5%, between 1% - 10%, between 5% - 10%, between 5% - 15%, between 10% - 15%, between 10% - 20%, between 15% - 20%, between 15% - 25%, between 20% - 25%, between 1% - 25%, between 1% - 20%, between 5% - 25%, between 5% - 20%, or between 5% - 15% higher than the growth rate of a control *L. crispatus* (under the same conditions).

**[0173]** In some embodiments, the *L. crispatus* that thrives in the presence of *L. jensenii* has a growth rate that is at least 1%, at least 2%, at least 3%, at least 4%, at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, at least 10%, at least 15%, at least 20%, or at least 25% higher when grown in the presence of *L. jensenii* than the growth rate of a control *L. crispatus* grown in the presence of *L. jensenii* (under the same conditions). In some embodiments, the *L. crispatus* that thrives in the presence of *L. jensenii* has a growth rate of between 1% - 5%, between 1% - 10%, between 5% - 10%, between 5% - 15%, between 10% - 15%, between 10% - 20%, between 15% - 20%, between 15% - 25%, between 20% - 25%, between 1% - 25%, between 1% - 20%, between 5% - 25%, between 5% - 20%, or between 5% - 15% higher when grown in the presence of *L. jensenii* than the growth rate of a control *L. crispatus* grown in the presence of *L. jensenii* (under the same conditions). In some embodiments, a microbial consortium of the present disclosure contains one or more *L. crispatus* bacteria that thrive in the presence of *L. jensenii*. In some embodiments, a microbial consortium of the present disclosure contains 1, 2, 3, 4, or 5 *L. crispatus* bacteria that thrive in the presence of *L. jensenii*.

**[0174]** In some embodiments, the *Lactobacillus crispatus* isolate that thrives in the presence of *L. jensenii* bacteria is obtained from a bacterial sample deposited as DSM Deposit Number 34772.

**[0175]** In some embodiments, the *Lactobacillus crispatus* isolate that thrives in the presence of *L. jensenii* bacteria is derived or propagated from a bacterial sample deposited as DSM 34770. In some embodiments, the genome of the *Lactobacillus crispatus* isolate that thrives in the presence of *L. jensenii* bacteria, when aligned with the genome of a bacterial sample deposited as DSM 34772, has a percent identity greater than or equal to a threshold value (e.g., a threshold value of 99.81%, 99.90%, or 99.96%).



[0176] Aspects of this disclosure provide, in part, an *L. crispatus* bacteria that thrives in the presence of *L. jensenii* that is capable of engrafting the female genitourinary tract of a subject for at least a pre-determined period of time in the presence of an *L. jensenii* bacteria.

[0177] The engraftment of an *L. crispatus* that thrives in the presence of *L. jensenii* in the female genitourinary tract can persist over a pre-determined period of time in the presence of *L. jensenii*. In some embodiments, the engraftment of an *L. crispatus* that thrives in the presence of *L. jensenii* persists for at least 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 2 weeks, 3 weeks, 4 weeks, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 1 year, 2 years, or more in the presence of *L. jensenii*. In some embodiments, the engraftment of an *L. crispatus* that thrives in the presence of *L. jensenii* persists between 1-7 days, between 1-2 weeks, between 1-3 weeks, between 1-4 weeks, between 2-4 weeks, between 1-2 months, between 2-4 months, between 4-6 months, between 6-8 months, between 8-10 months, between 10-12 months, between 1-6 months, between 6-12 months, between 3-9 months, between 6 months – 1 year, between 6 months – 2 years, or between 1-2 years in the presence of *L. jensenii*. The duration for which engraftment of an *L. crispatus* that thrives in the presence of *L. jensenii* persists in the presence of *L. jensenii* varies depending on several factors known to those of skill in the art, such as the specific medical condition being treated, individual variability (*e.g.*, lifestyle and diet), and underlying causes of the condition.

[0178] In some embodiments, an *L. crispatus* isolate of the present disclosure thrives independent of the presence of *L. jensenii*. In other words, an *L. crispatus* isolate can, in some embodiments, thrive regardless of whether an *L. jensenii* is present. A bacteria (*e.g.* an *L. crispatus* isolate) that thrives independent of another bacteria (*e.g.*, an *L. jensenii* isolate) is one that maintains a similar growth rate when grown in the presence or absence of the another bacteria (*e.g.*, an *L. jensenii* isolate).

[0179] In some embodiments, the *Lactobacillus crispatus* isolate that thrives independent of the presence of *L. jensenii* bacteria is obtained from a bacterial sample deposited as DSM Deposit Number 34771. In some embodiments, the *Lactobacillus crispatus* isolate that thrives independent of the presence of *L. jensenii* bacteria is obtained from a bacterial sample deposited as DSM Deposit Number 34773.

[0180] In some embodiments, the *Lactobacillus crispatus* isolate that thrives independent of the presence of *L. jensenii* bacteria is derived or propagated from a bacterial sample deposited as DSM 34771. In some embodiments, the genome of the *Lactobacillus*

*crispatus* isolate that thrives independent of the presence of *L. jensenii* bacteria, when aligned with the genome of a bacterial sample deposited as DSM 34771, has a percent identity greater than or equal to a threshold value (e.g., a threshold value of 99.81%, 99.90%, or 99.96%).

[0181] In some embodiments, the *Lactobacillus crispatus* isolate that thrives independent of the presence of *L. jensenii* bacteria are derived or propagated from a bacterial sample deposited as DSM 34773. In some embodiments, the genome of the *Lactobacillus crispatus* isolate that thrives independent of the presence of *L. jensenii* bacteria, when aligned with the genome of a bacterial sample deposited as DSM 34773, has a percent identity greater than or equal to a threshold value (e.g., a threshold value of 99.81%, 99.90%, or 99.96%).

[0182] In some embodiments, a microbial consortium of the present disclosure contains one or more *L. crispatus* bacteria that thrive independent of the presence of *L. jensenii*. In some embodiments, a microbial consortium of the present disclosure contains 1, 2, 3, 4, or 5 *L. crispatus* bacteria that thrive independent of the presence of *L. jensenii*. In some embodiments, a microbial consortium of the present disclosure contains two *L. crispatus* bacteria (e.g., two different *L. crispatus* bacteria) that thrive independent of the presence of *L. jensenii*.

[0183] In some embodiments, each *L. crispatus* bacteria that thrives independent of the presence of *L. jensenii* is capable of engrafting the female genitourinary tract of the subject for at least a pre-determined period of time. The engraftment of an *L. crispatus* isolate that thrives independent of the presence of *L. jensenii* in the female genitourinary tract can persist over a pre-determined period of time. In some embodiments, the engraftment of an *L. crispatus* isolate that thrives independent of the presence of *L. jensenii* persists for at least 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 2 weeks, 3 weeks, 4 weeks, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 1 year, 2 years, or more. In some embodiments, the engraftment of an *L. crispatus* isolate that thrives independent of the presence of *L. jensenii* persists between 1-7 days, between 1-2 weeks, between 1-3 weeks, between 1-4 weeks, between 2-4 weeks, between 1-2 months, between 2-4 months, between 4-6 months, between 6-8 months, between 8-10 months, between 10-12 months, between 1-6 months, between 6-12 months, between 3-9 months, between 6 months – 1 year, between 6 months – 2 years, or between 1-2 years. The duration for which engraftment of an *L. crispatus* that thrives independent of the presence of *L. jensenii* persists varies depending on several factors known to those of skill in the art, such

as the specific medical condition being treated, individual variability (e.g., lifestyle and diet), and underlying causes of the condition.

**[0184]** In some embodiments, the one or more *L. crispatus* isolates comprises a first and a second *L. crispatus* isolate. In some embodiments, the first *L. crispatus* isolates has a growth rate of about 0.28 hours<sup>-1</sup> (e.g., when grown in a selective medium starting at pH 4.7). In some embodiments, the first *L. crispatus* isolates has a growth rate of about 0.36 hours<sup>-1</sup> (e.g., when grown in selective medium starting at pH 5.2) In some embodiments, the first *L. crispatus* isolate has an acidification rate of about -0.006 hours<sup>-1</sup> (e.g., when grown in a selective medium starting at pH 4.7). In some embodiments, the first *L. crispatus* isolate has an acidification rate of about -0.007 hours<sup>-1</sup> (e.g., when grown in a selective medium starting at pH 5.2). In some embodiments, the second *L. crispatus* isolates has a growth rate of about 0.38 hours<sup>-1</sup> (e.g., when grown in a selective medium starting at pH 4.7). In some embodiments, the second *L. crispatus* isolate has a growth rate of about 0.4 hours<sup>-1</sup> (e.g., when grown in a selective medium starting at pH 5.2). In some embodiments, the second *L. crispatus* isolate has an acidification rate of about -0.008 hours<sup>-1</sup> (e.g., when grown in a selective medium starting at pH 4.7). In some embodiments, the second *L. crispatus* isolate has acidification rate of about -0.011 hours<sup>-1</sup> (e.g., when grown in a selective medium starting at pH 5.2).

**[0185]** In some embodiments, the one or more *L. crispatus* isolates comprises a first, a second, and a third *L. crispatus* isolate. In some embodiments, the first *L. crispatus* isolate has a growth rate of about 0.28 hours<sup>-1</sup> (e.g., when grown in a selective medium starting at pH 4.7) . In some embodiments, the first *L. crispatus* isolate has a growth rate of about 0.36 hours<sup>-1</sup> (e.g., when grown in selective medium starting at pH 5.2) In some embodiments, the first *L. crispatus* isolate has an acidification rate of about -0.006 hours<sup>-1</sup> (e.g., when grown in a selective medium starting at pH 4.7). In some embodiments, the first *L. crispatus* isolate has an acidification rate of about -0.007 hours<sup>-1</sup> (e.g., when grown in a selective medium starting at pH 5.2). In some embodiments, the second *L. crispatus* isolate has a growth rate of about 0.38 hours<sup>-1</sup> (e.g., when grown in a selective medium starting at pH 4.7). In some embodiments, the second *L. crispatus* isolate has a growth rate of about 0.4 hours<sup>-1</sup> (e.g., when grown in a selective medium starting at pH 5.2). In some embodiments, the second *L. crispatus* isolate has an acidification rate of about -0.008 hours<sup>-1</sup> (e.g., when grown in a selective medium starting at pH 4.7). In some embodiments, the second *L. crispatus* isolate has acidification rate of about -0.011 hours<sup>-1</sup> (e.g., when grown in a selective medium starting

at pH 5.2). In some embodiments, the third *L. crispatus* isolate has a growth rate of about 0.25 hours<sup>-1</sup> (e.g., when grown in a selective medium starting at pH 4.7). In some embodiments, the third *L. crispatus* isolate has a growth rate of about 0.32 hours<sup>-1</sup> (e.g., when grown in a selective medium starting at pH 5.2). In some embodiments, the third *L. crispatus* isolate has an acidification rate of about -0.007 hours<sup>-1</sup> (e.g., when grown in a selective medium starting at pH 4.7). In some embodiments, the third *L. crispatus* isolate has an acidification rate of about -0.011 hours<sup>-1</sup> (e.g., when grown in a selective medium starting at pH 5.2).

**[0186]** In some embodiments, different lactobacilli (e.g., *L. crispatus*, *L. jensenii*) included in microbial consortia of the present disclosure differ in their respective growth rates by at least 1%, at least 2%, at least 3%, at least 4%, at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, at least 10%, at least 15%, at least 20%, or at least 25%. For example, the growth rate of an *L. jensenii* isolate may differ by at least 1%, at least 2%, at least 3%, at least 4%, at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, at least 10%, at least 15%, at least 20%, or at least 25% relative to an *L. crispatus* isolate. In some embodiments, a first lactobacilli isolate (e.g., *L. crispatus* isolate, *L. jensenii* isolate) and a second lactobacilli isolate differ in their growth rates by between 1-2%, between 1-3%, between 1-4%, between 1-5%, between 1-6%, between 1-7%, between 1-8%, between 1-9%, between 1-10%, between 1-11%, between 1-12%, between 1-13%, between 1-14%, between 1-15%, between 2-3%, between 2-4%, between 2-5%, between 2-6%, between 2-7%, between 2-8%, between 2-9%, between 2-10%, between 2-11%, between 2-12%, between 2-13%, between 2-14%, between 2-15%, between 3-4%, between 3-5%, between 3-6%, between 3-7%, between 3-8%, between 3-9%, between 3-10%, between 3-11%, between 3-12%, between 3-13%, between 3-14%, between 3-15%, between 4-5%, between 4-6%, between 4-7%, between 4-8%, between 4-9%, between 4-10%, between 4-11%, between 4-12%, between 4-13%, between 4-14%, between 4-15%, between 5-6%, between 5-7%, between 5-8%, between 5-9%, between 5-10%, between 5-11%, between 5-12%, between 5-13%, between 5-14%, between 5-15%, between 6-7%, between 6-8%, between 6-9%, between 6-10%, between 6-11%, between 6-12%, between 6-13%, between 6-14%, between 6-15%, between 7-8%, between 7-9%, between 7-10%, between 7-11%, between 7-12%, between 7-13%, between 7-14%, between 7-15%, between 8-9%, between 8-10%, between 8-11%, between 8-12%, between 8-13%, between 8-14%, between 8-15%, between 9-10%, between 9-11%, between 9-12%, between 9-13%, between 9-14%, between 9-15%, between 10-11%, between 10-

12%, between 10-13%, between 10-14%, between 10-15%, between 11-12%, between 11-13%, between 11-14%, between 11-15%, between 12-13%, between 12-14%, between 12-15%, between 13-14%, between 13-15%, or between 14-15%.

**[0187]** In some embodiments, the growth rate of a lactobacilli isolate refers to its growth rate at a pH of around 3, around 3.5, around 4, around 4.5, around 5, around 5.5, around 6, or around 6.5. In some embodiments, the growth rate of a lactobacilli isolate refers to its growth rate at a pH of between 3-6.5, between 3-5.5, between 3-5, between 3-4.5, between 3-4, between 3.5-5.5, between 3.5-5, or between 3.5-4.5. In some embodiments, the growth rate of a lactobacilli isolate refers to its growth rate at a pH of about 4.7 or 5.2. In some embodiments, the growth rate of a lactobacilli isolate refers to its growth rate at a temperature of around 5°C, around 10°C, around 15°C, around 20°C, around 25°C, around 30°C, around 35°C, around 40°C, around 45°C, around 50°C, or around 55°C. In some embodiments, the growth rate of a lactobacilli isolate refers to its growth rate at a temperature of between 5°C – 15°C, between 10°C – 20°C, between 15°C – 25°C, between 20°C – 30°C, between 25°C – 35°C, between 30°C – 40°C, between 35°C – 44°C, between 40°C – 50°C, or between 45°C – 55°C. In some embodiments, the growth rate of a lactobacilli isolate refers to its growth rate at a temperature of about 37 °C.

**[0188]** In some embodiments, a *Lactobacillus* isolate of the present disclosure (*e.g.*, *L. jensenii* isolate, *L. crispatus* isolate) is capable of utilizing glycogen. A bacteria that is capable of utilizing glycogen is one that possesses the enzymatic machinery necessary to break down glycogen (*e.g.*, glycogen phosphorylase), enabling the bacteria to use glycogen as a source of energy and carbon for its metabolic processes. Lactobacilli that are capable of utilizing glycogen into glucose can then utilize glucose through fermentation pathways to produce lactic acid, which contributes to the maintenance of an acidic environment in the vagina (Amabebe E and Anumba DOC, The Vaginal Microenvironment: The Physiologic Role of lactobacilli, Front. Med. Vol. 8 (2018)). Methods to identify bacteria capable of utilizing glycogen are known to those in the art and may include, for example, culturing bacteria in a glycogen-rich agar or broth, performing a reducing sugar assay or growth inhibition assay, or evaluating polysaccharide breakdown products using electrospray ionization.

**[0189]** In some embodiments, a *Lactobacillus* isolate of the present disclosure (*e.g.*, *L. jensenii* isolate, *L. crispatus* isolate) is capable of utilizing a variety of carbohydrate substrates in order to grow and propagate. A *Lactobacillus* bacteria may be capable of

utilizing D-ribose, D-galactose, D-glucose, D-fructose, D-mannose, D-mannitol, Methyl  $\alpha$  D-glucopyranoside, N-acetylglucosamine, Amygdalin, Arbutin, Esculin ferric citrate, Salicin, D-cellobiose, D-maltose, D-lactose, D-saccharose (sucrose), D-trehalose, starch, glycogen, and/or gentiobiose in order to grow and propagate. In some embodiments, a *L. jensenii* bacteria is capable of utilizing D-ribose, D-galactose, D-glucose, D-fructose, D-mannose, D-mannitol, Methyl  $\alpha$  D-glucopyranoside, N-acetylglucosamine, Amygdalin, Arbutin, Esculin ferric citrate, Salicin, D-cellobiose, D-maltose, D-lactose, D-saccharose (sucrose), D-trehalose, Starch, Glycogen, and/or Gentiobiose in order to grow and propagate. In some embodiments, a *L. crispatus* bacteria is capable of utilizing D-ribose, D-galactose, D-glucose, D-fructose, D-mannose, D-mannitol, Methyl  $\alpha$  D-glucopyranoside, N-acetylglucosamine, Amygdalin, Arbutin, Esculin ferric citrate, Salicin, D-cellobiose, D-maltose, D-lactose, D-saccharose (sucrose), D-trehalose, Starch, Glycogen, and/or Gentiobiose in order to grow and propagate. A microbial consortia comprising one or more *Lactobacillus* bacteria (e.g., *L. jensenii*, *L. crispatus*) can grow and propagate in the presence of D-ribose, D-galactose, D-glucose, D-fructose, D-mannose, D-mannitol, Methyl  $\alpha$  D-glucopyranoside, N-acetylglucosamine, Amygdalin, Arbutin, Esculin ferric citrate, Salicin, D-cellobiose, D-maltose, D-lactose, D-saccharose (sucrose), D-trehalose, Starch, Glycogen, and/or Gentiobiose.

**[0190]** A *Lactobacillus* bacteria may include one or more genes that encode proteins that allow for the utilization of a variety of carbohydrate substrates. For example, a *Lactobacillus* bacteria may include one or more genes that encode proteins that allow for the utilization of D-ribose, D-galactose, D-glucose, D-fructose, D-mannose, D-mannitol, Methyl  $\alpha$  D-glucopyranoside, N-acetylglucosamine, Amygdalin, Arbutin, Esculin ferric citrate, Salicin, D-cellobiose, D-maltose, D-lactose, D-saccharose (sucrose), D-trehalose, Starch, Glycogen, and/or Gentiobiose.

**[0191]** In some embodiments, a *Lactobacillus* bacteria (e.g., a *L. crispatus* bacteria) includes the *pulA* gene (which encodes for pullanase) which allows for glycogen utilization in the bacteria. In some embodiments, a *Lactobacillus* bacteria (e.g., a *L. crispatus* bacteria) includes a full-length *pulA* gene (which encodes for pullanase) which allows for glycogen utilization in the bacteria. In some embodiments, a *Lactobacillus* bacteria (e.g., a *L. crispatus* bacteria) includes a *pulA* gene having a deletion or insertion in the *pulA* gene which renders the resulting protein at least partially dysfunctional.

[0192] Bacteria that possess the enzyme lactate dehydrogenase are capable of producing lactic acid (D-lactate and/or L-lactate), which results in the acidification of the environment and inhibits the growth of pathogenic bacteria and yeast in the vagina. Techniques to evaluate lactic acid production rates are known to those of skill in the art and may involve measuring the pH of a lactobacilli sample (e.g., a culture of lactobacilli) or by using a lactate assay kit (e.g., a colorimetric or fluorometric lactate assay kit) over time. In some embodiments, lactobacilli (e.g., *L. crispatus*, *L. jensenii*) of the present disclosure produces around 1 g/L, around 2 g/L, around 3 g/L, around 4 g/L, around 5 g/L, around 6 g/L, around 7 g/L, around 8 g/L, around 9 g/L, around 10 g/L, around 11 g/L, around 12 g/L, around 13 g/L, around 14 g/L, or around 15 g/L of lactic acid (D-lactate and/or L-lactate). In some embodiments, lactobacilli (e.g., *L. crispatus*, *L. jensenii*) of the present disclosure produces between 1-2 g/L, between 1-3 g/L, between 1-4 g/L, between 1-5 g/L, between 1-6 g/L, between 1-7 g/L, between 1-8 g/L, between 1-9 g/L, between 1-10 g/L, between 1-11 g/L, between 1-12 g/L, between 1-13 g/L, between 1-14 g/L, between 1-15 g/L, between 2-3 g/L, between 2-4 g/L, between 2-5 g/L, between 2-6 g/L, between 2-7 g/L, between 2-8 g/L, between 2-9 g/L, between 2-10 g/L, between 2-11 g/L, between 2-12 g/L, between 1-13 g/L, between 2-14 g/L, between 2-15 g/L, between 3-4 g/L, between 3-5 g/L, between 3-6 g/L, between 3-7 g/L, between 3-8 g/L, between 3-9 g/L, between 3-10 g/L, between 3-11 g/L, between 3-12 g/L, between 3-13 g/L, between 3-14 g/L, between 3-15 g/L, between 4-5 g/L, between 4-6 g/L, between 4-7 g/L, between 4-8 g/L, between 4-9 g/L, between 4-10 g/L, between 4-11 g/L, between 4-12 g/L, between 4-13 g/L, between 4-14 g/L, between 4-15 g/L, between 5-6 g/L, between 5-7 g/L, between 5-8 g/L, between 5-9 g/L, between 5-10 g/L, between 5-11 g/L, between 5-12 g/L, between 5-13 g/L, between 5-14 g/L, between 5-15 g/L, between 6-7 g/L, between 6-8 g/L, between 6-9 g/L, between 6-10 g/L, between 6-11 g/L, between 6-12 g/L, between 6-13 g/L, between 6-14 g/L, between 6-15 g/L, between 7-8 g/L, between 7-9 g/L, between 7-10 g/L, between 7-11 g/L, between 7-12 g/L, between 7-13 g/L, between 7-14 g/L, between 7-15 g/L, between 8-9 g/L, between 8-10 g/L, between 8-11 g/L, between 8-12 g/L, between 8-13 g/L, between 8-14 g/L, between 8-15 g/L, between 9-10 g/L, between 9-11 g/L, between 9-12 g/L, between 9-13 g/L, between 9-14 g/L, between 9-15 g/L, between 10-11 g/L, between 10-12 g/L, between 10-13 g/L, between 10-14 g/L, between 10-15 g/L, between 11-12 g/L, between 11-13 g/L, between 11-14 g/L, between 11-15 g/L, between 12-13 g/L, between 12-14 g/L, between 12-15 g/L, between 13-14 g/L, between 13-15 g/L, or between 14-15 g/L of lactic acid (D-lactate and/or L-lactate).

**[0193]** In some embodiments, lactobacilli (*e.g.*, *L. crispatus*, *L. jensenii*) of the present disclosure produces lactic acid (D-lactate and/or L-lactate) at a rate of about 0.1, about 0.2, about 0.3, about 0.4, about 0.5, about 0.6, about 0.7, about 0.8, about 0.9, or about 1 gram of lactic acid (D-lactate and/or L-lactate) per gram of biomass per hour (g/g/h). In some embodiments, lactobacilli (*e.g.*, *L. crispatus*, *L. jensenii*) of the present disclosure produces lactic acid (D-lactate and/or L-lactate) at a rate of between 0.1-0.2 g/g/h, between 0.1-0.3 g/g/h, between 0.1-0.4 g/g/h, between 0.1-0.5 g/g/h, between 0.1-0.6 g/g/h, between 0.1-0.7 g/g/h, between 0.1-0.8 g/g/h, between 0.1-0.9 g/g/h, between 0.1-1.0 g/g/h, between 0.2-0.3 g/g/h, between 0.2-0.4 g/g/h, between 0.2-0.5 g/g/h, between 0.2-0.6 g/g/h, between 0.2-0.7 g/g/h, between 0.2-0.8 g/g/h, between 0.2-0.9 g/g/h, between 0.3-0.4 g/g/h, between 0.3-0.5 g/g/h, between 0.3-0.6 g/g/h, between 0.3-0.7 g/g/h, between 0.3-0.8 g/g/h, between 0.3-0.9 g/g/h, between 0.3-1.0 g/g/h, between 0.4-0.5 g/g/h, between 0.4-0.6 g/g/h, between 0.4-0.7 g/g/h, between 0.4-0.8 g/g/h, between 0.4-0.9 g/g/h, between 0.4-1.0 g/g/h, between 0.5-0.6 g/g/h, between 0.5-0.7 g/g/h, between 0.5-0.8 g/g/h, between 0.5-0.9 g/g/h, between 0.5-1.0 g/g/h, between 0.6-0.7 g/g/h, between 0.6-0.8 g/g/h, between 0.6-0.9 g/g/h, between 0.6-1.0 g/g/h, between 0.7-0.8 g/g/h, between 0.7-0.9 g/g/h, between 0.7-1.0 g/g/h, between 0.8-0.9 g/g/h, between 0.8-1.0 g/g/h, or between 0.9-1.0 g/g/h.

**[0194]** Lactic acid can be composed of two enantiomers of lactate, D-lactate and L-lactate, and can be quantified using chiral chromatography (*e.g.*, chiral high performance liquid chromatography). In some embodiments, D-lactate represents 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% of total lactate. In some embodiments, D-lactate represents between 10% and 15%, between 10% and 20%, between 10% and 25%, between 10% and 30%, between 10% and 35%, between 10% and 40%, between 10% and 45%, between 10% and 50%, between 10% and 55%, between 10% and 60%, between 10% and 65%, between 10% and 70%, between 10% and 75%, between 10% and 80%, between 10% and 85%, between 10% and 90%, between 10% and 95%, between 10% and 100%, between 25% and 30%, between 25% and 35%, between 50% and 90%, between 50% and 95%, between 50% and 100%, between 55% and 60%, between 55% and 65%, between 55% and 70%, between 55% and 75%, between 55% and 80%, between 55% and 85%, between 55% and 90%, between 55% and 95%, between 55% and 100%, between 60% and 65%, between 60% and 70%, between 60% and 75%, between 60% and 80%, between 60% and 85%, between 60% and 90%, between 60% and 95%, between 60% and 100%, between 65% and 70%, between 65% and 75%, between 65%



and 80%, between 65% and 85%, between 65% and 90%, between 65% and 95%, between 65% and 100%, between 70% and 75%, between 70% and 80%, between 70% and 85%, between 70% and 90%, between 70% and 95%, between 70% and 100%, between 75% and 80%, between 75% and 85%, between 75% and 90%, between 75% and 95%, between 75% and 100%, between 80% and 85%, between 80% and 90%, between 80% and 95%, between 80% and 100%, between 85% and 90%, between 85% and 95%, between 85% and 100%, between 90% and 95%, between 90% and 100%, between 95% and 100% of total lactate. In some embodiments, L-lactate represents 0%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, or 50% of total lactate. In some embodiments, L-lactate represents between 0% - 5%, between 0% - 10%, between 0% - 15%, between 0% - 20%, between 0% - 25%, between 0% - 30%, between 0% - 35%, between 0% - 40%, between 0% - 45%, between 0% - 50%, between 5% - 10%, between 5% - 15%, between 5% - 20%, between 5% - 25%, between 5% - 30%, between 5% - 35%, between 5% - 40%, between 5% - 45%, between 5% - 50%, between 10% - 15%, between 10% - 20%, between 10% - 25%, between 10% - 30%, between 10% - 35%, between 10% - 40%, between 10% - 45%, between 10% - 50%, between 15% - 20%, between 15% - 25%, between 15% - 30%, between 15% - 35%, between 15% - 40%, between 15% - 45%, between 15% - 50%, between 20% - 25%, between 20% - 30%, between 20% - 35%, between 20% - 40%, between 20% - 45%, between 20% - 50%, between 25% - 30%, between 25% - 35%, between 25% - 40%, between 25% - 45%, between 25% - 50%, between 30% - 35%, between 30% - 40%, between 30% - 45%, between 30% - 50%, between 35% - 40%, between 35% - 45%, between 35% - 50%, between 40% - 45%, between 40% - 50%, or between 45% - 50% of total lactate.

**[0195]** The term “**acidification rate**,” as used herein, refers to the rate at which bacteria (*e.g.*, lactobacilli) lower the pH (acidity) of their surrounding environment, *e.g.*, through the production of lactic acid. The acidification rate can be monitored by measuring the change in pH over time (*e.g.*, between 0-8 hours, between 8-24 hours). In some embodiments, the natural log of the acidification rate is about  $-0.020 \text{ hour}^{-1}$ , about  $-0.015 \text{ hour}^{-1}$ , about  $-0.010 \text{ hour}^{-1}$ , or about  $-0.005 \text{ hour}^{-1}$ . In some embodiments, the acidification rate is between  $-0.020$  and  $-0.015 \text{ hour}^{-1}$ , between  $-0.020$  and  $-0.010 \text{ hour}^{-1}$ , between  $-0.020$  and  $-0.005 \text{ hour}^{-1}$ , between  $-0.020$  and  $0.000 \text{ hour}^{-1}$ , between  $-0.015$  and  $-0.010 \text{ hour}^{-1}$ , between  $-0.015$  and  $-0.005 \text{ hour}^{-1}$ , between  $-0.015$  and  $0.000 \text{ hour}^{-1}$ , between  $-0.010$  and  $-0.005 \text{ hour}^{-1}$ , between  $-0.010$  and  $0.000 \text{ hour}^{-1}$ , or between  $-0.005$  and  $0.000 \text{ hour}^{-1}$ .

**[0196]** The terms “**time to engraftment**” and “**successful engraftment at a pre-determined time period**,” as used herein, refer to the success and efficiency with which the transplanted bacteria (*e.g.*, lactobacilli) become established (*e.g.*, colonize and propagate) within a subject (*e.g.*, a subject’s genitourinary tract, *e.g.*, the cervix, vagina, uterus, and/or fallopian tubes of a subject). The “**female genitourinary tract**,” as used herein, refers to the female reproductive and urinary systems including ovaries, fallopian tubes, uterus, cervix vagina, external genitalia, and the urinary tract. Engraftment can be influenced by numerous factors, including the health of the subject’s vaginal environment, the quality and diversity of the transplanted bacteria (*e.g.*, lactobacilli), and the specific method used in the transplantation procedure. The time to engraftment can be evaluated by assessing over time the subject’s clinical symptoms, vaginal pH, microbiome composition and diversity (*e.g.*, using sequencing or 16S rRNA profiling), and/or abundance of lactobacilli (*e.g.*, using qPCR).

**[0197]** In some embodiments, a *Lactobacillus* isolate of the present disclosure has a time to engraftment of about 6, 12, 18, 24, 36, 48, or 72 hours. In other words, a *Lactobacillus* isolate may, in some embodiments, engraft in the genitourinary tract of a subject within 6, 12, 18, 24, 36, 48, or 72 hours following administration of the microbial consortium to the subject. In some embodiments, a *Lactobacillus* isolate of the present disclosure has a time to engraftment of about 1-6, 5-10, 6-12, 10-15, 12-18, 15-20, 18-24, 20-30, 24-36, 30-40, 36-48, or 36-72 hours.

**[0198]** In some embodiments, at least one *Lactobacillus* isolate of a microbial consortium of the present disclosure will have a successful engraftment at a pre-determined time period in the genitourinary tract of a subject administration of the microbial consortium. In some embodiments, a pre-determined time period is about 6, 12, 18, 24, 36, 48, or 72 hours. In some embodiments, a pre-determined time period is about 1-6, 5-10, 6-12, 10-15, 12-18, 15-20, 18-24, 20-30, 24-36, 30-40, 36-48, or 36-72 hours.

**[0199]** In some embodiments, a microbial consortium of the present disclosure is comprised of at least two lactobacilli isolates (*e.g.*, *L. jensenii* isolates, *L. crispatus* isolates) that differ in each of the functional characteristics (*e.g.*, growth rate, capability of utilizing a carbon substrate (*e.g.*, glycogen), rate of lactic acid production, acidification rate, and time to engraftment in the female genitourinary tract). In some embodiments, a microbial consortium of the present disclosure is comprised of at least two *L. crispatus* isolates that differ in each of the functional characteristics (*e.g.*, growth rate, capability of utilizing a carbon substrate (*e.g.*,

glycogen), rate of lactic acid production, acidification rate, and time to engraftment in the female genitourinary tract).

**[0200]** In some embodiments, a microbial consortium of the present disclosure is comprised of at least two lactobacilli isolates (*e.g.*, *L. jensenii* isolates, *L. crispatus* isolates) that differ in their growth rates. In some embodiments, a microbial consortium of the present disclosure is comprised of *L. jensenii* and at least one *L. crispatus* that differ in their growth rates. In some embodiments, a microbial consortium of the present disclosure is comprised of at least two *L. crispatus* that differ in their growth rates.

**[0201]** In some embodiments, at least two lactobacilli (*e.g.*, *L. crispatus* isolate, *L. jensenii* isolate) of the present disclosure differ in their acidification rates. In some embodiments, a first lactobacilli (*e.g.*, *L. crispatus* isolate, *L. jensenii* isolate) has an acidification rates that is at least 1%, at least 2%, at least 3%, at least 4%, at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, at least 10%, at least 11%, at least 12%, at least 13%, at least 14%, at least 15%, at least 20%, at least 25%, at least 35%, at least 50%, or at least 75% higher than the acidification rate of a second lactobacilli (*e.g.*, *L. crispatus* isolate, *L. jensenii* isolate). In some embodiments, a first lactobacilli (*e.g.*, *L. crispatus* isolate, *L. jensenii* isolate) has an acidification rates that is between 1-2%, between 1-3%, between 1-4%, between 1-5%, between 1-6%, between 1-7%, between 1-8%, between 1-9%, between 1-10%, between 1-11%, between 1-12%, between 1-13%, between 1-14%, between 1-15%, between 4-10%, between 4-11%, between 4-12%, between 4-13%, between 4-14%, between 4-15%, between 5-6%, between 5-7%, between 5-8%, between 5-9%, between 5-10%, between 5-11%, between 5-12%, between 5-13%, between 5-14%, between 5-15%, between 7-12%, between 7-13%, between 7-14%, between 7-15%, between 8-9%, between 8-10%, between 8-11%, between 8-12%, between 8-13%, between 8-14%, between 8-15%, between 9-15%, between 10-11%, between 10-12%, between 10-13%, between 10-14%, between 10-15%, between 11-12%, between 11-13%, between 11-14%, between 11-15%, between 12-13%, between 12-14%, between 12-15%, between 13-14%, between 13-15%, or between 10-40% higher than the acidification rate of a second lactobacilli (*e.g.*, *L. crispatus* isolate, *L. jensenii* isolate).

**[0202]** In some embodiments, the acidification rates are acidification rates *in vitro* (*e.g.*, in culture) and/or *in vivo*.

**[0203]** In some embodiments, at least two lactobacilli (*e.g.*, *L. crispatus* isolate, *L. jensenii* isolate) of the present disclosure differ in their capability of utilizing glycogen. In

some embodiments, the at least two lactobacilli (*e.g.*, *L. crispatus* isolate, *L. jensenii* isolate) differ in their capability of utilizing glycogen by at least 1%, at least 2%, at least 3%, at least 4%, at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, at least 10%, at least 15%, at least 20%, or at least 25%. In some embodiments, the at least two lactobacilli (*e.g.*, *L. jensenii* isolate, *L. crispatus* isolate) differ in their capability of utilizing glycogen by between 1-2%, between 1-3%, between 1-4%, between 1-5%, between 1-6%, between 1-7%, between 1-8%, between 1-9%, between 1-10%, between 1-11%, between 1-12%, between 1-13%, between 1-14%, between 1-15%, between 2-3%, between 2-4%, between 2-5%, between 2-6%, between 2-7%, between 2-8%, between 7-15%, between 8-9%, between 8-10%, between 8-11%, between 8-12%, between 8-13%, between 8-14%, between 8-15%, between 9-10%, between 9-11%, between 9-12%, between 9-13%, between 9-14%, between 9-15%, between 10-11%, between 10-12%, between 10-13%, between 10-14%, between 10-15%, between 11-12%, between 11-13%, between 11-14%, between 11-15%, between 12-13%, between 12-14%, between 12-15%, between 13-14%, between 13-15%, or between 10-40%.

**[0204]** In some embodiments, at least two lactobacilli (*e.g.*, *L. crispatus* isolate, *L. jensenii* isolate) of the present disclosure have different carbon utilization profiles. In some embodiments, the carbon utilization profile is selected from the group consisting of: a glycogen utilization profile; a glucose utilization profile; a fructose utilization profile; a mannose utilization profile; a maltodextrin utilization profile; and a mannitol utilization profile.

**[0205]** In some embodiments, the capability of utilizing glycogen is a capability of utilizing glycogen *in vitro* (*e.g.*, in culture) and/or *in vivo*.

**[0206]** In some embodiments, the capability of utilizing mannitol and glucose is a capability of utilizing mannitol and glucose *in vitro* (*e.g.*, in culture) and/or *in vivo*.

**[0207]** In some embodiments, the capability of utilizing glycogen, glucose, fructose, mannose, maltodextrin, or mannitol is a capability of utilizing glycogen, glucose, fructose, mannose, maltodextrin, or mannitol *in vitro* (*e.g.*, in culture) and/or *in vivo*.

**[0208]** In some embodiments, at least two lactobacilli (*e.g.*, *L. crispatus* isolate, *L. jensenii* isolate) of the present disclosure differ in their lactic acid production rates. In some embodiments, the at least two lactobacilli (*e.g.*, *L. crispatus* isolate, *L. jensenii* isolate) differ in their lactic acid production rates by at least 1%, at least 2%, at least 3%, at least 4%, at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, at least 10%, at least 15%, at least 20%, or at least 25%. In some embodiments, the at least two lactobacilli (*e.g.*, *L. crispatus*

isolate, *L. jensenii* isolate) differ in their lactic acid production rates by between 1-2%, between 1-3%, between 1-4%, between 1-5%, between 1-6%, between 1-7%, between 1-8%, between 1-9%, between 1-10%, between 1-11%, between 1-12%, between 1-13%, between 1-14%, between 1-15%, between 2-3%, between 2-4%, between 2-5%, between 2-6%, between 2-7%, between 2-8%, between 2-9%, between 2-10%, between 2-11%, between 2-12%, between 2-13%, between 2-14%, between 2-15%, between 3-4%, between 3-5%, between 3-6%, between 7-10%, between 7-11%, between 7-12%, between 7-13%, between 7-14%, between 7-15%, between 8-9%, between 8-10%, between 8-11%, between 8-12%, between 8-13%, between 8-14%, between 8-15%, between 9-10%, between 9-11%, between 9-12%, between 9-13%, between 9-14%, between 9-15%, between 10-11%, between 10-12%, between 10-13%, between 10-14%, between 10-15%, between 11-12%, between 11-13%, between 11-14%, between 11-15%, between 12-13%, between 12-14%, between 12-15%, between 13-14%, between 13-15%, or between 14-15%.

**[0209]** In some embodiments, at least two *L. crispatus* of the present disclosure differ in their lactic acid production rate. In some embodiments, the at least two *L. crispatus* differ in their lactic acid production rate by at least 1%, at least 2%, at least 3%, at least 4%, at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, at least 10%, at least 15%, at least 20%, or at least 25%. In some embodiments, the at least two *L. crispatus* differ in their lactic acid production rate by between 1-2%, between 1-3%, between 1-4%, between 1-5%, between 1-6%, between 1-7%, between 1-8%, between 1-9%, between 1-10%, between 1-11%, between 1-12%, between 1-13%, between 1-14%, between 1-15%, between 2-3%, between 2-4%, between 2-5%, between 2-6%, between 2-7%, between 2-8%, between 2-9%, between 2-10%, between 2-11%, between 2-12%, between 6-13%, between 6-14%, between 6-15%, between 7-8%, between 7-9%, between 7-10%, between 7-11%, between 7-12%, between 7-13%, between 7-14%, between 7-15%, between 8-9%, between 8-10%, between 8-11%, between 8-12%, between 8-13%, between 8-14%, between 8-15%, between 9-10%, between 9-11%, between 9-12%, between 9-13%, between 9-14%, between 9-15%, between 10-11%, between 10-12%, between 10-13%, between 10-14%, between 10-15%, between 11-12%, between 11-13%, between 11-14%, between 11-15%, between 12-13%, between 12-14%, between 12-15%, between 13-14%, between 13-15%, or between 14-15%.

**[0210]** In some embodiments, the lactic acid production rate is a lactic acid production rate *in vitro* (e.g., in culture) and/or *in vivo*.

[0211] In some embodiments, a rate of lactic acid production (*e.g.*, rate of D-lactic acid, rate of L-lactic acid) is an *in vitro* rate that is calculated after 24 hours of bacterial growth in culture. In some embodiments, a rate of lactic acid production is determined for a particular bacteria or consortia after an inoculation of MRS broth at 37 °C with 5 million CFU/mL. Rates of D-lactic acid production were determined according to the following equation: Rate of D-lactic acid production equals natural logarithm (Ln) of (final concentration of D-lactic acid divided by initial concentration of D-lactic acid) divided by (final time minus initial time). Rates of L-lactic acid production were determined according to the following equation: Rate of L-lactic acid production equals natural logarithm (Ln) of (final concentration of L-lactic acid divided by initial concentration of L-lactic acid) divided by (final time minus initial time).

[0212] In some embodiments, at least two lactobacilli (*e.g.*, *L. crispatus* isolate, *L. jensenii* isolate) of the present disclosure differ in their time to engraftment in the female genitourinary tract. In some embodiments, the at least two lactobacilli (*e.g.*, *L. crispatus* isolate, *L. jensenii* isolate) differ in their time to engraftment in the female genitourinary tract by about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 15, 18, or 24 hours. In some embodiments, the at least two lactobacilli (*e.g.*, *L. crispatus* isolate, *L. jensenii* isolate) differ in their time to engraftment in the female genitourinary tract by between 1-6, 5-10, 6-12, 10-15, 12-18, 15-20, 18-24, 20-30, or 24-36 hours.

[0213] In some embodiments, a *L. jensenii* and at least one *L. crispatus* of the present disclosure differ in their time to engraftment in the female genitourinary tract. In some embodiments, the *L. jensenii* and the at least one *L. crispatus* differ in their time to engraftment in the female genitourinary tract by about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 15, 18, or 24 hours. In some embodiments, the *L. jensenii* and the at least one *L. crispatus* differ in their time to engraftment in the female genitourinary tract by between 1-6, 5-10, 6-12, 10-15, 12-18, 15-20, 18-24, 20-30, or 24-36 hours.

[0214] In some embodiments, at least two *L. crispatus* of the present disclosure differ in their time to engraftment in the female genitourinary tract. In some embodiments, the at least two *L. crispatus* differ in their time to engraftment in the female genitourinary tract by about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 15, 18, or 24 hours. In some embodiments, the at least two *L. crispatus* differ in their time to engraftment in the female genitourinary tract by 1-6, 5-10, 6-12, 10-15, 12-18, 15-20, 18-24, 20-30, or 24-36 hours.

[0215] In some embodiments, the time to engraftment in the female genitourinary tract is determined *in vivo*.

[0216] In some embodiments, each *Lactobacillus* isolate of the consortium is obtained from a healthy human donor. The lactobacilli comprised in the defined population may be obtained from at least two different healthy human donors (*e.g.*, 2, 3, 4, 5, or more healthy human donors). In some embodiments, the lactobacilli comprised in the defined population are obtained from three different healthy human donors.

[0217] A *Lactobacillus* isolate of a microbial consortium may be obtained from the vaginal environment of a healthy human donor, *e.g.*, a human donor, *e.g.*, a female donor, with a eubiotic vaginal microbiome, *e.g.*, a *Lactobacillus*-dominated microbiome. A *Lactobacillus* isolate of a microbial consortium may be obtained using the steps as provided in **FIG. 1**. For example, in some embodiments, a *Lactobacillus* isolate of a microbial consortia is obtained by (1) administering a vaginal microbiome transplant (VMT) (comprising a substantially complete vaginal microbiome preparation (SCVMP) derived from a single healthy female donor) to the female genitourinary tract of a recipient subject, (2) collecting a sample from the genitourinary tract of the recipient subject and culturing the sample, and (3) collecting an isolate from the cultured sample. In some embodiments, a VMT is as described in DeLong, K. et al., *Front Cell Infect Microbiol.* 2019 Aug 28;9:306, the content of which is incorporated herein by reference. In some embodiments, a VMT is as described in Lev-Sagie, A. et al., *Nat Med.* 2019 Oct;25(10):1500-1504, the content of which is incorporated herein by reference. In some embodiments, a VMT is as described in Bosma, E. F. et al. Antibiotic-free vaginal microbiota transplantation (VMT) changes vaginal microbiota and immune profile in women with asymptomatic dysbiosis: reporting of a randomized, placebo-controlled trial. *medRxiv (Cold Spring Harbor Laboratory)* (2024), the content of which is incorporated herein by reference.

[0218] In some embodiments, a *Lactobacillus* isolate of a microbial consortium is obtained by (1) administering a substantially complete vaginal microbiome preparation derived from a single healthy female donor to the female genitourinary tract of a recipient subject, (2) collecting a sample from the genitourinary tract of the recipient subject and culturing the sample, and (3) collecting an isolate from the cultured sample.

**Microbial consortia**

[0219] Aspects of this disclosure provide, in part, microbial consortia comprised of a defined population of isolated lactobacilli (*e.g.*, *L. crispatus*, *L. jensenii*) configured to promote engraftment in a female genitourinary tract of a subject. In some embodiments, the defined population of isolated lactobacilli (*e.g.*, *L. crispatus*, *L. jensenii*) configured to promote engraftment in a female genitourinary tract of a subject comprises an *L. jensenii* bacteria, an *L. crispatus* bacteria that thrives in the presence of the *L. jensenii* bacteria, and at least one additional *L. crispatus* bacteria that thrives independent of the presence of the *L. jensenii* bacteria. In some embodiments, the at least one *L. crispatus* bacteria that thrives independent of the presence of the *L. jensenii* bacteria is capable of engrafting the female genitourinary tract of the subject for at least a pre-determined of time.

[0220] A microbial consortium of the present disclosure is designed, in some embodiments, to include specific *Lactobacillus* isolates having a diverse set of functional and genomic characteristics. A microbial consortium as described herein and having a diverse set of functional and genomic characteristics can engraft in the female genitourinary tract of a diverse set of subjects, each of whom have different vaginal environments. For example, a microbial consortium of the disclosure may include two or more *Lactobacillus* isolates, wherein each isolate has different functional characteristics (*e.g.*, different growth rates, different acidification rates, different carbohydrate utilization profiles, different rates of engraftment). A microbial consortium of the disclosure may include two or more *Lactobacillus* isolates, wherein each isolate has different genomic characteristics. For example, in determining a set of two or more *Lactobacillus* isolates to be included in a microbial consortium, *in silico* analysis can be used to identify a species-level pangenome comprising genes that are linked to engraftment (and beneficial effect on a subject, *e.g.*, reduction of (local) inflammation, *e.g.*, in the genitourinary tract). In some embodiments, *in silico* analysis can be used to identify a *Lactobacillus crispatus* pangenome comprising genes that are linked to engraftment (and beneficial effect on a subject, *e.g.*, reduction of (local) inflammation, *e.g.*, in the genitourinary tract). Isolates can be selected for inclusion in the microbial consortium such that a combination of isolates provides maximal coverage of a preselected portion (or all) of the pangenome (*e.g.*, the genes of the pangenome that are linked to engraftment). In other words, a combination of isolates can be combined into a microbial consortium such that the consortium encompasses a preselected portion (or all) of



the pangenome (*e.g.*, the genes of the pangenome that are linked to engraftment) even though any single isolate might not encompass the preselected portion by itself. In some embodiments, a microbial consortium is generated to encompass a maximal portion of the pangenome with the minimum number of necessary *Lactobacillus* isolates. Maximal coverage of a pangenome (*e.g.*, a *Lactobacillus crispatus* engrafting pangenome) within a microbial consortium allows for the consortium to be capable of successfully engrafting a diverse set of vaginal environments (*e.g.*, belonging to diverse set of subjects).

**[0221]** In some embodiments, an exemplary microbial consortium of the disclosure is comprised of a *Lactobacillus jensenii* isolate obtained from a bacterial sample deposited as DSM Deposit Number 34770; a *Lactobacillus crispatus* isolate obtained from a bacterial sample deposited as DSM Deposit Number 34771; a *Lactobacillus crispatus* isolate obtained from a bacterial sample deposited as DSM Deposit Number 34772; and a *Lactobacillus crispatus* isolate obtained from a bacterial sample deposited as DSM Deposit Number 34773.

**[0222]** In some embodiments, an exemplary microbial consortium of the disclosure is comprised of:

(a) *Lactobacillus jensenii* isolate having a genome that, when aligned with the genome of a bacterial sample deposited as DSM 34770, has a percent identity greater than or equal to a threshold value (*e.g.*, a threshold value of 99.81%, 99.90%, or 99.96%);

(b) *Lactobacillus crispatus* isolate having a genome that, when aligned with the genome of a bacterial sample deposited as DSM 34771, has a percent identity greater than or equal to a threshold value (*e.g.*, a threshold value of 99.81%, 99.90%, or 99.96%);

(c) *Lactobacillus crispatus* isolate having a genome that, when aligned with the genome of a bacterial sample deposited as DSM 34772, has a percent identity greater than or equal to a threshold value (*e.g.*, a threshold value of 99.81%, 99.90%, or 99.96%); and

**[0223]** (d) *Lactobacillus crispatus* isolate having a genome that, when aligned with the genome of a bacterial sample deposited as DSM 34773, has a percent identity greater than or equal to a threshold value (*e.g.*, a threshold value of 99.81%, 99.90%, or 99.96%).

**[0224]** In some embodiments, the microbial consortium is an *n*-member consortium comprising *n* bacterial isolates, in which *n* is an integer reflecting the number of different bacterial isolates in the consortium. In some embodiments, *n* is at least 2, at least 3 or at least 4. In some embodiments, *n* is in the range of 2 to 10, 2 to 6, or 2 to 4. In some embodiments, *n* is 2, 3, 4, 5, 6, 7, 8, 9, 10 or more. In some embodiments, the microbial consortium is a 2-member consortium comprising two bacterial isolates. In some embodiments, the microbial

consortium is a 3-member consortium comprising three bacterial isolates. In some embodiments, the microbial consortium is a 4-member consortium comprising four bacterial isolates.

[0225] In some embodiments, the defined population of isolated lactobacilli is comprised of an *L. jensenii*, an *L. crispatus* that thrives in the presence of the *L. jensenii*, and an additional *L. crispatus* that thrives independent of the presence of the *L. jensenii*, wherein the relative abundance of the *L. jensenii* is about 15%, about 20%, about 25%, about 30%, or about 35% of the total viable cells, the relative abundance of the *L. crispatus* that thrives in the presence of the *L. jensenii* is about 15%, about 20%, about 25%, about 30%, or about 35% of the total viable cells, and the additional *L. crispatus* that thrives independent of the presence of the *L. jensenii* is about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, or about 70% of the total viable cells. In some embodiments, the defined population of isolated lactobacilli is comprised of an *L. jensenii*, an *L. crispatus* that thrives in the presence of the *L. jensenii*, and an additional *L. crispatus* that thrives independent of the presence of the *L. jensenii*, wherein the relative abundance of the *L. jensenii* is between 15% - 20%, between 15% - 25%, between 15% - 30%, between 15% - 35%, between 20% - 25%, between 20% - 30%, between 20% - 35%, or between 30% - 35% of the total viable cells, the relative abundance of the *L. crispatus* that thrives in the presence of the *L. jensenii* is between 15% - 20%, between 15% - 25%, between 15% - 30%, between 15% - 35%, between 20% - 25%, between 20% - 30%, between 20% - 35%, or between 30% - 35% of the total viable cells, and the additional *L. crispatus* that thrives independent of the presence of the *L. jensenii* is between 30% - 35%, between 30% - 40%, between 30% - 45%, between 30% - 50%, between 30% - 55%, between 30% - 60%, between 30% - 65%, between 30% - 70%, between 35% - 40%, between 35% - 45%, between 35% - 50%, between 35% - 55%, between 35% - 60%, between 35% - 65%, between 35% - 70%, between 40% - 45%, between 40% - 50%, between 40% - 55%, between 40% - 60%, between 40% - 65%, between 40% - 70%, between 45% - 50%, between 45% - 55%, between 45% - 60%, between 45% - 65%, between 45% - 70%, between 50% - 55%, between 50% - 60%, between 50% - 65%, between 50% - 70%, between 55% - 60%, between 55% - 65%, between 55% - 70%, between 60% - 65%, between 60% - 70%, or between 65% - 75% of the total viable cells.

[0226] In some embodiments, the defined population of isolated lactobacilli is comprised of an *L. jensenii*, an *L. crispatus* that thrives in the presence of the *L. jensenii*, and

an additional *L. crispatus* that thrives independent of the presence of the *L. jensenii*, wherein the relative abundance of the *L. jensenii* is about 25% of the total viable cells, the relative abundance of the *L. crispatus* that thrives in the presence of the *L. jensenii* about 25% of the total viable cells, and the additional *L. crispatus* that thrives independent of the presence of the *L. jensenii* is about 50% of the total viable cells.

**[0227]** Relative abundance of *Lactobacillus* isolates within the defined population can be determined using an absorbance measurement (e.g. OD600 measurement of isolates in culture), a viable cell counting methodology, or by weight (e.g., weight of lyophilized isolates).

**[0228]** In some embodiments, the defined population of isolated lactobacilli is comprised of an *L. jensenii*, an *L. crispatus* that thrives in the presence of the *L. jensenii*, and an additional *L. crispatus* that thrives independent of the presence of the *L. jensenii*, the *L. jensenii* comprises a plurality of *L. jensenii*, the *L. crispatus* that thrives in the presence of the *L. jensenii* comprises a plurality of *L. crispatus* that thrives in the presence of the *L. jensenii*, and the at least one additional *L. crispatus* that thrives independent of the presence of the *L. jensenii* comprises a plurality of *L. crispatus* that thrives independent of the presence of the *L. jensenii*.

**[0229]** In some aspects, this disclosure provides a microbial consortium comprising a defined population of isolated lactobacilli configured to promote engraftment in a female genitourinary tract of a subject. In some embodiments, the defined population comprises an *L. jensenii*, an *L. crispatus* that thrives in the presence of the *L. jensenii*, and at least two additional *L. crispatus*, wherein the at least two *L. crispatus* have different growth rates (e.g., different growth rates *in vitro*).

**[0230]** In some embodiments, at least two of the *L. crispatus* differ in their growth rates, capability of utilizing glycogen, rate of producing lactic acid, acidification rate, and/or time to engraftment in the female urinary tract.

**[0231]** In some embodiments, the defined population comprises an *L. jensenii*, an *L. crispatus* that thrives in the presence of the *L. jensenii*, and at least two additional *L. crispatus*, wherein the at least two *L. crispatus* have different growth rates, wherein the relative abundance of the *L. jensenii* is about 15%, about 20%, about 25%, about 30%, or about 35% of the total viable cells, the relative abundance of the *L. crispatus* that thrives in the presence of the *L. jensenii* is about 15%, about 20%, about 25%, about 30%, or about 35% of the total viable cells, and the relative abundance of the at least two additional *L.*

*crispatus* is about 15%, about 20%, about 25%, about 30%, or about 35% of the total viable cells. In some embodiments, the defined population comprises an *L. jensenii*, an *L. crispatus* that thrives in the presence of the *L. jensenii*, and at least two additional *L. crispatus*, wherein the at least two *L. crispatus* have different growth rates, wherein the relative abundance of the *L. jensenii* is between 15% - 20%, between 15% - 25%, between 15% - 30%, between 15%-35%, between 20% - 25%, between 20% - 30%, between 20% - 35%, between 25% - 30%, between 25% - 35%, or between 30% - 35% of the total viable cells, the relative abundance of the *L. crispatus* that thrives in the presence of the *L. jensenii* is between 15% - 20%, between 15% - 25%, between 15% - 30%, between 15%-35%, between 20% - 25%, between 20% - 30%, between 20% - 35%, between 25% - 30%, between 25% - 35%, or between 30% - 35% of the total viable cells, and the relative abundance of the at least two additional *L. crispatus* is between 15% - 20%, between 15% - 25%, between 15% - 30%, between 15%-35%, between 20% - 25%, between 20% - 30%, between 20% - 35%, between 25% - 30%, between 25% - 35%, or between 30% - 35% of the total viable cells. In some embodiments, the defined population comprises an *L. jensenii*, an *L. crispatus* that thrives in the presence of the *L. jensenii*, and at least two additional *L. crispatus*, wherein the at least two *L. crispatus* have different growth rates, wherein the relative abundance of the *L. jensenii* is about 25% of the total viable cells, the relative abundance of the *L. crispatus* that thrives in the presence of the *L. jensenii* is about 25% of the total viable cells, and the relative abundance of the at least two additional *L. crispatus* is about 50% of the total viable cells.

**[0232]** In some embodiments, the defined population comprises an *L. jensenii*, an *L. crispatus* that thrives in the presence of the *L. jensenii*, and at least two additional *L. crispatus*, wherein the at least two *L. crispatus* have different growth rates, the *L. jensenii* comprises a plurality of *L. jensenii*, the *L. crispatus* that thrives in the presence of the *L. jensenii* comprises a plurality of *L. crispatus* that thrives in the presence of the *L. jensenii*, and each of the at least two additional *L. crispatus* comprises a plurality of *L. crispatus*.

**[0233]** Aspects of this disclosure provide, in part, a microbial consortium comprising a defined population of isolated lactobacilli (*e.g.*, *L. jensenii*, *L. crispatus*) configured to promote engraftment in a female genitourinary tract (*e.g.*, cervix, vagina, uterus, and/or fallopian tubes). In some embodiments, the defined population of isolated lactobacilli (*e.g.*, *L. jensenii*, *L. crispatus*) configured to promote engraftment in a female genitourinary tract is comprised of an *L. jensenii*, an *L. crispatus* that thrives in the presence of the *L. jensenii*, and at least two additional *L. crispatus*, wherein the at least two additional *L. crispatus* have

different acidification rates. In some embodiments, the difference in acidification rates between the at least two additional *L. crispatus* is at least 1%, at least 2%, at least 3%, at least 4%, at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, at least 10%, at least 15%, at least 20%, or at least 25%. In some embodiments, the difference in acidification rates between the at least two additional *L. crispatus* is between 1-10%, between 1-15%, between 1-20%, between 5-10%, between 5-15%, between 5-20%, between 10-20%, between 10-30%, between 10-40%, or between 10-50%.

**[0234]** In some embodiments, the defined population is comprised of an *L. jensenii*, an *L. crispatus* that thrives in the presence of the *L. jensenii*, and at least two additional *L. crispatus*, wherein the at least two additional *L. crispatus* have different acidification rates, at least two *L. crispatus* differ in their growth rates, capability of utilizing glycogen, rate of lactic acid production, and/or time to engraftment in the female genitourinary tract.

**[0235]** In some embodiments, the defined population is comprised of an *L. jensenii*, an *L. crispatus* that thrives in the presence of the *L. jensenii*, and at least two additional *L. crispatus*, wherein the at least two additional *L. crispatus* have different acidification rates, the relative abundance of *L. jensenii* is about 15%, about 20%, about 25%, about 30%, or about 35% of the total viable cells, the relative abundance of *L. crispatus* that thrives in the presence of the *L. jensenii* is about 15%, about 20%, about 25%, about 30%, or about 35% of the total viable cells, and the relative abundance of the at least two additional *L. crispatus* bacteria is about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, or about 70% of the total viable cells. In some embodiments, the defined population is comprised of an *L. jensenii*, an *L. crispatus* that thrives in the presence of the *L. jensenii*, and at least two additional *L. crispatus*, wherein the at least two additional *L. crispatus* have different acidification rates, the relative abundance of *L. jensenii* is between 15% - 20%, between 15% - 25%, between 15% - 30%, between 15% - 35%, between 20% - 25%, between 20% - 30%, between 20% - 35%, between 25% - 30%, between 25% - 35%, or between 30% - 35% of the total viable cells, the relative abundance of the *L. crispatus* that thrives in the presence of the *L. jensenii* is between 15% - 20%, between 15% - 25%, between 15% - 30%, between 15% - 35%, between 20% - 25%, between 20% - 30%, between 20% - 35%, between 25% - 30%, between 25% - 35%, or between 30% - 35% of the total viable cells, and the relative abundance of the at least two additional *L. crispatus* is between 30% - 35%, between 30% - 40%, between 30% - 45%, between 30% - 50%, between 30% - 55%, between 30% - 60%, between 30% - 65%, between 30% - 70%, between 35% - 40%,

between 35% - 45%, between 35% - 50%, between 35% - 55%, between 35% - 60%, between 35% - 65%, between 35% - 70%, between 40% - 45%, between 40% - 50%, between 40% - 55%, between 40% - 60%, between 40% - 65%, between 40% - 70%, between 45% - 50%, between 45% - 55%, between 45% - 60%, between 45% - 65%, between 45% - 70%, between 50% - 55%, between 50% - 60%, between 50% - 65%, between 50% - 70%, between 55% - 60%, between 55% - 65%, between 55% - 70%, between 60% - 65%, between 60% - 70%, or between 65% - 70% of the total viable cells. In some embodiments, the defined population is comprised of an *L. jensenii*, an *L. crispatus* that thrives in the presence of the *L. jensenii*, and at least two additional *L. crispatus*, wherein the at least two additional *L. crispatus* have different acidification rates, the relative abundance of the *L. jensenii* is about 25% of the total viable cells, the relative abundance of the *L. crispatus* that thrives in the presence of the *L. jensenii* is about 25% of the total viable cells, and the relative abundance of the at least two *L. crispatus* is about 50% of the total viable cells.

**[0236]** In some embodiments, the defined population is comprised of an *L. jensenii*, an *L. crispatus* that thrives in the presence of the *L. jensenii*, and at least two additional *L. crispatus*, wherein the at least two additional *L. crispatus* have different acidification rates, the *L. jensenii* comprises a plurality of *L. jensenii*, the *L. crispatus* that thrives in the presence of the *L. jensenii* comprises a plurality of *L. crispatus* that thrives in the presence of the *L. jensenii*, and each of the at least two additional *L. crispatus* comprises a plurality of *L. crispatus*.

**[0237]** In some embodiments, at least two lactobacilli (*e.g.*, *L. crispatus*, *L. jensenii*) of a microbial consortium described herein differ in their growth rates. In some embodiments, at least two lactobacilli (*e.g.*, *L. crispatus*, *L. jensenii*) of a microbial consortium described herein differ in their growth rates by at least 1%, at least 2%, at least 3%, at least 4%, at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, at least 10%, at least 15%, at least 20%, or at least 25%.

**[0238]** In some embodiments, an *L. jensenii* and at least one *L. crispatus* of a microbial consortium described herein differ in their growth rates. In some embodiments, an *L. jensenii* and at least one *L. crispatus* of a microbial consortium described herein differ in their growth rates by at least 1%, at least 2%, at least 3%, at least 4%, at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, at least 10%, at least 15%, at least 20%, or at least 25%.

**[0239]** In some embodiments, at least two *L. crispatus* of a microbial consortium described herein differ in their growth rates. In some embodiments, at least two *L. crispatus*

of a microbial consortium described herein differ in their growth rates by at least 1%, at least 2%, at least 3%, at least 4%, at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, at least 10%, at least 15%, at least 20%, or at least 25%.

**[0240]** In some embodiments, at least two lactobacilli (e.g., *L. crispatus*, *L. jensenii*) of a microbial consortium described herein differ in their acidification rates. In some embodiments, at least two lactobacilli (e.g., *L. crispatus*, *L. jensenii*) of a microbial consortium described herein differ in their acidification rates by at least 1%, at least 2%, at least 3%, at least 4%, at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, at least 10%, at least 15%, at least 20%, or at least 25%.

**[0241]** In some embodiments, an *L. jensenii* and at least one *L. crispatus* of a microbial consortium described herein differ in their acidification rates. In some embodiments, an *L. jensenii* and at least one *L. crispatus* of a microbial consortium described herein differ in their acidification rates by at least 1%, at least 2%, at least 3%, at least 4%, at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, at least 10%, at least 15%, at least 20%, or at least 25%.

**[0242]** In some embodiments, at least two *L. crispatus* of a microbial consortium described herein differ in their acidification rates. In some embodiments, at least two *L. crispatus* of a microbial consortium described herein differ in their acidification rates by at least 1%, at least 2%, at least 3%, at least 4%, at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, at least 10%, at least 15%, at least 20%, or at least 25%.

**[0243]** In some embodiments, at least two lactobacilli (e.g., *L. crispatus*, *L. jensenii*) of a microbial consortium described herein differ in their capability of utilizing glycogen. In some embodiments, at least two lactobacilli (e.g., *L. crispatus*, *L. jensenii*) of a microbial consortium described herein differ in their capability of utilizing glycogen by at least 1%, at least 2%, at least 3%, at least 4%, at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, at least 10%, at least 15%, at least 20%, or at least 25%.

**[0244]** In some embodiments, an *L. jensenii* and at least one *L. crispatus* of a microbial consortium described herein differ in their capability of utilizing glycogen. In some embodiments, an *L. jensenii* and at least one *L. crispatus* of a microbial consortium described herein differ in their capability of utilizing glycogen by at least 1%, at least 2%, at least 3%, at least 4%, at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, at least 10%, at least 15%, at least 20%, or at least 25%.

**[0245]** In some embodiments, at least two *L. crispatus* of a microbial consortium described herein differ in their capability of utilizing glycogen. In some embodiments, at least two *L. crispatus* of a microbial consortium described herein differ in their capability of utilizing glycogen by at least 1%, at least 2%, at least 3%, at least 4%, at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, at least 10%, at least 15%, at least 20%, or at least 25%.

**[0246]** In some embodiments, at least two lactobacilli (*e.g.*, *L. crispatus*, *L. jensenii*) of a microbial consortium described herein differ in their rate of lactic acid production. In some embodiments, at least two lactobacilli (*e.g.*, *L. crispatus*, *L. jensenii*) of a microbial consortium described herein differ in their rate of lactic acid production by at least 1%, at least 2%, at least 3%, at least 4%, at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, at least 10%, at least 15%, at least 20%, or at least 25%.

**[0247]** In some embodiments, an *L. jensenii* and at least one *L. crispatus* of a microbial consortium described herein differ in their rate of lactic acid production. In some embodiments, an *L. jensenii* and at least one *L. crispatus* of a microbial consortium described herein differ in their rate of lactic acid production by at least 1%, at least 2%, at least 3%, at least 4%, at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, at least 10%, at least 15%, at least 20%, or at least 25%.

**[0248]** In some embodiments, at least two *L. crispatus* of a microbial consortium described herein differ in their rate of lactic acid production. In some embodiments, at least two *L. crispatus* of a microbial consortium described herein differ in their capability of rate of lactic acid production by at least 1%, at least 2%, at least 3%, at least 4%, at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, at least 10%, at least 15%, at least 20%, or at least 25%.

**[0249]** In some embodiments, at least two lactobacilli (*e.g.*, *L. crispatus*, *L. jensenii*) of a microbial consortium described herein differ in their time to engraftment in the female genitourinary tract. In some embodiments, at least two lactobacilli (*e.g.*, *L. crispatus*, *L. jensenii*) of a microbial consortium described herein differ in their time to engraftment in the female genitourinary tract by about 1-6, 5-10, 6-12, 10-15, 12-18, 15-20, 18-24, 20-30, or 24-36 hours.

**[0250]** In some embodiments, an *L. jensenii* and at least one *L. crispatus* of a microbial consortium described herein differ in their time to engraftment in the female genitourinary tract. In some embodiments, an *L. jensenii* and at least one *L. crispatus* of a microbial



consortium described herein differ in their time to engraftment in the female genitourinary tract by about 1 day, about 2 days, about 3 days, about 4 days, about 5 days, about 6 days, or about 7 days.

**[0251]** In some embodiments, at least two *L. crispatus* of a microbial consortium described herein differ in their time to engraftment in the female genitourinary tract. In some embodiments, at least two *L. crispatus* of a microbial consortium described herein differ in their time to engraftment in the female genitourinary tract by about 1 day, about 2 days, about 3 days, about 4 days, about 5 days, about 6 days, or about 7 days.

**[0252]** In some embodiments, the isolates of the microbial consortium engraft equally in the female genitourinary tract of the subject. In some embodiments, each of the isolates of the microbial consortium engraft in the female genitourinary tract of the subject. In some embodiments, one, two, three, or four isolates of the microbial consortium engraft in the female genitourinary tract of the subject. In some embodiments, the *L. jensenii* of the microbial consortium is the first bacteria of the consortium to engraft in the female genitourinary tract of the subject.

**[0253]** In some embodiments, at least one of the lactobacilli bacteria of the microbial consortium is capable of engrafting the female genitourinary tract of the subject within 1, 2, 3, 4, 5, 6, 8, 10, 12, 15, 18, 24, 36, or 48 hours after administration of the consortium to the female genitourinary tract of the subject. In some embodiments, at least two or at least three of the lactobacilli bacteria of the microbial consortium is capable of engrafting the female genitourinary tract of the subject within 1, 2, 3, 4, 5, 6, 8, 10, 12, 15, 18, 24, 36, or 48 hours after administration of the consortium to the female genitourinary tract of the subject.

**[0254]** Engraftment of the female genitourinary tract can be quantified, *e.g.*, by counting the number of colony forming units (CFU)/gram and/or performing nucleic acid sequencing of microbes comprised in one or more samples of the female genitourinary tract. In some embodiments, engraftment of the female genitourinary tract is successful if at least one (at least two, or at least three) of the lactobacilli bacteria is detected within the female genitourinary tract after a certain period of time (*e.g.*, 6, 12, 24, 36, 48, or 72 hours). In some embodiments, engraftment of the female genitourinary tract is successful if the relative abundance of at least one of the lactobacilli bacteria is at least 1%, at least 5%, at least 10%, at least 20%, at least 25%, at least 30%, or at least 40% of the total microbial abundance in the genitourinary tract. In some embodiments, the integration of transplanted bacteria (*e.g.*, a microbial consortium or individual lactobacilli isolates) into a subject's existing microbial

community represents engraftment. The success of engraftment can be assessed through microbiome analysis (*e.g.*, 16S rRNA sequencing, metagenomic sequencing, or qPCR) or laboratory testing (*e.g.*, culture).

**[0255]** In some embodiments, at least one of the lactobacilli bacteria of the exemplary microbial consortium described herein is capable of persisting within the female genitourinary tract of the subject for at least 1, 2, 3, 4, 5, 6, 8, 10, 12, 15, 18, 20, 25, 30, or 35 days after administration to the female genitourinary tract of the subject. In some embodiments, at least one of the lactobacilli bacteria of the exemplary microbial consortium described herein is capable of persisting within the female genitourinary tract of the subject for at least 1, 2, 3, 4, 5, 6, or 8 weeks after administration to the female genitourinary tract of the subject.

**[0256]** In some embodiments, 1, 2, 3, or 4 of the lactobacilli bacteria of the exemplary microbial consortium described herein is capable of persisting within the female genitourinary tract of the subject for at least 1, 2, 3, 4, 5, 6, 8, 10, 12, 15, 18, 20, 25, 30, or 35 days after administration to the female genitourinary tract of the subject. In some embodiments, 1, 2, 3, or 4 of the lactobacilli bacteria of the exemplary microbial consortium described herein is capable of persisting within the female genitourinary tract of the subject for at least 1, 2, 3, 4, 5, 6, or 8 weeks after administration to the female genitourinary tract of the subject.

**[0257]** In some embodiments, the lactobacilli of the consortium are capable of competing with the resident microbiota (*e.g.*, resident lactobacilli and resident non-lactobacilli) within the female genitourinary tract of the subject, and, in embodiments, successfully engraft. In some embodiments, the lactobacilli of the consortium are capable of competing with the resident microbiota (*e.g.*, resident lactobacilli and resident non-lactobacilli) within the female genitourinary tract of the subject for nutrients (*e.g.*, carbohydrates, vitamins), space in the genitourinary tract, and/or growth, and, in embodiments, successfully engraft.

**[0258]** In some embodiments, the resident microbiota (*e.g.*, resident lactobacilli and resident non-lactobacilli) within the female genitourinary tract of the subject is at least partially removed by a local antiseptic or antibiotic treatment, *e.g.*, for 1, 2, 3, 4, 5, or 6 days, or 1, 2, or 3 weeks prior to administration of the microbial consortium, *e.g.*, to provide space within the genitourinary tract for engraftment. In some embodiments, an antiseptic is an antiseptic solution. An antiseptic solution may comprise chlorhexidine and/or povidone-

iodine. In some embodiments, an antiseptic solution comprises about 10% or 5-10% povidone-iodine. In some embodiments, an antiseptic solution comprises about 0.5% chlorhexidine. In some embodiments, an antibiotic is metronidazole or clindamycin.

**[0259]** The microbial consortium may include at least 1 million colony forming units (CFUs), at least 5 million CFU, at least 6 million CFU, at least 7 million CFU, at least 8 million CFU, or at least 10 million CFU of total bacteria. The microbial consortium may include at least 1 million CFU/mL, at least 5 million CFU/mL, at least 6 million CFU/mL, at least 7 million CFU/mL, at least 8 million CFU/mL, at least 9 million CFU/mL, or at least 10 million CFU/mL of total bacteria. In some embodiments, the microbial consortium includes 1-100 million colony forming units (CFUs), 20-100 million CFU, 50-100 million CFU, or 50-200 million CFU. In some embodiments, the microbial consortium comprises at least  $10^7$ ,  $10^8$ ,  $10^9$ ,  $10^{10}$ ,  $10^{11}$  or  $10^{12}$  CFU of total bacteria. In some embodiments, the microbial consortium comprises at least  $10^7$ ,  $10^8$ ,  $10^9$ ,  $10^{10}$ ,  $10^{11}$  or  $10^{12}$  CFU/mL of total bacteria.

**[0260]** In some embodiments, at least a subset of the lactobacilli comprised in the microbial consortia or related compositions described herein is capable of engrafting in a human vagina. In some embodiments, at least a subset of the individual lactobacilli isolates (*e.g.*, *L. crispatus* isolates, *L. jensenii* isolates) described herein is capable of engrafting in a human vagina. In some embodiments, engraftment occurs even during menstrual discharge. In some embodiments, engraftment is substantially maintained (stable) after one or more menstrual discharge. The lactobacilli comprised in the microbial consortia, the individual lactobacilli isolates (*e.g.*, *L. crispatus* isolates, *L. jensenii* isolates) described herein, or related compositions may continue to reside in the vagina after administration over several menstruation cycles. In some embodiments, the lactobacilli engraft in the vaginal microbial niche over one or more than one (*e.g.*, two, three, four, five or six) menstruation cycle upon vaginal administration. Residence time can be determined, *e.g.*, using nucleic acid sequencing, *e.g.*, for specific lactobacilli, *e.g.*, comparing sequencing results prior to and post administration of the microbial consortia, individual lactobacilli isolates (*e.g.*, *L. crispatus* isolates, *L. jensenii* isolates), or related compositions described herein, *e.g.*, to determine the identity of newly added members (*e.g.*, one or more lactobacilli) to the subject's microbial community, and then at predetermined time intervals (*e.g.*, prior or post a menstruation cycle, optionally, over a number of menstruation cycles) determine (*e.g.*, through nucleic acid sequencing) that all or a certain subset of the newly added members are still substantially present at the specific time interval. In some embodiments, the residence time of the subject

's microbiota may be compared to the donor's microbiota. The residence time may vary depending on various factors including hormone levels (*e.g.*, estrogen), diet, sexual activity, acidity status of the vagina, and the presence or absence of genital infections and other microbial perturbations, *e.g.*, treatment with antibiotics.

**[0261]** Successful engraftment of the lactobacilli comprised in the microbial consortia or related compositions, or of the individual lactobacilli isolates (*e.g.*, *L. crispatus* isolates, *L. jensenii* isolates) described herein, may be indicated by one or more of: decreased pH, increased lactic acid content, lower abundance of antibiotic resistance genes, decreased amount of fungal DNA, decreased toxin content, decreased pathogenicity factors, decreased inflammatory cytokines and chemokines, decreased immune cell infiltrates, decreased total bacterial DNA load, decreased total pathogenic DNA load, increased viscoelasticity, increased sialoglycan content, decreased relative or absolute abundance of pathobionts or pathogens, or any combination thereof in the vaginal cavity and the vaginal microbial niche (*e.g.*, when compared to baseline of the same subject (*e.g.*, prior to administration and engraftment) or a non-treated control subject).

**[0262]** In some embodiments, pathogens are not detectable in a microbial consortium of the present disclosure. A pathogen is any microorganism (*e.g.*, virus, bacteria, fungus, protozoa) that has the capability of causing disease or illness in a host. Non-limiting examples of pathogens include *Gardnerella vaginalis*, *Chlamydia trachomatis*, Herpes Simplex Virus, Human Papillomavirus, *Candida* species, and *Trichomonas vaginalis*. In some embodiments, other non-lactobacilli are not detectable. As used herein, the term “**other non-lactobacilli**” refers to microorganisms that may or may not be pathogenic (*e.g.*, may be commensal organisms or environmental contaminants) and are not part of the lactobacilli genus. Non-limiting examples of other non-lactobacilli include *Atopobium* species, *Prevotella* species, and *Streptococcus* species.

#### **Lactobacilli in the vaginal niche**

**[0263]** Lactobacilli are the predominant microorganisms in the vaginal microbial community, and they play a major role in maintaining a healthy urogenital tract, including the vaginal and/or endometrial niche. Lactobacilli are capable of preventing adhesion and growth of pathogenic microorganisms and/or overgrowth of pathobionts through mechanisms that appear to involve secretion of anti-adhesion factors, hydrogen peroxide, bacteriocins and fermenting the glycogen to lactic acid, thereby creating an acidic environment hostile to

pathogens and pathobionts. The genus *Lactobacillus* comprises a phenotypically heterogeneous group of Gram-positive, aerotolerant, lactic acid producing bacteria. Other typical characteristics include being catalase-negative and rod-shaped, and generally possess DNA with a low content of guanine (G) and cytosine (C), less than about 50%. They are members of the phylum Firmicutes, class Bacilli, order *Lactobacillales* and family *Lactobacillaceae*. One skilled in the art will be able to identify *Lactobacillus* species using standard techniques.

[0264] Species of lactobacilli can be identified phenotypically, as well as genetically, *e.g.*, on the basis of 16S rRNA (ribosomal RNA) sequence (or the DNA encoding the 16S rRNA, generally referred to as 16S rDNA). Genetic analysis can be performed using standard techniques, for example whole genome sequencing analysis as well as widely used typing approaches based on nucleotide variation in several hundred DNA sequences and a few gene fragments: Multi-locus Sequence Typing (MLST), Multi-locus Variable number of tandem repeats Analysis (MLVA), rMLST and cgMLST) discussed, *e.g.*, in Marcos Pérez-Losada, M. et al., “Microbial sequence typing in the genomic era”, *Infection, Genetics and Evolution*, Vol. 63, Sep. 2018, p. 346-359.

[0265] Other identification techniques include: Vaginal pH, Nugent Score, Whiff Test, gas liquid chromatographic analysis of glucose fermentation products, total anaerobe concentrations, total aerobe concentrations, enzymatic activity (*e.g.*, lipase, phospholipase A2 and phospholipase C, hydrogen peroxide production).

#### **Stability**

[0266] The lactobacilli comprised in the microbial consortia or related compositions comprising the same, are generally viable for up to one month if stored in the refrigerator at 4° C and up to several months (*e.g.*, 1, 2, 3, 4, 5, 6, 9, 12, 15, 18, or 24 months, or longer) if stored frozen at about –18° C, or preferably at about -80° C in solution or on agar plates. Individual lactobacilli isolates described herein (*e.g.*, *L. jensenii* isolates, *L. crispatus* isolates) are generally viable for up to one month if stored in the refrigerator at 4° C and up to several months (*e.g.*, 1, 2, 3, 4, 5, 6, 9, 12, 15, 18, or 24 months, or longer) if stored frozen at about –18° C, or preferably at about -80° C in solution or on agar plates.

[0267] The lactobacilli comprised in the microbial consortia or related compositions comprising the same, are generally viable for up to several months (*e.g.*, 1, 2, 3, 4, 5, 6, 9, 12, 15, 18, or 24 months, or longer) or years (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 years) if stored in

a lyophilized or freeze-dried format. Individual lactobacilli isolates described herein (*e.g.*, *L. jensenii* isolates, *L. crispatus* isolates) are generally viable for up to several months (*e.g.*, 1, 2, 3, 4, 5, 6, 9, 12, 15, 18, or 24 months, or longer) or years (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 years) if stored in a lyophilized or freeze-dried format. Viability decreases over time. The microbial consortia or related compositions are suitable for administration if they retain at least 30%, 40%, 50%, 60%, 70%, or at least 80% viable bacteria prior to use. Individual lactobacilli isolates described herein (*e.g.*, *L. jensenii* isolates, *L. crispatus* isolates) are suitable for administration if they retain at least 30%, 40%, 50%, 60%, 70%, or at least 80% viable bacteria prior to use. Viable cells are generally able to colonize and engraft. Percent viability refers to the percentage of viable bacteria in a population. In some embodiments, the microbial consortia, individual lactobacilli isolates (*e.g.*, *L. jensenii* isolates, *L. crispatus* isolates), or other related composition are further processed by adding a diluent, such as, *e.g.*, saline, *e.g.*, to formulate a pharmaceutical composition. In some embodiments, the microbial consortia, individual lactobacilli isolates (*e.g.*, *L. jensenii* isolates, *L. crispatus* isolates), one or more lactobacilli isolates (*e.g.*, *L. jensenii* isolates, *L. crispatus* isolates) identified by the method of the invention, or compositions thereof described herein are further processed to be lyophilized (*e.g.*, by freeze-drying, spray drying or other technologies known in the art), *e.g.*, for easy storage, packaging, formulated in, for instance, compositions, and transport, and can be rehydrated before administration.

#### **Acidifying Agents**

[0268] The microbial consortia, individual lactobacilli isolates (*e.g.*, *L. crispatus* isolates, *L. jensenii* isolates) described herein, one or more lactobacilli isolates identified by the method of the invention, or related compositions, may optionally comprise one or more acidifying agents, such as, *e.g.*, organic acids or salts thereof. In some embodiments, acidifying agents may be used to reduce the pH of the compositions, *e.g.*, to below pH 5.5 or 5.0, *e.g.*, to between pH 3.5 to 4.5, or pH 3.0 to 4.5. In decreasing pH such acidifiers may act as anti-microbial agents (*e.g.*, to inhibit *Candida* or pathogenic bacteria). Acidifying agents comprise, *e.g.*, lactic, acetic, ascorbic, citric, folic sorbic, or boric acid. In other embodiments, the acidifying agent is administered separate from the compositions comprising one or more lactobacilli isolates identified by the method of the invention, a microbial consortia, individual lactobacilli isolates described herein (*e.g.*, *L. jensenii* isolates, *L. crispatus* isolates), or related compositions; *e.g.* prior to, concurrent with, or after administration of the

composition (*e.g.*, in a different dosage form, such as, *e.g.* a suppository, cream, gel, powder, douche or similar), *e.g.*, for one to seven days, or one to ten days. In these embodiments, the acidifying agent may be used to promote the survival and engraftment of the one or more lactobacilli isolates identified by the method of the invention, the *Lactobacilli* comprised in the microbial consortia, individual lactobacilli isolates described herein (*e.g.*, *L. jensenii* isolates, *L. crispatus* isolates), or related compositions. Acidifying agents may also be useful as spermicides, *e.g.*, to kill or inactivate residual sperm that may be present in the preparation. In one embodiment, the spermicidal activity is contributed by adding lactic acid. In one embodiment, lactic acid may be provided as a racemic mixture of D- and L-isomers, or at different suitable ratio, including, *e.g.*, only D-lactate or only L-lactate.

[0269] In some embodiments, microbial consortia, compositions comprising individual lactobacilli isolates (*e.g.*, *L. jensenii* isolates, *L. crispatus* isolates), or related compositions are acidified by adding 0.1%, 0.5%, 1%, 1.5%, 2%, 2.5%, 3% or 5% of acidifier (*e.g.*, lactic acid). In preferred embodiments, the acidifier is added at a concentration of 0.5% to 1.5%, or 0.2% to 2% (w/w), or a similar concentration that matches a healthy vagina.

#### **Viscosity**

[0270] In some embodiments, microbial consortia, compositions comprising individual lactobacilli isolates (*e.g.*, *L. jensenii* isolates, *L. crispatus* isolates), or related compositions described herein may comprise one or more pharmaceutically acceptable buffer or diluent or other excipients. In embodiments, one or more excipients is used to formulate the composition in different dosage forms, such as, *e.g.*, suppositories, creams or dissolving films or tablets.

[0271] In some embodiments, the dosage form is liquid, solid or semi-solid. The solid dosage form preferably comprises a tablet, capsule, or a film. The semi-solid dosage form preferably comprises a suppository, ointment, gel, cream or rigid foam. In a preferred embodiment, the dosage form is a gel.

#### **Prebiotics**

[0272] A “prebiotic” as used herein is a growth substrate, which increases growth of bacteria (such as lactobacilli) comprised in a microbial consortia, individual lactobacilli isolates (*e.g.*, *L. jensenii* isolates, *L. crispatus* isolates) described herein, in a composition comprising one or more (isolated) bacterial isolates identified according to the method of the invention, or related compositions as could be measured, *e.g.*, *in vitro*. If desired, though it is

not generally necessary, a prebiotic may be added to the compositions described herein, *e.g.*, to create a symbiotic mixture, *e.g.*, to increase growth of the bacteria upon administration to the genitourinary tract of a female subject. This may, under certain circumstances, increase successful colonization and engraftment. In other embodiments, prebiotics may be used for the maintenance of an engrafted preparation and/or general vaginal health.

[0273] If a prebiotic is desired, it should be carefully chosen to not be greatly metabolizable by any yeast (*e.g.*, *Candida* species), pathobionts or pathogens (*e.g.*, *E. coli* and other Gram-negative bacteria) that may reside in the female subject's genitourinary tract (*e.g.*, to avoid promoting their growth). Prebiotics include, *e.g.*, lactitol, lactulose, and in some instances also other oligosaccharides and soluble fibers, *e.g.*, fructooligosaccharides (FOS), glucooligosaccharides (GOS), and inulin.

#### **Other Active Agents**

[0274] If desired, other active agents may be added to microbial consortia, *e.g.*, to address bacterial or fungal infections and/or sexually transmitted diseases in the female subject, for example antimicrobial agents, antifungal agents, antibacterial agents, antiviral agents, antibiotics, antiparasitic agents (*e.g.*, with activities against *Trichomonas vaginalis*), anti-inflammatory agents, and the like. Care must be taken when formulating these agents into the pharmaceutical composition so as to not substantially interfere with the activity and efficacy of the lactobacilli comprised in the preparations and compositions described herein.

[0275] In some embodiments, the microbial consortia comprise a form of estrogen. Adequate levels of estrogen play a role in the trophism of vaginal mucosa, and estrogens increase the cellular content of glycogen.

[0276] In some embodiments, the microbial consortia comprise thiosulfate, *e.g.*, to potentiate the anti-pathogenic effect of lactobacilli.

[0277] If desired, a treatment regimen could utilize an antibiotic administered to a subject prior to administration of the microbial consortia. An antibiotic may include metronidazole, or one or more antibiotics of the following classes: a macrolide (*e.g.*, azithromycin, clarithromycin and erythromycin), a tetracycline (*e.g.*, doxycycline, tigecycline), a fluoroquinolone (*e.g.*, levofloxacin, ciprofloxacin and mocifloxacin), a cephalosporin (*e.g.*, ceftriaxone, defotaxime, ceftazidime, cefepime), a penicillin (*e.g.*, amoxicillin, amoxicillin with clavulanate, ampicillin, piperacillin, and ticarcillin) optionally with a beta-lactamase inhibitor (*e.g.*, sulbactam, tazobactam and clavulanic acid), such as



ampicillin-sulbactam, piperacillin-tazobactam and ticarcillin with clavulanate, an aminoglycoside (*e.g.*, amikacin, arbekacin, gentamicin, kanamycin, neomycin, netilmicin, paromomycin, rhodostreptomycin, streptomycin, tobramycin, and apramycin), a penem or carbapenem (*e.g.*, doripenem, ertapenem, imipenem and meropenem), a monobactam (*e.g.*, aztreonam), an oxazolidinone (*e.g.*, linezolid), vancomycin, glycopeptide antibiotics (*e.g.*, telavancin), and the like.

[0278] In some embodiments, the pharmaceutical compositions can further contain an antimicrobial (an antibiotic or antifungal) selected from metronidazole, tinidazole, secnidazole, clindamycin, nystatin, azithromycin, erythromycin, ofloxacin, doxycycline, levofloxacin, amoxicillin, and fluconazole.

[0279] If desired, the microbial consortia can contain an agent for treating infections with mycobacteria. Suitable agents for treating infections with mycobacteria include an aminoglycoside (*e.g.*, capreomycin, kanamycin, streptomycin), a fluoroquinolone (*e.g.*, ciprofloxacin, levofloxacin, moxifloxacin), isozianid and isozianid analogs (*e.g.*, ethionamide), aminosalicylate, cycloserine, diarylquinoline, ethambutol, pyrazinamide, prothionamide, rifampin, and the like.

[0280] If desired, the microbial consortia can contain a suitable antiviral agent, such as remdesivir, foscavir, Cytogam® (cytomegalovirus immune globulin), pleconaril, rupintrivir, palivizumab, motavizumab, cytarabine, docosanol, denotivir, cidofovir, and acyclovir.

[0281] If desired, the microbial consortia can contain a suitable antifungal agent, such as polyene (*e.g.*, nystatin and natamycin) and imidazole antifungals (*e.g.*, flucanazole and clotrimazole).

[0282] If desired, the donor sample or compositions comprising one or more bacterial isolates identified by the inventive method, can contain one or more suitable steroids. For example, the donor sample or composition may include androgens/anabolic steroids, estrogen, progestogens, corticosteroids, neurosteroids, estradiol, estropipate, drospirenone, norethisterone, levonorgestrel, testosterone, fluoxymesterone, methylesterosterone, oxandrolone, and oxymetholone.

### **Dosage Forms and Formulations**

[0283] The microbial consortia, individual lactobacilli isolates (*e.g.*, *L. jensenii* isolates, *L. crispatus* isolates), or related compositions may be formulated into isolate preparations and (pharmaceutical) compositions and various dosage forms. In the following, the preferred

dosage forms are provided, wherein all dosage forms are equally suitable for the microbial consortia or individual lactobacilli isolates (*e.g.*, *L. jensenii* isolates, *L. crispatus* isolates).

**[0284]** Multiple dosage forms are contemplated herein, and include a suspension, spray, gel, cream, ointment, powder, (gelatin or vegetable cellulose) capsule, solution for lavages or douches, foams, films, ovules, a vaginal insert (*e.g.* tampon), tablets, disk, wafer (*e.g.*, drying on film, by vaporization), or a microencapsulated product employing excipients and formulation techniques known to those skilled in the art. Particularly preferred dosage forms include formed gels, lyophilized gels, tablets, frozen formulations and films. In one embodiment, the dosage form is a capsule.

**[0285]** A number of suitable excipients can be used to formulate the microbial consortia, compositions comprising individual lactobacilli isolates (*e.g.*, *L. jensenii* isolates, *L. crispatus* isolates), or related compositions such as bulking agents, polymers, carbon substrates, mucoadhesive agents, or pH modifiers and/or buffers.

**[0286]** The carbon substrate excipients may act as a carbon substrate for the microbiota contained in the microbial consortia, compositions comprising individual lactobacilli isolates (*e.g.*, *L. jensenii* isolates, *L. crispatus* isolates), or related compositions. Such carbon sources comprise mannitol, and Guar gum. Some excipients may further serve as mucoadhesive agents or as viscosity agents. Bulking agents may comprise one or more of mannitol, microcrystalline cellulose, guar gum, inulin, or alginic acid (*e.g.*, sodium alginate). Polymers may comprise structural polymers. In some embodiments, polymers comprise one or more of mucin, hyaluronic acid, polyvinyl alcohol, sodium CMC, polyvinylpyrrolidone, hydroxypropyl methylcellulose (*e.g.*, Carbopol 934), and poloxamer (*e.g.*, poloxamer 407). Mucoadhesive agents comprise, *e.g.*, alginic acid (sodium alginate) and sodium CMC. Viscosity agents comprise, *e.g.*, to Guar gum and Carbopol 934.

**[0287]** Some excipients serve as pH modifiers and/or buffers, such as lactic acid and acetate buffer.

**[0288]** Suitable formulations show little to no flow on suitable vertical surfaces and maintain high bacterial viability (*e.g.*, CFU count) both upon formulation and during (long-term) storage.

**[0289]** Desired formulation selection parameters include, for example, water activity / moisture content (*e.g.*, of dried formulations), *e.g.*, between 0.5 – 3% water (*e.g.*, for longer term dried formulation stability); microbial diversity, *e.g.*, absence of microorganisms such

as, *Pseudomonas aeruginosa*, *Candida albicans*, *Staphylococcus aureus*, Ph Eur criteria 5.1.4, 2.6.12 & 2.6.13).

[0290] Suitable testing methods include standard assays, such as, plate count (*e.g.*, MRS agar), *e.g.*, for life bacteria count, dose determination, shelf-life; rheometer, *e.g.*, for mucoadhesion and viscosity, pH meter, Karl Fisher / water activity meter, Ph Eur testing, *e.g.*, for microbial loads.

[0291] The one or more bacterial isolates identified by the methods of the invention, the microbial consortia, individual lactobacilli isolates (*e.g.*, *L. jensenii* isolates, *L. crispatus* isolates), or related compositions can be lyophilized and formulated into, *e.g.*, gels and tablets as well as other dosage forms that can be filled with lyophilized products, such as, *e.g.*, capsules. The microbial consortia, individual lactobacilli isolates (*e.g.*, *L. jensenii* isolates, *L. crispatus* isolates), or related compositions can also be formulated into gels that can be frozen, as well as into liquid media (*e.g.*, with glycerol) that can be frozen. The composition comprising one or more bacterial isolates identified by the methods of the invention, the microbial consortia, individual lactobacilli isolates (*e.g.*, *L. jensenii* isolates, *L. crispatus* isolates), or related compositions can also be formulated into (air-dried) films, that could be, *e.g.*, shaped like disks.

#### **Lyophilized Formulations**

[0292] The microbial consortia, composition comprising individual lactobacilli isolates (*e.g.*, *L. jensenii* isolates, *L. crispatus* isolates), or related compositions, can be lyophilized. The lyophilized preparation or lyophilized composition is stable and may be stored for extended periods of time at 2-8°C. The lyophilized preparation or lyophilized composition comprises the lyophilized microbial consortia or lyophilized individual lactobacilli isolates (*e.g.*, *L. jensenii* isolates, *L. crispatus* isolates). The lyophilized preparation or lyophilized composition may be supplied in a vial. The lyophilized preparation or lyophilized composition can be reconstituted prior to administration (*e.g.*, in a clinical or home setting) with a reconstitution agent. In some embodiments, the reconstitution agent comprises a gel, a gel forming agent, or a liquid. The liquid may comprise water, saline or another liquid suitable for reconstitution and subsequent administration to a subject.

[0293] Sequencing may be performed to assess the microbial community of the donor microbiota sample and to select suitable donor females.

[0294] Optionally, nucleic acid sequencing is performed to identify the presence of any antimicrobial resistance (AMR) genes in the donor sample, *e.g.*, to determine that a donor sample is substantially free of antimicrobial resistance (AMR) genes. Antimicrobial resistance (AMR) genes include genes that confer resistance to one or more antibiotics, including, *e.g.*, aminoglycosides, beta-lactams, tetracyclines, and sulfonamides (*e.g.*, as described and cataloged in the NCBI National Database of Antibiotic Resistant Organisms (NDARO)). It will be appreciated that due to extensive use of antibiotics the cut-off is not zero. Some reasonable allowance for the presence of AMR genes is made. The precise cut-off can be determined by one of ordinary skill, based on, *e.g.*, the nature of the AMR genes (*e.g.*, degree of health concern) and public health recommendations.

#### **Methods of Administration**

[0295] Aspects of this disclosure provide, in part, methods for vaginal administration to a human female subject of a microbial consortia, individual lactobacilli isolates (*e.g.*, *L. jensenii* isolates, *L. crispatus* isolates), or related compositions described herein. Further aspects of this disclosure provide, in part, a method of increasing the relative abundance of lactobacilli (*e.g.*, *L. crispatus*, *L. jensenii*) in the female genitourinary tract of a subject comprising administering a microbial consortium described herein to the genitourinary tract of the subject.

[0296] In some embodiments, the female genitourinary tract is dysbiotic. In some embodiments, the genitourinary tract of the subject exhibits dysbiosis. Further, the invention provides the microbial consortium for use in therapy. In one embodiment, the invention provides the microbial consortium described herein for use in a method of treating dysbiosis of the genitourinary tract of a female subject, said method comprising administering the microbial consortium described herein to the genitourinary tract of the subject.

[0297] In some embodiments, the female genitourinary tract of the subject comprises a bacterial population having a relative abundance of *Lactobacillus* that is less than 90% of the total bacterial population prior to administration of a microbial consortium of the present disclosure. In some embodiments, the female genitourinary tract of the subject comprises a bacterial population having a relative abundance of *Lactobacillus* that is less than 85%, less than 80%, less than 75%, less than 70%, less than 65%, less than 60%, less than 55%, less than 50%, less than 45%, less than 40%, less than 35%, less than 30%, less than 25%, or less than 20% of the total bacterial population prior to administration of a microbial consortium of

the present disclosure. In some embodiments, the female genitourinary tract of the subject comprises a bacterial population having a relative abundance of *Lactobacillus* that is between 20% - 25%, between 20% - 30%, between 20% - 35%, between 20% - 40%, between 20% - 45%, between 20% - 50%, between 20% - 55%, between 20% - 60%, between 20% - 65%, between 20% - 70%, between 20% - 75%, between 20% - 80%, between 20% - 85%, between 20% - 90%, between 25% - 30%, between 25% - 35%, between 25% - 40%, between 25% - 45%, between 25% - 50%, between 25% - 55%, between 25% - 60%, between 25% - 65%, between 25% - 70%, between 25% - 75%, between 25% - 80%, between 25% - 85%, between 25% - 90%, between 30% - 35%, between 30% - 40%, between 30% - 45%, between 30% - 50%, between 30% - 55%, between 30% - 60%, between 30% - 65%, between 30% - 70%, between 30% - 75%, between 30% - 80%, between 30% - 85%, between 30% - 90%, between 35% - 40%, between 35% - 45%, between 35% - 50%, between 35% - 55%, between 35% - 60%, between 35% - 65%, between 35% - 70%, between 35% - 75%, between 35% - 80%, between 35% - 85%, between 35% - 90%, between 60% - 65%, between 60% - 70%, between 60% - 75%, between 60% - 80%, between 60% - 85%, between 60% - 90%, between 65% - 70%, between 65% - 75%, between 65% - 80%, between 65% - 85%, between 65% - 90%, between 70% - 75%, between 70% - 80%, between 70% - 85%, between 70% - 90%, between 75% - 80%, between 75% - 85%, between 75% - 90%, between 80% - 85%, between 80% - 90%, or between 85% - 95% of the total bacterial population prior to administration of a microbial consortium of the present disclosure.

**[0298]** In some embodiments, the female genitourinary tract of the subject comprises a bacterial population having a relative abundance of *Lactobacillus* that is greater than 70% of the total bacterial population following administration of a microbial consortium of the present disclosure. In some embodiments, the female genitourinary tract of the subject comprises a bacterial population having a relative abundance of *Lactobacillus* that is greater than 75%, greater than 80%, greater than 85%, greater than 90%, greater than 95%, or greater than 98% of the total bacterial population following administration of a microbial consortium of the present disclosure. In some embodiments, the female genitourinary tract of the subject comprises a bacterial population having a relative abundance of *Lactobacillus* that is between 70% - 75%, between 70% - 80%, between 70% - 85%, between 70% - 90%, between 70% - 95%, between 70% - 100%, between 75% - 80%, between 75% - 85%, between 75% - 90%, between 75% - 95%, between 75% - 100%, between 80% - 85%, between 80% - 90%,

between 80% - 95%, between 80% - 100%, between 85% - 90%, between 85% - 95%, between 85% - 100%, between 90% - 95%, between 90% - 100%, or between 95% - 100% of the total bacterial population following administration of a microbial consortium of the present disclosure.

**[0299]** In some embodiments, the relative abundance of *Lactobacillus* in the bacterial population of the female genitourinary tract of the subject is increased by at least 5% following administration of a microbial consortium of the present disclosure. In some embodiments, the relative abundance of *Lactobacillus* in the bacterial population of the female genitourinary tract of the subject is increased by at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, or at least 60% following administration of a microbial consortium of the present disclosure. In some embodiments, the relative abundance of *Lactobacillus* in the bacterial population of the female genitourinary tract of the subject is increased by between 5% - 10%, between 5% - 15%, between 5% - 20%, between 5% - 25%, between 5% - 30%, between 5% - 35%, between 5% - 40%, between 5% - 45%, between 5% - 50%, between 5% - 55%, between 5% - 60%, between 10% - 15%, between 10% - 20%, between 10% - 25%, between 10% - 30%, between 10% - 35%, between 10% - 40%, between 10% - 45%, between 10% - 50%, between 10% - 55%, between 10% - 60%, between 15% - 20%, between 15% - 25%, between 15% - 30%, between 15% - 35%, between 15% - 40%, between 15% - 45%, between 15% - 50%, between 15% - 55%, between 15% - 60%, between 20% - 25%, between 20% - 30%, between 20% - 35%, between 20% - 40%, between 20% - 45%, between 20% - 50%, between 20% - 55%, between 20% - 60%, between 35% - 60%, between 40% - 45%, between 40% - 50%, between 40% - 55%, between 40% - 60%, between 45% - 50%, between 45% - 55%, between 45% - 60%, between 50% - 55%, between 50% - 60%, or between 55% - 60% following administration of a microbial consortium of the present disclosure.

**[0300]** Vaginal dysbiosis refers to an imbalance or disruption in the normal microbial community within the vagina. It is often characterized by a decrease in the population of *Lactobacillus* species and an overgrowth of other microorganisms, which may include pathogenic bacteria, anaerobic bacteria, or yeasts. This shift in microbial composition can result in an increase in vaginal pH, a decrease in lactic acid production, and alterations in the overall ecosystem of the vagina (Lev-Sagie A, *et al.* The Vaginal Microbiome: II. Vaginal Dysbiotic Conditions, J Low Genit Tract Dis 26(1): 79-84 (2022); Saraf VS, *et al.* Vaginal

microbiome: normalcy vs dysbiosis, Archives of Microbiology 203: 3793-3802 (2021); van de Wijert JHHM, *et al.* The Vaginal Microbiota: What Have We Learned after a Decade of Molecular Characterization, PLoS One 9(8): e105998 (2014)). Method for treating vaginal dysbiosis are known in the art and may include administration of probiotics (*e.g.*, as oral supplements or applied directly to the vagina using suppositories or creams), administration of antibiotics (*e.g.*, in cases where vaginal dysbiosis is associated with a bacterial infection, such as bacterial vaginosis), administration of antifungal medications (*e.g.*, in cases where yeast, such as *Candida*, is the cause of dysbiosis), hormone replacement therapy or local estrogen therapy, administration of a vaginal microbiome transplant (*e.g.*, as a suppository or gel), or administration of acidifying agents or gels.

**[0301]** The methods may include using a device for administration, *e.g.*, these methods would normally be carried out by a healthcare provider (*e.g.*, in a clinic). Alternatively, the methods include using an alternative dosage form described herein, such as, *e.g.*, a suppository, tablet, capsule, film, cream, etc. The methods can, if desired, be carried out by the subject herself, *e.g.*, by self-administration (*e.g.*, at home). The methods may also include other healthcare related activities, such as diagnosing a health issue and providing standard of care in addition to providing a microbial consortia, composition comprising individual lactobacilli isolates (*e.g.*, *L. jensenii* isolates, *L. crispatus* isolates), or related composition described herein. The activities can include one or more combination therapies provided herein. For example, the methods for administration described herein may further comprise administering antimicrobial agents, antifungal agents, antibacterial agents, antiviral agents, antibiotics, antiparasitic agents (*e.g.*, with activities against *Trichomonas vaginalis*), anti-inflammatory agents, and the like.

**[0302]** In embodiments in which administration is carried out using a device, the device typically includes an open end (*e.g.*, a tip) for insertion into the vaginal cavity, and a dispensing end (*e.g.*, a plunger or piston) to expel the composition through the open end. The administration steps include: a) introducing the open end into a vaginal cavity, b) expelling the composition into the vaginal cavity, c) removing the device from the vaginal cavity (after administering the desired dose). Administration is preferably carried out with the subject being in a lithotomy position, *e.g.*, with the female subject in a lithotomy position. In some embodiments, the subject is to remain in a lithotomy position for at least 5 minutes, at least 10 minutes, at least 20 minutes, at least 30 minutes before returning to an upright position to allow the microbial consortia, individual lactobacilli isolates (*e.g.*, *L. jensenii* isolates, *L.*

*crispatus* isolates), or related composition sufficient residence time in the vaginal cavity, *e.g.*, sufficient contact time with the mucosal or endometrial surfaces of the vagina. In some embodiments, the administration is carried out targeting areas high in the vaginal cavity, *e.g.*, near the vaginal fornices.

**[0303]** If desired, the menstrual cycle of the subject female is taken into account when determining the timing of administration. For example, administration may be avoided during menstrual discharge. In some embodiments, a time other than during menstrual discharge is preferred for carrying out the administration, including, *e.g.*, during a time window that includes prior to ovulation and prior to menstrual discharge.

**[0304]** A microbial consortium may be administered to a subject as necessary. In some embodiments, a microbial consortium is administered to a subject one, two, or three times within 3-21 days. In some embodiments, a microbial consortium is administered to a subject once daily for 3, 5, 10, 15 or 20 days. In some embodiments, a microbial consortium is administered to a subject once daily for 5 days. In some embodiments, a microbial consortium is administered to a subject once daily for 15 days. In some embodiments, a microbial consortium is administered to a subject once every one to four weeks for a period of one to six months. In some embodiments, a microbial consortium is administered to a subject twice weekly, three times weekly or four times weekly for 1, 2, 3, 4, 5, 6, 7, or 8 weeks.

**[0305]** In some embodiments, the precise steps, timing, and length of the administration varies between subjects (and is, *e.g.*, determined by a healthcare provider) in order to provide optimal conditions for the bacteria (*e.g.*, lactobacilli) comprised in the microbial consortia or related compositions to colonize and become established (engrafted) in the vagina of the female subject.

#### **Health Symptoms and Biomarker Assessment**

**[0306]** In some embodiments, successful colonization and engraftment is associated with changes in (a) the composition of the microbiota of the female subject's genitourinary tract (*e.g.*, the vaginal and or endometrial tract) and/or (b) one or more disease-associated health symptoms over a predetermined time period. These desired changes may be included as additional selection criteria for the one or more isolates that are identified as being capable of colonization and engraftment. In a preferred embodiment, the one or more isolates identified by the methods described herein are not only capable of colonizing and engrafting in the



urogenital tract but also are associated with desired changes in the composition of the microbiota of the female subject's genitourinary tract and/or (b) one or more disease-associated health symptoms. Such isolates may preferably be selected for isolation, propagation and formulation into preparations and (pharmaceutical) compositions.

**[0307]** Desirable changes of the composition of the microbiota of the female subject's genitourinary tract that can be determined include, *e.g.*, a reduction in the relative abundance of pathogen and/or pathobiont residing in the genitourinary tract (*e.g.*, less than 10% relative abundance of pathogen and/or pathobiont), and/or an increase in relative abundance of *Lactobacillus* above 50% (above 60%, or above 70%).

**[0308]** Desirable changes in one or more disease-associated health symptoms that can be determined include, *e.g.*, (a) a reduction in one or more pro-inflammatory markers (*e.g.*, local or systemic cytokines and chemokines) and/or decreased immune cell infiltrates; (b) a lowering of vaginal pH (*e.g.*, by at least pH 0.3, 0.5, 1.0, or 1.5) and/or increased lactic acid content of the vaginal tract; and/or (c) a change in grade based on the Ison-Hay scoring system (*e.g.*, to grade 0 or grade I), when compared to the values of (a), (b), and/or (c) determined in the same subject prior to administration of one or more isolates capable of colonization and engraftment.

**[0309]** Other desirable changes in one or more disease-associated health symptoms that can be determined include, lower abundance of antibiotic resistance genes, decreased amount of fungal DNA, decreased toxin content, and/or decreased pathogenicity and/or virulence factors.

**[0310]** The vaginal health status of a female subject can be assessed prior to and/or after administration at predetermined intervals. Testing may include using one or more of: Amsel Criteria, Nugent Gram-stain scoring system, and Hay-Ison Criteria.

**[0311]** Amsel Criteria include the presence of three of the following four symptoms: (a) thin homogeneous malodorous discharge; (b) vaginal pH fluid >4.5; (c) an amine odor from vaginal fluid when 10% KOH is added; and (d) the presence of "clue" cells (vaginal epithelial cells with adherent bacteria that obscure cell margins) (Amsel et al., Am. J. Med. 74:14-22 (1983)).

**[0312]** The Nugent Gram-stain scoring system involves assessment of a normally prepared Gram stain for relative abundance of three morphotypes of bacteria, and then calculating the so-called Nugent score based on the amounts of large Gram-positive rods (lactobacilli morphotype; decrease in lactobacilli is scored as 0 to 4), small Gram-negative

and variable rods (*Bacteroides* and *Gardnerella* morphotype; scored as 0 to 4), and curved gram-variable rods (*Mobiluncus* spp. morphotype; scored as 0 to 2). The Nugent score can range from 0 to 10, with scores of 0-3 deemed normal (non-BV), 4-6 intermediate, and 7-10 positive for BV.

**[0313]** Hay-Ison Criteria (alternatively Ison-Hay scoring system) suggests five grades of flora: a) Grade 0, epithelial cells with no bacteria; b) Grade I, normal vaginal flora (*Lactobacillus* morphotypes alone); c) Grade II, reduced numbers of *Lactobacillus* morphotypes with a mixed bacterial flora; d) Grade III, mixed bacterial flora only, few or absent *Lactobacillus* morphotypes; e) Grade IV, Gram positive cocci only.

**[0314]** Grades 0, I, and IV are found in women without BV. Grade II is intermediate and not found in women with BV as defined by Amsel criteria. Grade III is consistent with BV as diagnosed by Amsel criteria. Grade III flora are indicative of BV (C.A. Ison and P.E. Hay, Sex Transm. Infect. 2002 Dec;78(6):413-5).

**[0315]** Further desirable changes in one or more disease-associated health symptoms that can be determined include beneficial immune response, which may be assessed by e.g. proteomics (e.g., Olink) and transcriptomics (e.g., Fluidigm or RNAseq).

**[0316]** In some embodiments, the desired changes associated with successful colonization and engraftment in the female subject's genitourinary tract are assessed within one, two, three, four, six, eight, ten, or twelve week(s), or 6 months, 8 months, 10 months, 12 months, 18 months, or within 24 months post administration of the donor sample.

**[0317]** In some embodiments, the desired changes associated with successful colonization and engraftment in the female subject's genitourinary tract are assessed within 1, 2, 3, 4, 5, 6 or 7 day(s) post administration of the donor sample.

**[0318]** In some embodiments, the desired changes associated with successful colonization and engraftment in the female subject's genitourinary tract are detectable within 1, 2, 3, 4, 5, 6, or 7 day(s) post administration of the donor sample.

**[0319]** In some embodiments, the desired changes associated with successful colonization and engraftment in the female subject's genitourinary tract are detectable within one, two, three, four, six, eight, ten, or twelve week(s), or 6 months, 8 months, 10 months, 12 months, 18 months, or within 24 months post administration of the donor sample.

### **Nucleic Acid Sequencing**

**[0320]** In one embodiment, methods include sequencing of nucleic acids in the sample to identify the bacterial taxa present in the sample, *e.g.*, a sample from the female subject's genitourinary tract prior to and after administration of the donor sample, or a composition comprising one or more bacterial isolates. Genetic information from the sample can be obtained by nucleic acid extraction from the sample. Methods for extracting nucleic acid from a sample are known in the art. For sequencing, nucleic acid is extracted from the bacterial samples collected. Numerous standard DNA extraction protocols exist. Preferably, extraction protocols are used that specifically enrich for microbial DNA (*e.g.*, protocols that reduce the amount of human DNA in the sample). Exemplary commercial kits include MoLysis Complete5 kit, and Qiagen DNAeasy Blood & Tissue kit. In a preferred embodiment, human DNA is removed prior to sequencing. DNA may be extracted using the Molysis Complete5 kit (MolZym), which uses a differential lysis method to extract microbial DNA and remove human DNA.

**[0321]** Nucleic acid sequencing may be conducted by any method known in the art, including whole genome sequencing or other standard nucleic acid techniques, such as Southern blot, sequence analysis, electrophoresis, and PCR (including quantitative PCR (qPCR)). Sequences can be determined, *e.g.*, by Maxam-Gilbert sequencing, Sanger sequencing, capillary array DNA sequencing, thermal cycle sequencing, solid-phase sequencing, sequencing with mass spectrometry such as matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF/MS), and sequencing by hybridization. Examples of electrophoretic analysis include slab gel electrophoresis such as agarose or polyacrylamide gel electrophoresis, capillary electrophoresis, and denaturing gradient gel electrophoresis.

**[0322]** Other exemplary sequencing methods include: dideoxy sequencing reactions (Sanger method) using labeled terminators or primers and gel separation in slab or capillary, shotgun sequencing, polymerase chain reaction (PCR), real-time polymerase chain reaction (qPCR), reverse transcription PCR (RT-PCR), multiplex PCR, ligase chain reaction, pyrosequencing, sequencing by synthesis, sequencing by ligation, massively parallel signature sequencing, polony sequencing, SOLiD sequencing, DNA nanoball sequencing, mass spectrometry sequencing, microfluidic sequencing, high-throughput sequencing, Illumina sequencing, HiSeq sequencing, MiSeq sequencing, 16S ribosome sequencing,

sequencing by chain termination and gel separation, as described by Sanger et al., PNAS, 74(12): 5463-67 (1977); chemical degradation of nucleic acid fragments. See, Maxam et al., PNAS, 74: 560-564 (1977); sequencing by hybridization. See, *e.g.*, Harris et al., (U.S. patent application number 2009/0156412); Illumina (MiSeq and HiSeq), Helicos True Single Molecule Sequencing (tSMS) (Helicos Biosciences) See Harris T. D. et al. (2008) Science 320:106-109; see also, *e.g.*, Lapidus et al. (U.S. Pat. No. 7,169,560), Lapidus et al. (U.S. patent application number 2009/0191565), Quake et al. (U.S. Pat. No. 6,818,395), Harris (U.S. Pat. No. 7,282,337), Quake et al. (U.S. patent application number 2002/0164629), and Braslowsky, et al., PNAS, 100: 3960-3964 (2003); 454 sequencing, *e.g.*, Roche 454 GS FLX (Roche) (Margulies, M et al. 2005, Nature, 437, 376-380); SOLiD technology, *e.g.*, SOLiD 5500 series (Applied Biosystems); Ion Torrent/IonProton/Ion Proton sequencing, *e.g.*, PGM, Proton, S5 Series, ; single molecule, real-time (SMRT) technology (Pacific Biosciences); nanopore sequencing (Oxford Nanopore Technologies) (Soni G V and Meller A. (2007) Clin Chem 53: 1996-2001); Qiagen Gene Reader; chemical-sensitive field effect transistor (chemFET) arrays (See *e.g.*, US Patent Application Publication No. 2009/0026082); and use of an electron microscope (Moudrianakis E. N. and Beer M. PNAS USA. 1965 March; 53:564-71), or combinations thereof, incorporated by reference herein. Exemplary next generation sequencing methods are described, *e.g.*, in Muneer Ahmad Malla et al., “Exploring the Human Microbiome: The Potential Future Role of Next-Generation Sequencing in Disease Diagnosis and Treatment”, Front. Immunol., 07 January 2019, incorporated by reference herein). If desired, the extracted nucleic acids can be amplified. Suitable amplification methods include polymerase chain reaction (PCR), ligase chain reaction (LCR), loop-mediated isothermal amplification (LAMP), strand displacement amplification (SDA), replicase-mediated amplification, Immuno-amplification, nucleic acid sequence-based amplification (NASBA), self-sustained sequence replication (3SR), rolling circle amplification, and transcription-mediated amplification (TMA). For example, the PCR can be real-time PCR. In some embodiments, the PCR is quantitative real-time PCR (QRT-PCR).

**[0323]** If desired, nucleic acids synthesized as the result of gene transcription and/or metagenomic molecules can be detected. For example, in the case of the 16S rRNA gene, genomic DNA corresponding, in whole or part, to regions of the 16S rRNA gene, messenger RNA (mRNA) transcripts, in whole or part, of the 16S rRNA gene, and/or functional 16S

rRNA may be detected and used to enumerate the abundance of a microbial taxon, *e.g.*, characterized by sequence homology of a particular 16S rRNA gene sequence.

[0324] Genetic analysis can be performed using standard techniques, for example whole genome sequencing analysis as well as widely used typing approaches based on nucleotide variation in several hundred DNA sequences and a few gene fragments: Multi-locus Sequence Typing (MLST), Multi-locus Variable number of tandem repeats Analysis (MLVA), rMLST and cgMLST) (discussed, *e.g.*, in Marcos Pérez-Losada, M. et al., “Microbial sequence typing in the genomic era”, *Infection, Genetics and Evolution*, Vol. 63, Sep. 2018, p. 346-359).

[0325] Sequencing by any of the methods described above and known in the art produces sequence reads. Sequence reads can be analyzed according to any number of methods known in the art to identify microbes (including bacterial taxa) in a sample, *e.g.*, a sample of vaginal fluid from a healthy donor.

[0326] Identification of microorganisms and sorting of them into taxa may also be achieved by other means such as analyzing proteomes, transcriptomes, metabolomes, or combinations thereof. For example, microbial RNA transcripts, proteins, non-16S genes, etc. may be profiled.

[0327] Other methods that may be employed to identify microbes include microarrays or other oligonucleotide probe-based technology (DNA or RNA-based) or antibody-based detection methods, including enzyme-linked immunosorbent assay (ELISA), western blot, immunohistochemistry, immunocytochemistry, flow cytometry and fluorescence-activated cell sorting (FACS), immunoprecipitation, and enzyme linked immunospot (ELISPOT).

#### **Bacterial Isolate Isolation and Propagation**

[0328] The methods for identifying bacterial isolates capable of colonizing and engrafting the female genitourinary tract optionally further comprise one or more steps to isolate, test, propagate and formulate (*e.g.*, dosage forms, preparations, compositions, such as, *e.g.*, pharmaceutical compositions) the one or more isolates that are identified using the methods described herein. As described herein, one or more isolates capable of colonizing and engrafting the female genitourinary tract can be identified by comparing the constituents (bacterial taxa) of the donor female with those of the female subject (*e.g.*, by nucleic acid sequencing), after a predetermined amount of time and/or after determining a desired outcome (*e.g.*, changes in dysbiosis or reduction in one or more disease-associated

symptoms). Isolates identified from the sample that match one or more isolates from the donor sample by a predetermined degree of sequence identity are thought to be capable of colonization and engraftment, and, in some embodiments, associated with resolution of dysbiosis and/or disease-associated health symptoms, or another desired outcome. Upon obtaining the identification of the isolates that are successful in colonizing and engrafting the microbial niche *in vivo*, the isolates so identified are then isolated from the healthy donor sample comprising the healthy donor microbial community.

[0329] Isolation and propagation of bacterial isolates are known in the art and a variety of methods exist to accomplish bacterial isolation and propagation *in vitro*.

[0330] For example, the lactic acid producing bacteria (*e.g.*, from the donor sample comprising the donor (vaginal) microbial community) or the sample comprising the subject (vaginal) microbial community) are isolated, *e.g.*, from human vaginal fluid/cervicovaginal secretions, selected and cultured *in vitro* in a suitable nutrient medium providing at least one isolated bacterial isolate. The at least one isolated isolate is then optionally tested for its ability to colonize and engraft by administering the isolated isolate to a female subject. The at least one isolate is then tested for residence time in the microbial niche of the urogenital tract of the subject and/or its associating with one or more health-related outcomes, *e.g.*, resolution of dysbiosis or reduction of one or more disease-associated symptoms exhibited by the female subject prior to administration of the one or more isolated isolates.

[0331] Alternatively, or in addition, the isolated isolates are tested for the presence of antimicrobial resistance (AMR) genes. Preferably, the isolated isolates comprise a low abundance (or are substantially free) of AMR genes. Antimicrobial resistance (AMR) genes include genes that confer resistance to one or more antibiotics, including, *e.g.*, aminoglycosides, beta-lactams, tetracyclines, and sulfonamides (*e.g.*, as described and cataloged in the NCBI National Database of Antibiotic Resistant Organisms (NDARO)). It will be appreciated that due to extensive use of antibiotics the cut-off is not zero. Some reasonable allowance for the presence of AMR genes is made. The precise cut-off can be determined by one of ordinary skill, based on, *e.g.*, the nature of the AMR genes (*e.g.*, degree of health concern) and public health recommendations.

[0332] Alternatively, or in addition, the isolated isolates are tested for the presence of one or more pathogenicity factors/ bacterial virulence factors, and/or colonization factors.

[0333] Further, if desired, though not generally necessary for the methods described herein, the one or more isolated isolates can undergo one or more *in vitro* functionality tests

(either prior to, concurrent with or after administration to a female subject), *e.g.*, to supplement or complete the data obtained from *in vivo* testing, including a) growth in a culture medium, b) antagonism of pathogens (bacterial, viral, and/or fungal) that inhabit the vaginal mucosa, c) high viability during fermentation, d) persistent colonization of mucosal surfaces (*e.g.*, as judged by *in vitro* adherence assays, *e.g.*, VEC), e) high lactic acid production, f) genetic stability upon repeated cultivation and *in vivo* upon vaginal administration, and/or g) high viability after final processing, *e.g.*, preserved in a diluent, in lyophilized (desiccated) or (micro)encapsulated form, and storage. Each of the distinguishing characteristics described herein can be detected according to standard methods known in the art.

[0334] For example, antagonism to pathogens may be determined by measuring the production of hydrogen peroxide by the isolated isolate *in vitro*, *e.g.*, by colorimetric assay (*e.g.*, using tetramethylbenzidine-containing medium or using commercially available hydrogen peroxide detection strips). Alternatively, or in addition antagonism to pathogens may be determined by measuring plating assays with the isolated isolate(s) and one or more pathogens and determining the size (*e.g.*, diameter) and number of the inhibition zones around the colonies of the isolated isolate(s). For example, rapid growth can be measured, *e.g.*, by determining the growth rate (population doubling) into late log phase or stationary phase, *e.g.*, in comparison to a comparator isolate or known growth rates of other members of the same species. For example, a high rate of viability and percent viability (*e.g.*, after culturing or desiccation) can be measured, *e.g.*, by determining the growth rate and/or number of dead/live cells, after exposure to stress, *e.g.*, variations in culturing temperature, pH (*e.g.*, low pH, high pH) richness of the growth medium, concentration of buffering agents, etc., and exposure to desiccation (*e.g.*, freeze-drying, lyophilization) and storage (and measuring the percent viable cells at predetermined intervals). Life/dead cell counts can be performed visually, *e.g.*, with a microscope, *e.g.*, with a dark field condenser and a Petroff-Hausser counting chamber to obtain a total cell count, or by colony forming units (CFU) on appropriate media plates, and the Live/Dead ratio calculated as CFU per ml/total cells per ml. For example, lactic acid levels can be determined, *e.g.*, in culture media, using a commercially available lactate assay or using common chemical separation techniques such as capillary electrophoresis and chromatography (*e.g.*, HPLC). For example, the ability of the isolated isolate(s) to adhere to the vaginal mucosa *in vivo* may be assessed *in vitro* using vaginal epithelial cells (VEC), cultured Caco-2 cells or other cell lines (*e.g.*, HeLa) by

determining an adherence value, *e.g.*, by calculating the percent vaginal epithelial cell (VEC) cohesion value, defined as the percentage of VECs, where at least one bacterial cell is adhered to in the total number of VECs. Alternatively, the adherence is determined by calculating the percent vaginal epithelial cell (VEC) cohesion value, defined as the percentage of VECs, where at least one bacterial cell is adhered in the total number of VECs. Another measure of *in vitro* adherence is to count the average number of microbial cells adhered to a pre-defined number of epithelial cells. For example, the stability of the bacterial genetic profile of the isolated isolate(s) upon repeated cultivation in large-scale production, and during manufacturing processes of, for instance, (pharmaceutical) compositions may be tested. In one embodiment, the isolated isolate(s) preserve their genetic profile upon at least 10 repeated cultivations.

**[0335]** The isolate(s) are preferably evaluated and selected as to genetic stability *in vivo* upon vaginal administration, *e.g.*, they are genetically stable *in vivo* for at least 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 8 months, ten months, or for at least 12 months.

**[0336]** The isolated isolate(s) are preferably evaluated and selected as to their viability upon cryo-preservation (*e.g.*, freezing) and storage, as well as freeze-drying/lyophilization. The isolated isolate(s) are preferably viable for up to several months (*e.g.*, 1, 2, 3, 4, 5, 6, 9, 12, 15, 18, or 24 months, or longer) if stored at 4° C, frozen at about -18° C, or at about -80° C. Viability decreases over time. The isolated isolate(s) are suitable for use if they retain at least 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, or at least 80% viable bacteria prior to use. In some embodiments, the isolated isolate(s) retain at least 30%, 40%, 50%, 60%, 70%, or at least 80% viable bacteria prior to use. Viable cells are generally able to colonize and engraft. Percent viability refers to the percentage of viable bacteria in a population. If desired, the one or more isolated isolates are further processed to be freeze-dried (lyophilized) and/or pelleted, *e.g.*, for easy storage, packaging, formulated in, for instance, compositions, and transport, and can be rehydrated before administration.

**[0337]** The bacterial isolates capable of colonizing and engrafting the female genitourinary tract, *e.g.*, isolated by the methods described herein can be propagated by any means known in the art. For example, lactic acid producing isolates (including, *e.g.*, lactobacilli isolates) can be grown in a number of different types of media, in either liquid, semisolid (*e.g.*, agar), or solid form. The culturing conditions can be similar to the natural environment inhabited by the bacteria.



[0338] Standard bacterial media generally include salts, a source of carbohydrate, and a pH buffer. For *Lactobacillus*, pH is generally maintained in the range of about 4.5 to about 7.0, preferably between pH 5.5-6.5 with the addition of sodium phosphate, arginine, ammonium hydroxide, sodium hydroxide, potassium hydroxide, etc. Vitamins and growth agents, including amino acid formulations, can also be added. Bacterial media are known and commercially available (*e.g.*, from Sigma-Aldrich), including, *e.g.*, MRS (including *Lactobacillus*-MRS Agar (De Man, J. et al., J. Appl. Bact. 23:130-135, 1960)), Rogosa, NZM, YM, M17, Thayer-Martin, Trypticase Soy, CDM (Geshnizgani A.M., and Onderdonk A.B., (J Clin Microbiol. 1992 May;30(5):1323-6)), and Brain-Heart infusion (BHI) broth. Carbohydrates useful for growing the present isolate include D-galactose, D-glucose, D-fructose, D-mannose, D-mannitol, N-acetylglucosamine, amygdalin, arbutin, esculin, salicin, D-cellobiose, D-maltose, sucrose, D-trehalose, amidon, and glycogen. Some of the media can be made selective for lactic acid producing bacteria, such as lactobacilli, *e.g.*, by using a low pH, and addition of certain additives, *e.g.*, sodium acetate and ammonium citrate, *e.g.*, to inhibit other flora and fungi. In some embodiments, the medium comprises Brucella blood agar with hemin and vitamin K, which enables growth of a wide range of vaginal bacteria.

[0339] Lactobacilli are aerotolerant organisms highly directed towards fermentation. Lactobacilli naturally colonize mammalian mucosal surfaces, and generally grow optimally at about body temperature, *e.g.*, 37° C, though they also grow at lower (*e.g.*, 30° C) and higher temperatures (*e.g.*, 40° C). The growth rate can be controlled by changing the temperature.

[0340] After reaching the desired bacterial cell density, the bacterial cells can be harvested using any suitable method to remove the cells from the culture medium. Non-limiting exemplary methods for harvesting the cultured cells include filtration, centrifugation, and sedimentation. In some embodiments, the cell biomass is washed at least once using a physiologically buffered solution. In some embodiments, the wash solution may contain additional components, such as, *e.g.*, glucose. In some embodiments, the wash solution comprises a buffer or one or more buffering agents. Exemplary buffering agents that may be used or added to the wash solution include phosphate salts, (*e.g.*, Na<sub>2</sub>HPO<sub>4</sub>, NaH<sub>2</sub>PO<sub>4</sub>, NaHCO<sub>3</sub>, and arginine).

#### **Formulation into Preparations and Pharmaceutical Compositions**

[0341] The one or more isolates capable of colonizing and engrafting identified by the methods described herein can be formulated into isolate preparations and (pharmaceutical)

compositions and various dosage forms, *e.g.*, using one or more carriers, excipients, diluents and/or buffers, that can optionally, be pharmaceutically acceptable. In some embodiments, the dosage form can be an applicator or dispenser, *e.g.*, those commonly used in medicine and science. In some embodiments, the dosage form can be a vaginal suppository that will remain in the vaginal cavity until it is dissolved.

[0342] Other dosage forms suitable for formulation include a suspension, spray, gel, cream, powder, (gelatin or vegetable cellulose) capsule, solution for lavages, douches, ovules, a vaginal insert, tablets, disk, wafer (*e.g.*, drying on film, by vaporization), or a microencapsulated product employing excipients and formulation techniques known to those skilled in the art. Suitable dosage forms are formulated to readily dissolve and/or disperse inside the vaginal cavity.

[0343] Other dosage forms suitable for formulation include absorbent products comprising the bacterial composition, such as, *e.g.*, a feminine hygiene diaper, panty liners, sanitary napkin, tampon, panty guard or an incontinence guard.

[0344] The one or more isolates capable of colonizing and engrafting identified by the methods described herein can be formulated in dry form, *e.g.*, lyophilized or spray dried and then optionally formulated into a dosage form described herein, or reconstituted, *e.g.*, with sterile water, a weak acidic solution, gel, or buffer prior to use. If the composition is not reconstituted, depending on the precise formulation of the dosage form, rehydration may also be achieved inside the vaginal cavity, *e.g.*, aided by resident vaginal fluid. Methods of preserving viable bacteria by lyophilization can promote long-term preservation of the microorganism. One skilled in the art will be able to lyophilize bacteria using standard techniques.

[0345] In all of these embodiments, dyes, perfumes, pH buffering agents, drying or resuspending agents, or other standard materials for drug formulation can be incorporated into the compositions and dosage forms.

[0346] In all of these embodiments, devices and compositions described herein may be packed in a suitable packaging, for example in a bottle, flacon, blister pack, cartridge, applicator or dispenser.

#### **Assembly of a Microbial Consortium**

[0347] The inventors have recognized that assembling a microbial consortium for therapeutic use may require monitoring the genetic sequence of bacteria for use in the

consortium over time. For example, genetic drift may cause the bacteria to no longer exhibit desirable therapeutic properties if the bacteria's genetic sequence is impacted by too many mutations (*e.g.*, SNVs, SNPs, or other changes to the genetic sequence). Accordingly, the inventors have developed techniques for computationally identifying whether two bacterial isolates are substantially similar to be considered a same bacteria. **FIG. 13** is a flowchart of an illustrative process 1300 for assembling a microbial consortium including two or more bacteria belonging to one or more bacterial species.

**[0348]** Various (*e.g.*, some or all) acts of process 1300 may be implemented using any suitable computing device(s). For example, in some embodiments, one or more acts of the illustrative process 1300 may be implemented in a clinical or laboratory setting. For example, one or more acts of the process 1300 may be implemented on a computing device that is located within the clinical or laboratory setting. In some embodiments, the computing device may directly obtain genetic sequencing data from a sequencing apparatus located within the clinical or laboratory setting. For example, a computing device included in the sequencing apparatus may directly obtain the genetic sequencing data from the sequencing apparatus. In some embodiments, the computing device may indirectly obtain genetic sequencing data from a sequencing apparatus that is located within or external to the clinical or laboratory setting. For example, a computing device that is located within the clinical or laboratory setting may obtain genetic sequencing data via a communication network, such as the Internet, a cloud computing environment, a local area network (LAN), wireless local area network (WLAN), wide area network (WAN), or any other suitable network, as aspects of the technology described herein are not limited to any particular communication network.

**[0349]** Additionally or alternatively, one or more acts of the illustrative process 1300 may be implemented in a setting that is remote from a clinical or laboratory setting. For example, the one or more acts of process 1300 may be implemented on a computing device that is located externally from a clinical or laboratory setting. In this case, the computing device may indirectly obtain genetic sequencing data that is generated using a sequencing apparatus located within or external to a clinical or laboratory setting. For example, the genetic sequencing data may be provided to computing device via a communication network, such as the Internet, a cloud computing environment, a WAN, or any other suitable network.

**[0350]** It should be appreciated that, in some embodiments, not all acts of process 1300, as illustrated in **FIG. 13**, may be implemented using one or more computing devices. For

example, the act 1312 of assembling the microbial consortium may be implemented manually (*e.g.*, by a clinician or manufacturer).

**[0351]** In some embodiments, the process 1300 may be used to determine whether a purported sample of a bacteria is substantially identical to a reference sample of the bacteria. In determining that the purported sample is substantially identical to the reference sample, it may further be determined that the purported sample and the reference sample can be considered a same bacteria such that the purported sample may be considered suitable for use in the microbial consortium.

**[0352]** Process 1300 begins at act 1302, where genetic sequencing data obtained from the purported sample of a bacteria for use in the microbial consortium may be obtained. Process 1300 also includes act 1304, which may be performed before, simultaneously with, or after act 1302, and includes obtaining genetic sequencing data from the reference sample of the bacteria for use in the microbial consortium. In some embodiments, the purported sample and/or the reference sample of the bacteria may be a sample of lactobacilli. In some embodiments, the purported sample and/or the reference sample of the bacteria may be a sample of *Lactobacilli crispatus*.

**[0353]** In some embodiments, the genetic sequencing data may be obtained by sequencing a biological sample (*e.g.*, an isolate of the purported sample and/or an isolate of the reference sample) obtained using any suitable sequencing technique. The genetic sequencing data may include sequencing data of any suitable type, from any suitable source, and be in any suitable format. In some embodiments, the sequencing apparatus used to sequence the biological sample may be selected from any suitable sequencing apparatus known in the art including, but not limited to, Illumina™, SOLid™, Ion Torrent™, PacBio™, a nanopore-based sequencing apparatus, a Sanger sequencing apparatus, or a 454™ sequencing apparatus. In some embodiments, sequencing apparatus used to sequence the biological sample is an Illumina sequencing (*e.g.*, NovaSeq™, NextSeq™, HiSeq™, MiSeq™, or MiniSeq™) apparatus.

**[0354]** As one illustrative example, in some embodiments, the genetic sequencing data may comprise bulk sequencing data. The bulk sequencing data may comprise at least 1 million reads, at least 5 million reads, at least 10 million reads, at least 20 million reads, at least 50 million reads, or at least 100 million reads. In some embodiments, the sequencing data comprises bulk RNA sequencing (RNA-seq) data, single cell RNA sequencing (scRNA-

seq) data, or next generation sequencing (NGS) data. In some embodiments, the sequencing data comprises microarray data.

**[0355]** In some embodiments, the genetic sequencing data may be obtained by accessing and/or receiving the genetic sequencing data after it had been obtained by sequencing a biological sample. For example, the genetic sequencing data may be obtained by accessing genetic sequencing data stored in computer-readable memory (*e.g.*, locally stored and/or remotely stored genetic sequencing data). As another example, the genetic sequencing data may be obtained by receiving the genetic sequencing data in a transmission from a remote computer (*e.g.*, via a network transmission over a network including but not limited to the Internet, a cloud computing environment, and/or a local or wide area network).

**[0356]** In some embodiments, after obtaining the genetic sequencing data of the purported and reference samples, process 1300 may proceed to act 1306, where the genetic sequencing data from the two samples may be aligned. Any suitable method of pairwise alignment may be used to align the obtained genetic sequencing data. For example, the NUCmer command with the `–maxmatch` flag, as implemented in MUMmer v3.23 or later, may be used to perform pairwise alignment of the obtained genetic sequencing data. Additional aspects related to the MUMmer package are described in S. Kurtz, et al., *Versatile and open software for comparing large genomes*, Genome Biology 5:R12 (2004).

**[0357]** After aligning the obtained genetic sequencing data, process 1300 may proceed to act 1308, where a percent identity value may be calculated using the aligned genetic sequencing data. The percent identity value describes a percent of the aligned genetic sequencing data that is identical between the two sets of genetic sequencing data, and may be written as:

$$PI = 100 \times \frac{N_{\text{align}} - N_{\text{SNV}}}{N_{\text{align}}}$$

where  $N_{\text{align}}$  is the total number of identical basepairs and  $N_{\text{SNV}}$  is the number of SNVs in the genetic sequencing data of the purported sample relative to the genetic sequencing data of the reference sample. Any suitable technique may be used to determine the values of  $N_{\text{align}}$  and  $N_{\text{SNV}}$ . For example, the `dnadiff` command, as implemented in MUMmer v3.23 or later, as described above, may be used to determine the values of  $N_{\text{align}}$  and  $N_{\text{SNV}}$ .

**[0358]** After determining the percent identity, process 1300 may proceed to act 1310, where it may be determined that the purported sample and the reference sample are samples of a same bacteria. This determination may be made by determining that the calculated

percent identity value is greater than or equal to a threshold value. The threshold value may define a distinction between different bacteria of a bacterial species. For example, a calculated percent identity that is smaller than the threshold value may indicate that the purported sample includes a large number of SNVs relative to the reference sample such that the purported sample and the reference sample may not be considered the same bacteria. A calculated percent identity that is greater than or equal to the threshold value may therefore indicate that the purported sample includes an acceptable number of SNVs relative to the reference sample such that the purported sample and the reference sample may be considered the same bacteria.

**[0359]** In some embodiments, the process 1300 may optionally include determining the threshold value. For example, the threshold value may be determined using genetic sequencing data obtained from two or more samples of a known same bacteria. Percent identities may then be calculated for pairs of the two or more samples using the obtained genetic sequencing data, as described herein (*e.g.*, by aligning the pairs of genetic sequencing data, determining values of  $N_{\text{align}}$  and  $N_{\text{SNV}}$ , and calculating the percent identities for each pair). The threshold value may then be determined using a largest value of the calculated percent identities, as this largest value indicates a smallest difference between genetic sequences of the same bacteria (*e.g.*, due to genetic drift or other factors that may introduce SNVs).

**[0360]** In some embodiments, the threshold value may be 99.81%. In some embodiments, the threshold value may be 99.90%. In some embodiments, the threshold value may be 99.96%. Additional aspects of the determination of the threshold value are described herein in connection with **FIG. 3**.

**[0361]** After determining that the purported sample and the reference sample are samples of the same bacteria, the process 1300 may proceed to act 1312, in which the microbial consortium may be assembled using the purported sample. The microbial consortium may be assembled by combining multiple lactobacilli bacteria together, including the purported sample. Identification of lactobacilli bacteria for inclusion in the microbial consortium can be determined using a number of different *in vitro*, *in vivo*, and *in silico* characteristics of potential bacteria, such as engraftment capability, growth and acidification rates *in vitro* and coverage across a species-level pangenome (see, *e.g.*, Examples 2-5).

**[0362]** The assemblies of the purported samples can be aligned against one or more of the publicly available *L. crispatus* RefSeq assemblies known to a person of ordinary skill,

including GCF\_000091765, GCF\_000160515, GCF\_000161915, GCF\_000162255,  
GCF\_000162315, GCF\_000165885, GCF\_000176975, GCF\_000301115, GCF\_000301135,  
GCF\_000466885, GCF\_001434005, GCF\_001541515, GCF\_001546025, GCF\_001563615,  
GCF\_002088015, GCF\_002218645, GCF\_002218695, GCF\_002218815, GCF\_002218845,  
GCF\_002218855, GCF\_002218885, GCF\_002218895, GCF\_002218945, GCF\_002218965,  
GCF\_002218975, GCF\_002219005, GCF\_002219015, GCF\_002219045, GCF\_002219055,  
GCF\_002219085, GCF\_002811165, GCF\_002861765, GCF\_002861775, GCF\_002861815,  
GCF\_002863245, GCF\_002863485, GCF\_002863505, GCF\_003795065, GCF\_003971565,  
GCF\_004361125, GCF\_004361175, GCF\_004361185, GCF\_004361195, GCF\_004361245,  
GCF\_004361295, GCF\_004361345, GCF\_004361375, GCF\_004361445, GCF\_004361465,  
GCF\_004361515, GCF\_004361555, GCF\_004361565, GCF\_004361575, GCF\_008694745,  
GCF\_009730275, GCF\_009769205, GCF\_009933525, GCF\_013456995, GCF\_013487905,  
GCF\_013778545, GCF\_015669875, GCF\_016767795, GCF\_018885325, GCF\_019537355,  
GCF\_020042005, GCF\_020042225, GCF\_020887095, GCF\_021278925, GCF\_021278945,  
GCF\_022453955, GCF\_022453975, GCF\_022454015, GCF\_022454025, GCF\_022454055,  
GCF\_022454075, GCF\_022454115, GCF\_022454135, GCF\_022454155, GCF\_022454175,  
GCF\_022454205, GCF\_022454275, GCF\_022454315, GCF\_022454335, GCF\_022454355,  
GCF\_022454375, GCF\_022454385, GCF\_022454395, GCF\_022454435, GCF\_022454495,  
GCF\_022454535, GCF\_022454555, GCF\_022454565, GCF\_022454615, GCF\_022454695,  
GCF\_022454705, GCF\_022454735, GCF\_022454755, GCF\_022454765, GCF\_022454795,  
GCF\_022454815, GCF\_022454845, GCF\_022454895, GCF\_022454915, GCF\_022454935,  
GCF\_022454955, GCF\_022454965, GCF\_022455005, GCF\_022455035, GCF\_022455065,  
GCF\_022455155, GCF\_022455175, GCF\_022455185, GCF\_022455205, GCF\_022455255,  
GCF\_022455295, GCF\_022455315, GCF\_022455325, GCF\_022455375, GCF\_022455395,  
GCF\_022455455, GCF\_022455475, GCF\_022455485, GCF\_022455515, GCF\_022455535,  
GCF\_022455555, GCF\_022455575, GCF\_022455595, GCF\_022455615, GCF\_022455635,  
GCF\_022455655, GCF\_022455675, GCF\_022455695, GCF\_022455705, GCF\_022455755,  
GCF\_022455855, GCF\_022455875, GCF\_022455915, GCF\_022455955, GCF\_022455975,  
GCF\_022455985, GCF\_022456015, GCF\_022456035, GCF\_022456175, GCF\_022456215,  
GCF\_022456295, GCF\_022456575, GCF\_022456595, GCF\_022456605, GCF\_022456655,  
GCF\_022456695, GCF\_022456705, GCF\_022456755, GCF\_022456795, GCF\_022456805,  
GCF\_022456835, GCF\_022456855, GCF\_022456875, GCF\_022456895, GCF\_022456935,  
GCF\_022456975, GCF\_022456995, GCF\_022457275, GCF\_022457405, GCF\_022457425,

GCF\_022457445, GCF\_022457465, GCF\_022457495, GCF\_022457545, GCF\_022458295, GCF\_022458335, GCF\_022458395, GCF\_022458415, GCF\_022458425, GCF\_025194045, GCF\_026740115, GCF\_027680585, GCF\_027682305, GCF\_027695305, GCF\_029011155, GCF\_029011475, GCF\_029011495, GCF\_029011595, GCF\_029011765, and GCF\_904382375.

**[0363]** FIG. 14 is a diagram of an illustrative computer system on which embodiments described herein (*e.g.*, such as the method of FIG. 13) may be implemented. The computer system 1400 may include one or more processors 1410 and one or more articles of manufacture that comprise tangible (*e.g.*, non-transitory) computer-readable storage media (*e.g.*, memory 1420 and one or more non-volatile storage media 1430). The processor 1410 may control writing data to and reading data from the memory 1420 and the non-volatile storage device 1430 in any suitable manner, as aspects of the technology described herein are not limited to any particular techniques for writing or reading data. To perform any of the functionality described herein, the processor 1410 may execute one or more processor-executable instructions stored in one or more non-transitory computer-readable storage media (*e.g.*, the memory 1420), which may serve as non-transitory computer-readable storage media storing processor-executable instructions for execution by the processor 1410.

**[0364]** Computing device 1400 may also include a network input/output (I/O) interface 1440 via which the computing device may communicate with other computing devices (*e.g.*, over a network), and may also include one or more user I/O interfaces 1450, via which the computing device may provide output to and receive input from a user. The user I/O interfaces may include devices such as a keyboard, a mouse, a microphone, a display device (*e.g.*, a monitor or touch screen), speakers, a camera, and/or various other types of I/O devices.

**[0365]** The above-described embodiments can be implemented in any of numerous ways. One or more aspects and embodiments of the present disclosure involving the performance of processes or methods may utilize program instructions executable by a device (*e.g.*, a computer, a processor, or other device) to perform, or control performance of, the processes or methods. In this respect, various inventive concepts may be embodied as a computer readable storage medium (or multiple computer readable storage media) (*e.g.*, a computer memory, one or more floppy discs, compact discs, optical discs, magnetic tapes, flash memories, circuit configurations in Field Programmable Gate Arrays or other semiconductor devices, or other tangible computer storage medium) encoded with one or



more programs that, when executed on one or more computers or other processors, perform methods that implement one or more of the various embodiments described above. The computer readable medium or media can be transportable, such that the program or programs stored thereon can be loaded onto one or more different computers or other processors to implement various ones of the aspects described above. In some embodiments, computer readable media may be tangible (*e.g.*, non-transitory) computer readable media. In some embodiments, the computer readable media may comprise a persistent memory.

[0366] In this respect, it should be appreciated that one implementation of the embodiments described herein comprises at least one computer-readable storage medium (*e.g.*, RAM, ROM, EEPROM, flash memory or other memory technology, CD-ROM, digital versatile disks (DVD) or other optical disk storage, magnetic cassettes, magnetic tape, magnetic disk storage or other magnetic storage devices, or other tangible, non-transitory computer-readable storage medium) encoded with a computer program (*i.e.*, a plurality of executable instructions) that, when executed on one or more processors, performs the above-discussed functions of one or more embodiments. The computer-readable medium may be transportable such that the program stored thereon can be loaded onto any computing device to implement aspects of the techniques discussed herein. In addition, it should be appreciated that the reference to a computer program which, when executed, performs any of the above-discussed functions, is not limited to an application program running on a host computer. Rather, the terms “**computer program**” and “**software**” are used herein in a generic sense to reference any type of computer code (*e.g.*, application software, firmware, microcode, or any other form of computer instruction) that can be employed to program one or more processors to implement aspects of the techniques discussed herein.

[0367] It will be apparent that example aspects, as described above, may be implemented in many different forms of software, firmware, and hardware in the implementations illustrated in the figures. Further, certain portions of the implementations may be implemented as a “**module**” that performs one or more functions. This module may include hardware, such as a processor, an application-specific integrated circuit (ASIC), or a field-programmable gate array (FPGA), or a combination of hardware and software.

[0368] The terms “**program**” or “**software**” are used herein in a generic sense to refer to any type of computer code or set of computer-executable instructions that can be employed to program a computer or other processor to implement various aspects as described above. Additionally, it should be appreciated that according to one aspect, one or more computer

programs that when executed perform methods of the present disclosure need not reside on a single computer or processor but may be distributed in a modular fashion among a number of different computers or processors to implement various aspects of the present disclosure.

**[0369]** Computer-executable instructions may be in many forms, such as program modules, executed by one or more computers or other devices. Generally, program modules include routines, programs, objects, components, data structures, etc. that perform particular tasks or implement particular abstract data types. Typically, the functionality of the program modules may be combined or distributed as desired in various embodiments.

**[0370]** When implemented in software, the software code can be executed on any suitable processor or collection of processors, whether provided in a single computer or distributed among multiple computers.

**[0371]** Further, it should be appreciated that a computer may be embodied in any of a number of forms, such as a rack-mounted computer, a desktop computer, a laptop computer, a tablet computer, a quantum computer, or a photonic computer as non-limiting examples. Additionally, a computer may be embedded in a device not generally regarded as a computer but with suitable processing capabilities, including a Personal Digital Assistant (PDA), a smartphone, or any other suitable portable or fixed electronic device.

**[0372]** Also, a computer may have one or more input and output devices. These devices can be used, among other things, to present a user interface. Examples of output devices that can be used to provide a user interface include printers or display screens for visual presentation of output and speakers or other sound generating devices for audible presentation of output. Examples of input devices that can be used for a user interface include keyboards, and pointing devices, such as mice, touch pads, and digitizing tablets. As another example, a computer may receive input information through speech recognition or in other audible formats.

**[0373]** Such computers may be interconnected by one or more networks in any suitable form, including a local area network or a wide area network, such as an enterprise network, and intelligent network (IN) or the Internet. Such networks may be based on any suitable technology and may operate according to any suitable protocol and may include wireless networks, wired networks or fiber optic networks.

**[0374]** Also, as described, some aspects may be embodied as one or more methods. The acts performed as part of the method may be ordered in any suitable way. Accordingly, embodiments may be constructed in which acts are performed in an order different than

illustrated, which may include performing some acts simultaneously, even though shown as sequential acts in illustrative embodiments.

## DEFINITIONS

[0375] The definitions hereinafter apply to all aspects and embodiments disclosed herein. Unless otherwise explained, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. It is further to be understood that methods and materials similar or equivalent to those described herein can be used in the practice or testing of this disclosure. The materials, methods, and examples are illustrative only and not intended to be limiting. Other features of the disclosure are apparent from the following detailed description and the claims.

[0376] In a preferred embodiment, the term “**about**” shall allow a deviation of  $\pm 10\%$ , and even more preferably of  $\pm 5\%$  from an indicated numerical value. Unless otherwise indicated, all numbers expressing quantities of components, times, and so forth, as used in the specification or claims are to be understood as being modified by the term “**about**.” Accordingly, unless otherwise implicitly or explicitly indicated, or unless the context is properly understood by a person of ordinary skill in the art to have a more definitive construction, the numerical parameters set forth are approximations that may depend on the desired properties sought and/or limits of detection under standard test conditions/methods as known to those of ordinary skill in the art.

[0377] The terms “**contacting**”, “**administering**” or “**subjecting**” and more specifically, “**vaginal application**” or “**vaginal administration**”, are used interchangeably herein and relate to a subject (*e.g.*, a female subject) or a specific organ or other physiological site (*e.g.*, the urogenital tract or vaginal cavity or a subpart thereof, *e.g.*, to a site on a vaginal wall (mucosal or endometrial surfaces) or vaginal fornices) and a dosage form, preparation or (pharmaceutical) composition that is provided or given to the subject or site, *e.g.*, for the purpose of colonizing and engrafting a desired bacterial community. In one embodiment, administering is performed locally. In one embodiment, administering is performed topically (*e.g.*, vaginally).

[0378] The terms “**bacterial isolate**” or “**isolate**” refers to isolated bacteria of the same species that share defined functional and/or genotypic characteristics. In some embodiments,

a bacterial isolate is a *Lactobacillus crispatus* isolate. In some embodiments, a bacterial isolate is a *Lactobacillus jensenii* isolate. In some embodiments, a bacterial isolate has been separated from other material (*e.g.*, from a vaginal bacterial community, *e.g.*, derived from a sample of cervicovaginal secretions or vaginal fluid). In some embodiments, a bacterial isolate may be propagated *in vitro*. A bacterial isolate is, in some embodiments, substantially free of contaminants or components that accompany the material it was derived from in its native state (*e.g.*, such as, vaginal mucus and epithelial cells). In some embodiments, a bacterial isolate is characterized by its genomic haplotypes. In some embodiments, two different bacterial isolates do not share a sequence identity of greater than a threshold value (*e.g.*, a threshold value of 99.81%, 99.90% or 99.96%) across their pairwise aligned genomes. In some embodiments, functional characteristics include growth rates, acidification rates, and rates of carbohydrate utilization, among others. In some embodiments, two or more different bacterial isolates (*e.g.*, obtained from different sources, *e.g.*, obtained from different donors) may be combined to generate a microbial consortium.

**[0379]** As used herein the term “**bacterial sample**” or “**microbial sample**” means a sample comprising bacteria. The sample can be, *e.g.*, vaginal fluid/discharge.

**[0380]** The term “**carbon substrate utilization profile**” or “**carbon utilization profile**” is an indicator (*e.g.*, a composite measure or set of measurements) reflecting the extent to which a bacterial isolate grows on or consumes different carbon substrates selected from a set of one or more (or two or more) carbon substrates, such as mannitol and glucose. In some embodiments, a carbon substrate utilization profile comprises a measure or measures of growth rate and/or acidification rate during log phase growth on two or more carbon substrates.

**[0381]** The term “**comprising**” shall be understood to simultaneously also disclose the term “**consisting**” as a preferred option. For example, if a composition is said to comprise three components, this also discloses a composition consisting of these three components as preferred embodiment.

**[0382]** If the term “**comprising**” is used when referring to (a) pharmaceutically active compound(s), bacterium(a), and the like, this shall be understood to simultaneously also disclose that the pharmaceutically active compound(s), bacterium(a), and the like is/are preferably the sole pharmaceutically active compound(s), bacterium(a), and the like. For example, if a microbial consortium is said to comprise *Lactobacillus crispatus* and

*Lactobacillus jensenii*, this simultaneously and preferably discloses that a donor sample contains *Lactobacillus crispatus* and *Lactobacillus jensenii* as the sole bacterial species.

[0383] The term “**consortium**” or “**microbial consortium**” is a defined mixture of bacteria that does not exist together naturally, i.e., is non-naturally occurring and/or synthetic, and that has emergent properties not possessed by any of the constituent bacteria individually. (“Non-naturally occurring” consortia).

[0384] “**Culture-independent method**” means methods not involving isolation and/or in vitro propagation of bacterial isolates, *e.g.*, in cultures.

[0385] The terms “**distinct**” or “**phenotypically distinct**” refer to isolates having different phenotypes. In some embodiments, distinct or phenotypically distinct isolates have different growth rates. In some embodiments, distinct or phenotypically distinct isolates have different acidifications rates. In some embodiments, distinct or phenotypically distinct isolates have different carbon substrate utilization profiles. In some embodiments, distinct or phenotypically distinct isolates have different carbon substrate utilization capabilities.

[0386] The terms “**dose**” and “**dosage**” are used interchangeably herein. A dose refers to the amount of active ingredient given to an individual at each administration. The dose will vary depending on a number of factors, including frequency of administration (*e.g.*, daily, one or more times per week, per month, or per 3 months); size and tolerance of the individual; severity of the condition; intended result (*e.g.*, treatment, prophylaxis, modulation or restoration of the microbial community), and the route of administration. A baseline dose can be administered and modified based on the initial response of the subject. For example, a single dose of the compositions comprising the substantially complete vaginal microbiota preparations described herein can be in the range of  $10^4$  to  $10^{10}$  colony forming units (per dose). In other embodiments, a single dose of the composition can be in the range of  $10^3$  to  $10^{12}$ ,  $10^4$  to  $10^9$ ,  $10^5$  to  $10^9$ ,  $10^5$  to  $10^8$ ,  $10^6$  to  $10^9$ ,  $10^7$  to  $10^9$ , or  $10^7$  to  $10^{10}$  colony forming units (per dose).

[0387] A “**dosage form**” refers to a particular physical form of a composition or preparation and depends, *e.g.*, on the desired amount of the material to be administered and on the route of administration, *e.g.*, oral or topical (*e.g.*, vaginal). For example, a dosage form can be in a suppository, a tablet, a capsule, a film, a cream, etc., or a device, such as, *e.g.*, an applicator or dispenser, *e.g.*, for vaginal administration. Dosage forms may be single or multiple-use dosage forms.

[0388] “**Dysbiosis**” as used herein means a microbial imbalance, i.e. an aberration of the healthy state, where normally predominant species are diminished in abundance and less predominant species become more abundant and/or predominant. Vaginal dysbiosis is a microbial imbalance in the vagina, wherein the vaginal imbalance may, in embodiments, extend to the endometrium. Dysbiosis is generally associated with one or more of: (a) qualitative and quantitative changes in the content or amount of the microbiota itself, (b) changes in their metabolic activities; and/or (c) changes in their local distribution (*e.g.*, inside a niche). A dysbiotic human microbiota (or microbiome) refers to a population of microbes that promotes inflammation of a tissue of the urogenital tract (*e.g.*, the vagina) and/or that contributes to or establishes an environment that permits or promotes the colonization or growth of one or more pathogenic microbes. Dysbiosis also refers to a perturbation of the urogenital (*e.g.*, vaginal) homeostasis. In some embodiments, a dysbiotic vaginal microbiota will generally result in increased pH relative to a healthy microbiota, *e.g.*, a pH above 4.5, *e.g.*, a pH of 5.0, 5.5, 6.0, 6.5, 7.0 or higher. In some embodiments, vaginal dysbiosis is characterized by a reduction of *Lactobacillus* spp., and an increased diversity of vaginal anaerobic bacteria. In some embodiments, vaginal dysbiosis is associated with infections, inflammation, and increased risk of sexual transmitted diseases. Dysbiosis may be characterized by the relative amount of selected pathogens, such as >20% selected pathogens, and the relative low abundance of vaginal *lactobacilli*, *e.g.* <10% vaginal *lactobacilli* (*L. crispatus*, *L. iners*, *L. jensenii*, *L. gasseri*).

[0389] “**Dysbiotic subjects**” as used herein comprise subjects having vaginal symptoms (symptomatic subjects) and subjects not having any vaginal symptoms (asymptomatic subjects), wherein symptoms are characterized by Amsel’s criteria, Nugent score and/or Hay/Ison score.

[0390] A “**desired change**” of dysbiosis may thus comprise prophylaxis and/or treatment of any of the above mentioned symptoms associated with dysbiosis, *e.g.* reduction of inflammation or infection of the urogenital tract, or the restoration and/or rebalancing of the vaginal microbiota (*e.g.*, to achieve homeostasis). The desired change of dysbiosis may alleviate at least one or more symptoms, delay the development of a symptom, alter the course of a symptom (*e.g.*, slowing the progression of a symptom), or reverse a symptom, wherein the symptom may include an increase of beneficial *Lactobacillus* spp., a reduction of infections, a reduction of the relative amount of selected pathogens, or a reduction in (local) inflammation.

[0391] As used herein, the term “**effective amount**” means the amount (*e.g.*, of a microbial consortia, individual *lactobacilli* isolates (*e.g.*, *L. jensenii* isolates, *L. crispatus* isolate), or related compositions) to be administered to a typical subject (*e.g.*, a female subject) that is sufficient to lead to a desired beneficial or therapeutic effect in the subject. The desired beneficial or therapeutic effect includes prophylaxis and/or treatment, *e.g.*, of dysbiosis, inflammation or an infection or urogenital tract, and also includes the restoration and/or rebalancing of the vaginal microbiota (*e.g.*, to achieve homeostasis), *e.g.*, an anti-inflammatory and/or anti-pathogenic state of the urogenital tract and the vaginal microbiota. An effective amount, for example, is the amount sufficient (*e.g.*, at dosages and for periods of time necessary) to alleviate at least one or more symptom, delay the development of a symptom, alter the course of a symptom (*e.g.*, slowing the progression of a symptom), or reverse a symptom. Effective amounts generally cause statistically significant, measurable changes.

[0392] As used herein, the term “**engraftment**” or “**colonization**” refers to the colonization of an environment (*e.g.*, a microbial niche or microbial community), *e.g.*, the female genitourinary tract of a subject, by a microbe, *e.g.*, a bacterium (*e.g.*, *lactobacilli*), such that the viable population of that microbe continues to reside, *e.g.*, in the niche or community, for a certain period of time (*e.g.*, a pre-determined period of time). Engraftment can be transient or stable depending on the period of time that the microbe continues to reside in the niche. Engraftment can be quantified, *e.g.*, by counting the number of colony forming units (CFU)/gram and/or performing nucleic acid sequencing of microbes comprised in one or more vaginal samples that are taken over a certain period of time. In some embodiments, successful engraftment of a microbe refers to colonization of the microbe in an environment such that the microbe can be detected within the environment after a certain period of time. In some embodiments, successful engraftment of a microbe refers to colonization of the microbe in an environment such that the relative abundance of the microbe is at least 5% or at least 10% of the total microbial abundance in the environment (*e.g.*, relative abundance of *lactobacilli* in the female genitourinary tract of a subject is at least 5% or at least 10% of the total microbial abundance). In some embodiments, a microbial consortium of the present disclosure promotes engraftment of at least a subset of the defined population (*e.g.*, at least one *Lactobacillus* isolate) in a female genitourinary tract of a subject. In some embodiments, a microbial consortium of the present disclosure promotes engraftment of the *lactobacilli* of the defined population in a female genitourinary tract of a subject. The defined population

may comprise a *Lactobacillus jensenii* isolate, a *Lactobacillus crispatus* isolate that thrives in the presence of *Lactobacillus jensenii*, and at least one additional *Lactobacillus crispatus* isolate that thrives independent of the presence of *Lactobacillus jensenii*.

[0393] In one embodiment, the term “**promotes engraftment**” refers to the engraftment of *Lactobacillus jensenii* isolate. In one embodiment, the term “promotes engraftment” refers to the engraftment of *Lactobacillus crispatus* isolate that thrives in the presence of *Lactobacillus jensenii*. In one embodiment, the term “promotes engraftment” refers to the engraftment of *Lactobacillus crispatus* isolate that thrives independent of the presence of *Lactobacillus jensenii*. In one embodiment, the term “promotes engraftment” refers to the engraftment of *Lactobacillus crispatus* isolate that thrives in the presence of *Lactobacillus jensenii* and *Lactobacillus crispatus* isolate that thrives independent of the presence of *Lactobacillus jensenii*. In one embodiment, the term “promotes engraftment” refers to the engraftment of *Lactobacillus jensenii* isolate and *Lactobacillus crispatus* isolate that thrives in the presence of *Lactobacillus jensenii*. In one embodiment, the term “promotes engraftment” refers to the engraftment of *Lactobacillus jensenii* isolate and *Lactobacillus crispatus* isolate that thrives independent of the presence of *Lactobacillus jensenii*. In one embodiment, the term “promotes engraftment” refers to the engraftment of the defined population, e.g., *Lactobacillus jensenii* isolate, *Lactobacillus crispatus* isolate that thrives in the presence of *Lactobacillus jensenii*, and *Lactobacillus crispatus* isolate that thrives independent of the presence of *Lactobacillus jensenii*.

[0394] As a non-limiting example, *L. crispatus* bacteria that thrives in the presence of *L. jensenii* is capable of engrafting the female genitourinary tract of a subject for at least a pre-determined period of time (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, or 20 days) in the presence of an *L. jensenii* bacteria.

[0395] The term “**engraftment potential**” refers to a latent capacity to engraft in a recipient. In some embodiments, engraftment potential refers to a quality of an isolate which has been isolated from a donor SCVMP and which has demonstrated successful engraftment in a recipient subject. In some embodiments, FBS-003 has engraftment potential. In some embodiments, FBS-004 has engraftment potential. In some embodiments, FBS-005 has engraftment potential. In some embodiments, FBS-006 has engraftment potential.

[0396] The terms “**eubiosis**”, “**eubiotic**”, and “**eubiotic microbiome**” refer to a healthy vaginal environment wherein the resident bacteria comprise a high relative abundance of *Lactobacillus* species. A eubiotic vaginal microbiome may be characterized by the relative



high abundance (e.g., greater than 70%, greater than 80%, greater than 90%, or greater than 95%) of healthy vaginal *Lactobacillus* species (*L. crispatus*, *L. iners*, *L. jensenii*, *L. gasseri*) and a relatively low abundance of selected pathogens. In some embodiments, eubiosis is characterized by a “*Lactobacillus*-dominant” vaginal microbiome. In some embodiments, a eubiotic microbiome is stable, meaning the microbiome persists in the vaginal environment over time (e.g., for 1 month, for 2 months, for 3 months, for 4 months, for 5 months, for 6 months, or for 12 months). In some embodiments, a eubiotic microbiome is stable, meaning the microbiome persists in the vaginal environment across menstrual cycles (e.g., for 1 menstrual cycle, for 2 menstrual cycles, for 3 menstrual cycles, for 4 menstrual cycles, for 5 menstrual cycles, for 6 menstrual cycles, or for 12 menstrual cycles).

[0397] The phrases “**excipient**” “**pharmaceutically acceptable carrier**” “**diluent**” or “**buffer**” as used herein mean a non-active, pharmaceutically acceptable material, ingredient, composition or vehicle that is added to form part of the final formulation and/or maintains a drug or other agent in a form for delivery to a subject. Each carrier preferably is compatible with the other ingredients of the formulation, for example the carrier does not decrease the impact of an active ingredient or agent upon the treatment, *e.g.*, the carrier is pharmaceutically inert. In some embodiments a pharmaceutically acceptable carrier can be a carrier other than water (including, *e.g.*, a cream, emulsion, gel, liposome, nanoparticle, film, ointment and/or vaginal device). In some embodiments, a pharmaceutical composition is provided comprising the substantially complete vaginal microbiota preparations together with a pharmaceutically acceptable carrier and/or diluent (*e.g.*, saline). These compositions allow the easy administration of the preparations by means known to the person skilled in the art. In some embodiments, a buffering agent is added, *e.g.*, a weak acid or base that maintains the acidity at a chosen level (*e.g.*, between pH 3.5 and 4.5) and prevents a rapid change in acidity.

[0398] The term “**haplotype**” refers to a set of DNA variations or polymorphisms, *e.g.* single nucleotide polymorphisms (SNPs) or single nucleotide variants (SNVs), on the same chromosome or in defined genetic regions and that are inheritable. In some embodiments, a haplotype can be used as a defining indicator of a particular bacteria or isolate.

[0399] The terms “**increased**”, “**increase**”, “**enhance**”, or “**activate**” can mean an increase of at least 10% as compared to a reference level, for example an increase of at least about 20%, or at least about 30%, or at least about 40%, or at least about 50%, or at least about 60%, or at least about 70%, or at least about 80%, or at least about 90% or up to and

including a 100% increase or any increase between 10-100% as compared to a reference level, or at least about a 2-fold, or at least about a 3-fold, or at least about a 4-fold, or at least about a 5-fold or at least about a 10-fold increase, or any increase between 2-fold and 10-fold or greater as compared to a reference level.

[0400] As used herein, the term “**isolated nucleic acids**” refers to the purification of nucleic acids from one or more cellular components. Isolated nucleic acids can be prepared from specimens using any acceptable method known in the art. For example, cells can be lysed using known lysis agents, and nucleic acids can be purified or partially purified from other cellular components.

[0401] As used herein the term “**lactic acid producing bacteria**” means bacteria that produce lactic acid by fermentation.

[0402] The terms “**lower**”, “**reduced**”, “**reduction**” or “**decrease**”, “**down-regulate**” or “**inhibit**” mean a decrease by at least 10% as compared to a reference level, for example a decrease by at least about 20%, or at least about 30%, or at least about 40%, or at least about 50%, or at least about 60%, or at least about 70%, or at least about 80%, or at least about 90% or up to and including a 100% decrease (i.e. absent level (or levels below the limit of detection) as compared to a reference sample), or any decrease between 10-100% as compared to a reference level.

[0403] A “**lyophilized**”, “**spray-dried**” or “**freeze-dried**” composition refers to a composition from which moisture has been removed, *e.g.*, for easy storage and transport. Such compositions can be rehydrated before use (*e.g.*, administration). The lyophilized, spray-dried, or freeze-dried composition may be further pelleted or packaged for easier storage and transportation.

[0404] The term “**microbe**” is used synonymously with the term “**microorganism**” and includes bacteria (Archaea, Eubacteria), yeast, fungi, and viruses. The term “species” is used herein to refer to a taxonomically and/or genetically distinct group of microorganisms. Species may include one or more distinguishable (*e.g.*, by sequencing) isolates.

[0405] The term “**microbiota**” refers to a community of microorganism localized to a distinct shared environment (a “**microbial niche**”). For example, “**vaginal microbiota**” is a community of one or more species of microorganisms that are localized to, or found in, a vagina. The term “**microflora**” or “**flora**” is used synonymously with the term “**microbiota**.” Healthy or normal microbiota denominates the community of commensal microorganisms that colonize (inhabit) a particular microbial niche of the host, such as the vagina. Bacteria

are the most numerous microbial components of the normal flora. “**Microbiome**” refers to the totality of microbes (bacteria, archaea, yeast, fungi and phages) and their genomes.

[0406] A “**microbial profile**” is a set of the species and/or isolates of microorganisms present in a sample of microorganisms. To the extent that a sample of microorganisms is obtained from, and corresponds to the species found in, a shared environment, the microbial profile details the species present in a microbial community. The term “**profile**” as used herein refers to both an individual profile and an aggregate profile over a plurality of individual profiles, depending on the context of its use. In some embodiments, an aggregate profile may include, *e.g.*, a pangenome (sequence), *e.g.*, all the genes (or genomes) of all individual members (*e.g.*, isolates) within a particular grouping (*e.g.*, clade).

[0407] “**Mucosa**” as used herein indicates a mucous membrane. Mucus is a secretion produced by, and covering, mucous membranes. Mucous fluid is viscous and typically produced from mucous cells (*e.g.*, goblet cells) found in mucous membranes and submucosal glands, and rich in antiseptic enzymes (such as lysozyme), immunoglobulins, inorganic salts, proteins such as lactoferrin, and glycoproteins (mucins). Mucosal surfaces include epithelial linings of the reproductive tract (vagina) and, *e.g.*, *lactobacilli* are capable of colonizing the vaginal mucosal surfaces.

[0408] In contrast, the term “**normal**” or “**healthy**” “**vaginal flora**” or “**vaginal niche**” “**microbiota**” “**urogenital tract**” or “**community state**” or similar terms connote that a woman has no vaginal complaints and does not exhibit a vaginal pathology (*e.g.*, no sign or symptom corresponding to or resulting from a pathology of the vagina), and the condition of the vagina is such of a relatively low susceptibility to sexually transmitted diseases and pathogens, and generally of low pH, *e.g.*, less than or equal to pH 4.5, *e.g.*, between 3.2 and 4.5; and generally dominated by lactic acid producing bacteria (*e.g.*, *Lactobacillus* spp.). Normal vaginal microbiota or normal flora are a community of microorganisms that localize to the vagina in a normal, healthy, that is, a non-pathological, non-pathogenic and/or non-dysbiotic, state.

[0409] “**Cervicovaginal secretions**” or “**vaginal fluid**” refers to the mixture of mucus secreted by the cervix, shed epithelial cells, vaginal transudate, and bacteria found in the vagina of a woman.

[0410] “**Endometrial fluid**” refers to a fluid accumulation within the endometrial cavity that contains bacterial cells, host cells and microbe and host derived proteins, nucleotides and metabolites. Typically, the endometrial fluid is obtained by aspiration for further analysis.

[0411] As used herein, a “**nucleic acid**” includes DNA and RNA (*e.g.*, mRNA, rRNA, or tRNA) and analogs thereof, including nucleic acids comprising conventional nitrogenous bases (*e.g.*, A, G, C, T, U), base analogs (*e.g.*, inosine), derivatives of purine or pyrimidine bases and any substitutions

[0412] The term “**rationometrically equal**” refers to a combination of isolates wherein each isolate is approximately equal in proportions or wherein each isolate is present at about the same relative abundance as each other isolate. The ratiometric proportions of isolates in a consortium can be determined by measuring CFUs, by qPCR, dry weight, or volume. In some embodiments, a consortium comprising 2 isolates will comprise about 50% of each isolate. In some embodiments, a consortium comprising 3 isolates will comprise about 33% of each isolate. In some embodiments, a consortium comprising 4 isolates will comprise about 25% of each isolate.

[0413] As used herein, “**sequence identity**” or “**percent identical**” as applied to nucleic acid molecules is the percentage of nucleic acid residues in a candidate nucleic acid molecule sequence that are identical with a subject nucleic acid molecule sequence, after aligning the sequences to achieve the maximum percent identity, and not considering any nucleic acid residue substitutions as part of the sequence identity. Nucleic acid sequence identity can be determined using any method known in the art, for example CLUSTALW, T-COFFEE, BLASTN.

[0414] As used herein, “**percentage of sequence identity**” is determined by comparing two optimally aligned sequences over a comparison window, wherein the portion of the polynucleotide sequence in the comparison window may comprise additions or deletions (*i.e.*, gaps) as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid base or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison and multiplying the result by 100 to yield the percentage of sequence identity.

[0415] As used herein, the term “**substantial identity**” of polynucleotide sequences means that a polynucleotide comprises a sequence that has at least about 95% identity to a given reference sequence. Percent identity can be anywhere from 90% to 100%. Most embodiments include at least: 95%, 96%, 97%, 98%, 99% or 99.9% compared to a reference sequence using the programs described herein (*e.g.*, BLAST, using standard parameters).

[0416] The term “**subject**” refers to a human (*e.g.*, a human female). In one embodiment, the subject is 18 years or older. In one embodiment, the subject is in puberty. In one embodiment, the subject is pregnant. In one embodiment, the subject is of childbearing age. In one embodiment, the subject is pre-menopausal. In one embodiment, the subject is post-menopausal. In one embodiment, the subject is administered the microbial consortia, individual *lactobacilli* isolates (*e.g.*, *L. jensenii* isolates, *L. crispatus* isolates), or related compositions described herein. In some embodiments, a subject is a human female exhibiting a clinical condition related to a microbial imbalance (*e.g.*, a dysbiosis).

[0417] The term “**substantially**” means to a great or significant extent. For example in the case of a sample that is “**substantially free of**” pathogens, the term means the sample is, for the most part, or essentially, but possibly not completely, void of a pathogen. A sample that is substantially free of selected pathogens can refer to a sample that comprises about 5%, <5%, <4%, <3%, <2%, or <1% of the selected pathogens. In a particular embodiment, the term “**substantially free**” comprises <5%.

[0418] As used herein the term “**suitable nutrient medium**” means a medium in which the bacteria might be cultivated.

[0419] As used herein, the terms “**treat**,” “**treatment**,” “**treating**” refer to prophylactic and therapeutic treatments, wherein the object is to prevent, reverse, alleviate, ameliorate, inhibit, slow down or stop the progression or severity of a condition or symptom of a condition associated with a disease or disorder. Treatment includes the improvement of symptoms or markers (of the disease or condition) and the cessation or at least slowing of progress or worsening of symptoms that would be expected in absence of treatment. Treatment effectiveness can be measured by monitoring one or more symptoms or clinical markers and is compared, *e.g.*, to the subject’s condition and symptoms before administration or to a control subject not undergoing treatment. In one example, treating a vaginal infection refers to reducing the amount of the infective agent (*e.g.*, number of cells or viral particles), reducing the severity of symptoms, and/or reducing the frequency of symptoms. A treatment that reduces the level of a pathogen to one which is kept in check by the immune system or by the state established by a healthy vaginal microbiota (*e.g.*, the infection is no longer detectable, *e.g.*, by symptoms or general diagnostic techniques) is considered effective as the term is used herein.

[0420] “**Genitourinary tract**” or “**urogenital tract**” as used herein as used herein includes the uterus, fallopian tubes, ovaries, vagina, cervix, vulva, and urinary tract. In some

instances, used herein are teachings and exemplifications specifically calling out the “**vaginal tract**”, “**vaginal cavity**”, “**vaginal lumen**”, or “**vagina**”. One of ordinary skill will appreciate that these exemplifications and teachings are illustrative only and non-limiting and, thus also apply, where appropriate, to other anatomical sites of the genitourinary tract or urogenital tract, not just to the vagina.

**[0421]** As used herein, the term “**viable**” refers to a cell (*e.g.*, a bacterial cell) that is able to survive in a given condition (*e.g.*, storage for a certain period of time under particular storage conditions, *e.g.*, including, temperature, humidity) and is generally able to colonize and reproduce (*e.g.*, in the urogenital tract) after exposure to the condition. Percent viability refers to the percentage of viable cells in a population. For example, percent viability can refer to the percentage of lactobacilli in a pharmaceutical composition that will survive (*e.g.*, refrigeration, freezing and/or storage) and colonize upon application to a vaginal mucosal surface. Viability can be evaluated by, *e.g.*, CFU, VCC or Bactobox methods. In the CFU method, dilutions series of the samples are plated on suitable medium for the microorganisms of interest, after which the number of colonies is counted after an outgrowth period at suitable temperature. In the VCC method, samples are stained with a live/dead stain and counted in *e.g.*, a counting chamber. The live/dead stain can be a fluorescent dye that stains cells with a compromised membrane (and hence decreased or no viability) red, and intact (live) cells green. In the Bactobox method, total particles/ml and alive, intact cell concentrations (ICC) are enumerated with an impedance flow cytometer, of which only ICC are used to initiate cultures at approximately 5 million CFU/ml.

**[0422]** The abbreviation “**CFU**” means “colony-forming unit”.

**[0423]** The abbreviation “**VCC**” means viable cell count (as determined by life cell staining).

### EXAMPLES

[0424] The present technology is further illustrated by the following Examples, which should not be construed as limiting in any way. The examples herein are provided to illustrate advantages of the present technology and to further assist a person of ordinary skill in the art with preparing or using the compositions and systems of the present technology. The examples should in no way be construed as limiting the scope of the present technology, as defined by the appended claims. The examples can include or incorporate any of the variations, aspects, or embodiments of the present technology described above. The variations, aspects, or embodiments described above may also further each include or incorporate the variations of any or all other variations, aspects or embodiments of the present technology.

[0425] In one aspect, the below Examples describe the inventors' development and characterization of a previously unknown bacterial consortium that may be administered as a live biotherapeutic product to women to resolve and/or treat vaginal dysbiosis and consequently to improve overall vaginal health. As described and demonstrated in the various Examples presented herein, a microbial consortium was developed using three phylogenetically distinct *Lactobacillus crispatus* isolates and one *Lactobacillus jensenii* isolate, each of which were discovered from healthy donors with a stably *Lactobacillus*-dominant vaginal environment, and which stably engrafted by vaginal microbiota transplantation (VMT) in a subject presenting with vaginal dysbiosis. As discussed in the Examples, the isolates were phenotypically characterized with respect to various properties, including growth rate, acidification rates, and carbohydrate utilization at one or more relevant pHs characteristic of a dysbiotic vaginal environment and compared to the characteristics of the 4-member consortium, as well as to bi-isolate and tri-isolate versions of the 4-member consortium. Overall, the data show that the isolates synergistically cooperate to exhibit characteristics and colonization activities required for successful and robust engraftment in a host dysbiotic vagina, and may be used effectively as a live biotherapeutic product to help women be treated for vaginal dysbiosis and conditions associated therewith.

**Example 1. Isolation of bacterial isolates from healthy donor eubiotic vaginal environments for building a therapeutic multi-isolate microbial consortium**

[0426] This Example describes how single *Lactobacillus* isolates were isolated from substantially complete vaginal microbiota preparations (SCVMPs) derived from healthy female donors. A schematic representation is provided in **FIG. 1**.

[0427] An SCVMP derived from a single healthy female donor was administered to the female genitourinary tract of a recipient subject.

[0428] To start the isolation, the microbiota composition of the SCVMP was analyzed on both the species and the haplotype level. The isolation process was aimed at maximizing the number of isolated lactobacilli with the exception of *L. iners*. For all *Lactobacillus* species, MRS medium was used. The minimum number of colonies to be picked was based on haplotypes found in the microbiome: for each major haplotype (present in high relative abundance), three colonies were picked, and for each minor haplotype (present in low relative abundance), five colonies were picked. This approach ensures that the number of colonies picked reflects the diversity in a preparation and hence maximizes the number of isolated lactobacilli. Generally, for each morphology 3 colonies were picked. If the number of morphologies was less than the number of haplotypes, more colonies were picked.

[0429] The starting point for isolation was a 50 µL aliquot of the SCVMP. A dilution series of the SCVMP was plated on the selected medium (which was chosen based on the species present according to the sequencing analysis) and grown for 3 days at 37 °C in an anaerobic jar with gaspack. All plates were counted and closely observed to identify different colony morphologies. All work was performed under sterile conditions. Single colonies were described, picked and transferred to new plates of the same medium and grown under the same conditions until colonies were observed that can be picked (normally 1-3 days). Once grown on the second plate, colonies were again described and transferred to a third plate in the same way as with the second. If the colony morphology was not pure, a new lineage was started. After growth on the third plate single colonies were transferred to liquid medium of the same type as the plates. The liquid cultures were incubated at 37°C in an anaerobic box with a gaspack. Once grown (normally 1-5 days), glycerol stocks were prepared, the pH was measured, and an aliquot was taken to be used for DNA extraction and sequencing.

[0430] The single isolate genomes were compared to the original metagenome of the donor's sample that the isolate was derived from. If this showed that not all of the



metagenome of the original preparation was covered, more colonies were sent for sequencing.

### **Example 2. Selection and characterization of isolates for clinical development**

**[0431]** This Example describes the selection and characterization procedure of how isolates isolated from the SCVMPs are selected for clinical development and inclusion in microbial consortia. A simplified schematic of the selection process described herein is depicted in **FIG. 2**. Further Examples describe several of the steps of the selection process in more detail.

**[0432]** To select isolate candidates for clinical use (hereafter simply referred to as ‘isolate’ or ‘candidate isolate’), a selection process as outlined below was followed. Step 1 was the key step in the selection process. One or more subsequent steps, in any combination, may be performed. This approach is different from previously described isolate development programs, as it takes an in-human first approach and starts with a sample comprising isolates that successfully engrafted in a recipient (“forward screen”), meaning that all microbial consortia that comprise one or more isolates identified using this method should include isolates that are configured to promote engraftment of the consortia. Strategies in the art typically start with an isolate collection (with no prior knowledge of any isolate collection member’s ability to engraft from one person to another), wherein the collection comprises isolates which are tested *in vitro* regarding specific parameters, *e.g.*, growth at low pH, followed by the transfer of selected isolates into a human subject and evaluation of engraftment (“reverse screen”). In contrast, the strategy described herein starts with isolates that have shown clinical engraftment first (*e.g.*, wherein a change of dysbiosis after transfer from a donor to a recipient as part of an SCVMP may be used as a readout), and wherein the isolate(s) are isolated and identified second. This increases the chance of identifying isolates from SCVMPs that are capable of engrafting and effecting a desired outcome (*e.g.*, a change in vaginal dysbiosis and/or inflammation), compared to existing methods that attempt to determine engraftment chances of the isolated isolates through *in vitro* approaches.

**[0433]** Seven healthy female donors were selected. An SCVMP was collected from each donor at least one time across multiple visits.

**[0434]** One or more of the following steps may be included in the selection process as desired:

[0435] (1) Clinical engraftment: The first step and criterion for candidates was clinical engraftment of the isolate(s) after administration of a donor's SCVMP to a recipient, optionally a recipient exhibiting dysbiosis. Suitable isolate candidates engraft in at least one recipient and preferably more than one recipient, *e.g.*, two, three or more different recipients.

[0436] (2) Isolate context: The isolate context of the candidate isolates was then evaluated. SCVMPs differ in their complexity; wherein some may contain many species that all transfer to a recipient, other SCVMPs may contain only a few *Lactobacillus* species. On haplotype/isolate level, there is variation in the number of isolates for each species present in a SCVMP. For creating an isolate-based product for clinical use, one option is to combine all the isolates originally present in the engrafting SCVMP. As consortia production is increasingly complex with larger numbers of isolates/species in the product, SCVMPs with a simpler isolate context (*e.g.*, fewer total isolates or species) are preferred over SCVMPs with a more complex isolate context (*e.g.*, many isolates/species). Another option for creating an isolate-based product for clinical use is to create a mixture of isolates derived from one or multiple donors that together cover the full or a large part of a reference genome, *e.g.*, a "pangenome" (and its functionality), in which case it is the broader genomic isolate context that is used for selection rather than the single-SCVMP isolate context. This approach is further described in point 6 in this Example.

[0437] (3) *In silico* selection: *In silico* analysis can be performed on both functional and genomic/haplotype level, to identify genes and isolates that are linked to, *e.g.*, success of changing dysbiosis (*e.g.*, a reversion from dysbiosis to a healthy vaginal microbiome, and/or a reduction of inflammation in the reproductive tract) in clinical trials conducted in humans with the SCVMPs from which the isolates are derived. These data may, for example, reveal genes or metabolites that are required for engraftment success. Moreover, these data may be used to create a pangenome (*e.g.*, for each species), which provides the sequence of all genes of the engrafting isolates of said species. The pangenome is the entire set of genes present in a selected set of organisms (*e.g.*, a selected set of bacteria). Isolates can subsequently be selected that cover (or are homologous to) a preselected portion (or all) of the pangenome which can be combined into a microbial consortium. In other words, a combination of isolates can be combined into a microbial consortium such that the consortium encompasses a preselected portion (or all) of the pangenome even though any single isolate might not encompass the preselected portion by itself.

[0438] (4) *In vitro* and *in silico* characterization: *In silico* characterization of candidate isolates can include genome assembly and functional annotation, determining the donor isolate diversity (*i.e.*, number of isolates present per donor), as well as comparisons with other isolates both from isolations from SCVMPs as well as public databases. *In vitro* assays on the candidate isolates include, for example, determining D- and L-lactic acid production, growth rate, acidification rate, pH optimum and pH tolerance, pathogen inhibition and survival, ability to culture and propagate, and detectability, stability, carbon substrate utilization capacities, and requirements for, *e.g.*, metals, vitamins, amino acids. The *in vitro* assay outcomes can be compared to engraftment data and engraftment pharmacodynamics. Isolates that engraft in multiple recipients and/or engraft faster than others are preferred for use in microbial consortia. Then, one or more *in vitro* assays can be consulted to further stratify the isolates in terms of preferability, *e.g.*, based on how the isolates performed in these assays.

[0439] (5) Immune response: The immune response in a recipient after administration of the SCVMP (that the isolate is derived from) can be evaluated, using, for example, proteomics (*e.g.*, Olink) and transcriptomics (*e.g.*, Fluidigm or RNAseq) approaches. This can be done both on the level of the SCVMP and also on the species-level using correlation matrices. Species/isolates that result in upregulation of anti-inflammatory markers and/or downregulation of pro-inflammatory markers represent preferred potential candidate isolates.

[0440] (6) Safety profile: Safety can be evaluated at the level of the SCVMPs, *e.g.*, as tested in human clinical trials. For example, in the event that an isolate originates from a SCVMP that showed product-related adverse events in a human trial, the isolate is non-preferred. A safety profile of the isolates can additionally be assessed *in silico* and *in vitro*, by testing *e.g.*, the presence of toxin genes and antibiotic resistance genes. Isolates with a better safety profile (*e.g.*, fewer resistances and/or toxin genes) are preferred.

[0441] (7) Competition assay: *In vitro* competition assays can be performed with a recipient's or dysbiotic subject's CVS material against isolates and/or SCVMP derived from a donor. An example of a set up for an *in vitro* competition assay is a well-diffusion assay, where recipient/dysbiotic material is grown on an agar plate, and donor-derived material (*e.g.*, one or more isolates from a SCVMP) or isolates isolated therefrom can be added to small wells punched into the plate. After several days of incubation, the plate is visually checked for clearing zones around the wells. It is thought that the larger the area

where the (dysbiotic) recipient material has not grown around that well, the larger the inhibitory capacity of the donor-derived isolate or preparation. Isolates that produce large clearing zones (*e.g.*, relative to other isolates) and/or isolates that show SCVMPs clearing zones with multiple different (dysbiotic) recipient materials are preferred. As a control for this assay, a donor SCVMP-recipient CVS pair is used that has been tested in the clinic.

[0442] (8) Pre-clinical *in vitro* model tests can be performed as further desired. These may include, for example, animal skin tolerance models and immune assays on cell lines.

### **Example 3. Engraftment analysis based on isolate genomes**

[0443] In order to determine if a single donor contained the same isolate across multiple CVS donation visits, a dissimilarity analysis of the genomic assembly of all the isolates was performed based on their SNV profiles (instead of selecting an arbitrary cutoff in similarity or number of SNVs). A map of SNVs was identified across the assemblies using Parsnp to align and compare each isolate's assembly to a reference assembly. Parsnp specifically identifies SNVs in the core alignment of the assemblies, the region where the assemblies best align. In this way, SNVs are more likely to represent genetic drift and mutations – more SNVs between two isolates suggests that the species have a further back common ancestor than isolate pairs having fewer SNVs. Using the SNV map, Parsnp counted the number of SNVs pair-wise between each isolate assembly. A dissimilarity matrix was created for all the isolates using the Euclidean distance between each isolate pair based on the number in their SNV profiles with every other isolate. Isolates that were closer together in the matrix had more similar SNV profiles across all the isolates.

[0444] Hierarchical clustering of the dissimilarity matrix was used to generate a phylogenetic tree of all of the isolates. Although the tree reveals which isolates are more similar to each other based on their SNV profile, it does not reveal where the tree should be cut to designate which isolates are the same isolate. Gap statistic analysis was used with increasing number of cuts to determine which number of cuts best minimized the within-isolate cluster dispersion, which identified 25 separate isolates across 8 donors. For each group containing more than one isolate, a representative isolate was selected that was used for further *in silico* analysis.

[0445] Bacteria having the same genomic characteristics were determined as having a set of bacterial isolates whose SNV profiles across a range of other isolates, including from other donors, are more similar to each other, and form distinct clusters using appropriate clustering approaches. The tree generated with the above-described method was very similar to a tree based on protein presence-absence, indicating validity of the method. The dissimilarity was used to create groups of isolates that were considered identical and hence the same isolate.

[0446] For the *in silico* analysis (**FIGs. 13-14**), Nucmer alignments of assemblies from 124 *L. crispatus* isolates were performed. Percent identity was calculated as the percent of the aligned assemblies that were identical to each other. The analysis showed that the maximum percent identity between isolates from different individuals is 0.9996 (**FIG. 3**). Therefore, assemblies with a percent identity greater than 0.9996 can be defined as being the same bacteria. Only isolates from the same individual share a percent identity greater than 0.9996. Of the 202 publicly available *L. crispatus* RefSeq assemblies, ranging from scaffold to chromosome levels of completeness, none share a percent identity with the tested isolates above 0.9996. In fact, the highest percent identity between the 202 RefSeq assemblies and the tested isolates was 0.9981.

[0447] This analysis showed that 1) with only very few exceptions, the isolates cluster together per donor, with some donors containing separate clusters of isolates; and, 2) the isolates within one donor are the same across all visits (although they can fluctuate in relative abundance) (**FIG. 4**). Accordingly, all visits from one donor could be regarded as engrafting isolates even if some visits engrafted and others did not, because all visits contained the same isolate sequences. The reason for lack of engraftment of some visits is hence not the isolates, but likely factors on the recipient side. This enables the selection of isolates from any visit from a donor for a live bacterial product. This also supports making a microbial consortium derived from different donors, where the strains are selected such that they comprise as much of the total donor gene pool as possible and vary in characteristics. Some donors engraft more often than others (**Table 1**), which implies that some isolates are better at engrafting than others.

[0448] In addition to the two example donors shown in **FIG. 4** (Donor 5 and Donor 7), stable isolate composition was shown across donation visits for five additional donors (data not shown). Across these five additional donors, there were an additional 15+ isolates. Most of these isolates were present in all donations.

[0449] Similar patterns for relative abundance were observed for the donor material in the recipient after administration of said donor material to recipient subjects (FIG. 5), indicating that the isolates work together as a consortium. Material from Donor 5 was administered to Recipients 1 and 2; material from Donor 7 was administered to Recipients 3 and 4. There was resident *L. crispatus* present in the vaginal tract of Recipient 4 at the time of baseline, which was outcompeted by the donor *L. crispatus* isolates. Note that in instances in which the *L. crispatus* bars in the graph do not add up to 100%, there were other species present (e.g., *L. jensenii*).

**Table 1.** Number of Engraftments/recipients per donor, for *L. crispatus*-dominant donors.

| Donor    | Total recipients this donor was used for, n | Full/stable engraftment, n | Partial or fluctuating engraftment, n | No engraftment, n |
|----------|---------------------------------------------|----------------------------|---------------------------------------|-------------------|
| <u>1</u> | 4                                           | 2                          | 0                                     | 2                 |
| <u>2</u> | 3                                           | 0                          | 1                                     | 2                 |
| <u>3</u> | 3                                           | 0                          | 2                                     | 1                 |
| <u>4</u> | 2                                           | 0                          | 1                                     | 1                 |
| <u>5</u> | 4                                           | 2                          | 0                                     | 2                 |
| <u>6</u> | 4                                           | 1                          | 2                                     | 1                 |
| <u>7</u> | 4                                           | 1                          | 2                                     | 1                 |

Donors in bold or underlined are those from whom the final selected bacteria for the exemplary 4-member consortium were isolated, with bold being the donors of the *L. crispatus* isolates (donors 5 and 7), and underlined the donor for the *L. jensenii* isolate (donor 1).

**Example 4. In silico selection: pangenome and specific analyses**

[0450] The pangenome may be, in some embodiments, a set of genes present in a selected set of lactobacilli. An “engrafting pangenome” is a set of genes present in a selected set of lactobacilli that are capable of engraftment. The genomes of all *L. crispatus* isolates that had engrafted into recipient subjects in Example 3 (as members of an SCVMP) were aligned (FIG. 6). The combined shared genome across all the isolates capable of engraftment was determined to be the “engrafting pangenome”, which can also be called the

“engraftome”. Each isolate was then ranked based on the number of unique genes that an isolate contributed to the engrafting pangenome. Results for the top-ranked isolates are shown in **Table 2**. This ranking was then used to prioritize particular isolates for *in vitro* testing. A combination of *in silico* and *in vitro* characterizations were ultimately used to select particular isolates for an exemplary 4-member consortium of the disclosure.

**Table 2.** Results of analysis of pangenome contribution and *pulA* gene for key isolates.

| Isolate                     | Species             | No. of unique genes contributed to engrafting pangenome <sup>1,2</sup> | Coverage of pangenome (%) <sup>2</sup> | <i>pulA</i> present and intact <sup>2</sup> | No. of successful / partially successful recipients <sup>3</sup> |
|-----------------------------|---------------------|------------------------------------------------------------------------|----------------------------------------|---------------------------------------------|------------------------------------------------------------------|
| <b>FBS-003</b>              | <i>L. jensenii</i>  | NA                                                                     | NA                                     | NA                                          | 2 / 0                                                            |
| <b>FBS-004</b>              | <i>L. crispatus</i> | 81                                                                     | 67                                     | -                                           | 1 / 2                                                            |
| <b>FBS-005</b>              | <i>L. crispatus</i> | 49                                                                     | 66                                     | +                                           | 2 / 0                                                            |
| <b>FBS-006</b>              | <i>L. crispatus</i> | 14                                                                     |                                        | +                                           | 1 / 2                                                            |
| <b>Comparator isolate 1</b> | <i>L. crispatus</i> | 53                                                                     | 69                                     | -                                           | 0 / 2                                                            |
| <b>Comparator isolate 2</b> | <i>L. crispatus</i> | 125                                                                    | 74                                     | -                                           | 0 / 2                                                            |
| <b>Comparator isolate 3</b> | <i>L. crispatus</i> | 31                                                                     | 75                                     | +                                           | 0 / 2                                                            |
| <b>Comparator isolate 4</b> | <i>L. crispatus</i> | 26                                                                     | 66                                     | +                                           | 1 / 2                                                            |
| <b>Comparator isolate 5</b> | <i>L. crispatus</i> | 31                                                                     | 65                                     | +                                           | 2 / 0                                                            |
| <b>Comparator isolate 6</b> | <i>L. crispatus</i> | 102                                                                    | 64                                     | +                                           | 0 / 1                                                            |
| <b>Comparator isolate 7</b> | <i>L. crispatus</i> | 51                                                                     | 64                                     | +                                           | 0 / 1                                                            |
| <b>Comparator isolate 8</b> | <i>L. crispatus</i> | ND                                                                     | 70                                     | +                                           | 2 / 0                                                            |

|                             |             |    |    |  |       |
|-----------------------------|-------------|----|----|--|-------|
| <b>Comparator isolate 9</b> | L. jensenii | NA | NA |  | 2 / 0 |
|-----------------------------|-------------|----|----|--|-------|

*pulA* = glycogen utilization gene; ND = no data; NA = not applicable.

FBS-003, FBS-004, FBS-005, and FBS-006 were selected for the exemplary 4-member consortium.

<sup>1</sup> The total engrafting pangenome consists of 3767 genes.

<sup>2</sup> Only for *L. crispatus*.

<sup>3</sup> For the donor, not only the isolate's visit.

<sup>4</sup> Low pangenome contribution but selected for other reasons.

**[0451]** Furthermore, the presence or absence of selected genes and functionalities was evaluated *in silico*. All isolates were tested for antimicrobial resistance and virulence genes using the Abricate tool with utilizing Resfinder and VFDB as a reference database with default parameters (% nucleotide identity > 80% and % query coverage > 80%). Using these tools and settings, no such genes were found in any of the isolates.

**[0452]** While glycogen has historically been considered to be the main carbon substrate in the vagina, studies on the carbon utilization of lactobacilli in the vagina have been sparse. The pullanase *pulA* gene was recently identified as the first gene related to glycogen utilization in *L. crispatus*. Evaluation of *pulA* in the *L. crispatus* isolates showed that several isolates had a deletion in the *pulA* gene that may affect gene functionality. It was also shown that for donor 7 (of whom two isolates were selected for the exemplary 4-member consortium), *pulA*<sup>+</sup> (FBS-005) and *pulA*<sup>-</sup> (FBS-004) isolates co-exist together, and also transfer to a recipient together during administration of SCVMP (**FIGs. 4 and 5**). This suggests that these isolates “benefit” from each other, and in general that having a mixture of carbohydrate substrate preferences in the microbial consortium would benefit engraftment, as this enables the isolates to thrive in recipients and environments with different carbohydrate availability.

**[0453]** Accordingly, in the exemplary 4-member consortium, both *pulA*<sup>+</sup> and *pulA*<sup>-</sup> bacteria were used. As the donor 7 mixture showed that the *pulA*<sup>+</sup> and *pulA*<sup>-</sup> bacteria stay together, in both the donor and recipient subject after administration of the SCVMP, the *pulA*<sup>+</sup> and *pulA*<sup>-</sup> bacteria from the same donor 7 were used in the exemplary 4-member consortium (instead of mixing a *pulA*<sup>+</sup> and *pulA*<sup>-</sup> isolate from two different donors).



**Example 5. *In vitro* selection through laboratory tests**

[0454] *In vitro* lab tests were performed to evaluate a number of selected bacteria for acidification rates, growth rates, pH tolerance, and lag phase after transfer.

[0455] Briefly, the experiments were conducted as follows with all growth performed in MRS media at pH 4.7. First, isolates were grown overnight from glycerol stock and transferred to fresh medium with a starting OD<sub>600</sub> of 0.1. Multiple tubes were made so that one tube per time point could be taken out for sampling without disturbing or aerating the other cultures. Two tubes were taken out every 1.5 hours for the first 8 hours, and then twice a day after that, to measure the OD<sub>600</sub> and pH. Isolate resilience was tested by transferring the isolate in late stationary phase (at around 48 hours) to new medium with a starting OD<sub>600</sub> of 0.5, and measuring again every 1.5 hours for 8 hours. Finally, lactic acid was measured at 3 selected time points.

[0456] As shown in **FIGs. 7A-7B**, different phenotypes were observed across the isolates.

[0457] The growth rate between 0 and 8 hours varied from 0.22-0.68 h<sup>-1</sup>, which corresponds to doubling times of approximately 2-4.5 hours. Acidification rates between 0 and 8 hours varied from 0.01-0.05, which corresponds to a final pH in exponential phase between 4.3 and 4.6. The majority of acidification takes place after the exponential growth phase, with isolates reaching a final pH (for as long as measurements were performed, which is not yet the end point for most isolates) between 3.5 and 4.1. For most isolates, this corresponded fairly well with the pH of the donor vaginal environment from which they were isolated, with isolates isolated from lower pH environments also showing a lower final pH than isolates isolated from environments with a relatively higher pH (*e.g.*, with pH typically being between 3.6 and 4.3). This indicated that the *in vitro* assay reflects the phenotypes of the isolates in the vaginal environment. After transfer to fresh medium from stationary phase, isolates that acidified the most showed a marginally longer lag time after transfer, but also eventually took up the same pattern as before transfer (with the exception of isolate FBS-005). Both total lactate production and the ratio between D- and L-lactate varied across isolates. Both *L. jensenii* isolates produced barely any L-lactate, while all *L. crispatus* isolates except one of the comparator isolates produced a mixture, with slightly more D-lactate (**Table 3**).

**[0458]** In combination with the *in silico* data in Example 4, the *in vitro* data of this Example led to the identification of an exemplary 4-member consortium. It was hypothesized by the inventors that combining bacterial isolates with unique and different phenotypes into a microbial consortium would increase the range of environments into which bacteria of the consortium can engraft, as each isolate could occupy their own niche, and help the other bacteria in sequential engraftment and acidification. Using this strategic approach, an exemplary 4-member consortium comprising a *Lactobacillus jensenii* isolate (FBS-003; DSM 34770), a *Lactobacillus crispatus* isolate that was isolated from a donor having *L. jensenii* (FBS-005; DSM 34772) (a bacteria thrives in the presence of *Lactobacillus jensenii* bacteria), and two additional bacteria that were isolated from a donor lacking *L. jensenii* (FBS-004; DSM 34771) (FBS-006; DSM 34773) (*Lactobacillus crispatus* isolate that thrive independent of the presence of *Lactobacillus jensenii*) (**FIG. 15**).

**Table 3.** Overview of properties for isolate selection.

| Isolate | Species             | pul<br>A | $\mu$ avg<br>0-8h<br>(h <sup>-1</sup> ) | Doubli<br>ng time<br>(hh:m<br>m) | Highes<br>t<br>measur<br>ed<br>OD <sub>600</sub> | Acidifica<br>tion rate<br>0-8h<br>(h <sup>-1</sup> ) | Final<br>pH<br>expone<br>ntial<br>phase | Final<br>pH<br>station<br>ary<br>phase <sup>1</sup> | CVS<br>pH |
|---------|---------------------|----------|-----------------------------------------|----------------------------------|--------------------------------------------------|------------------------------------------------------|-----------------------------------------|-----------------------------------------------------|-----------|
| FBS-003 | <i>L. jensenii</i>  | NA       | 0.40                                    | 02.30                            | 4.8                                              | -0.04                                                | 4.4                                     | 3.9                                                 | 4.1       |
| FBS-004 | <i>L. crispatus</i> | -        | 0.68 <sup>2</sup>                       | 01.53                            | 3.7                                              | -0.02                                                | 4.5                                     | 3.9                                                 | 3.9       |
| FBS-005 | <i>L. crispatus</i> | +        | 0.50                                    | 01.59                            | 4.5                                              | -0.05                                                | 4.3                                     | 3.7                                                 | 3.9       |
| FBS-006 | <i>L. crispatus</i> | +        | 0.23                                    | 04.16                            | 5.9                                              | -0.02                                                | 4.4                                     | 3.6                                                 | 3.9       |

|                       |  |  |      |       |     |       |     |     |     |
|-----------------------|--|--|------|-------|-----|-------|-----|-----|-----|
| Minimum (14 isolates) |  |  | 0.22 | 01.53 | 2.2 | -0.05 | 4.3 | 3.5 | 3.7 |
| Maximum (14 isolates) |  |  | 0.68 | 04.29 | 8.5 | -0.01 | 4.6 | 4.1 | 4.2 |
| Average (14 isolates) |  |  | 0.37 | 02.58 | 5.0 | -0.03 | 4.5 | 3.7 | 3.9 |

\*ND = no data; CVS = Cervicovaginal secretions; *pulA* + means intact *pulA*; *pulA* – means deletion in *pulA*. The minimum, maximum, and average values are for a total of 14 isolates (the 4 selected FBS isolates and 10 comparator isolates).

**Table 3. (continued)**

| Isolate               | Species             | Time to growth after transfer (h) | Total lactate 47h (g/L) | D/L lactate ratio 8h | D/L lactate ratio 23h | D/L lactate ratio 47h |
|-----------------------|---------------------|-----------------------------------|-------------------------|----------------------|-----------------------|-----------------------|
| FBS-003               | <i>L. jensenii</i>  | 1.5                               | 15.0                    | 27.0                 | 17.6                  | 12.0                  |
| FBS-004               | <i>L. crispatus</i> | 3.0                               | 14.9                    | 4.7                  | 2.4                   | 2.0                   |
| FBS-005               | <i>L. crispatus</i> | 4.5                               | 25.2                    | 7.0                  | 4.0                   | 3.5                   |
| FBS-006               | <i>L. crispatus</i> | 3.0                               | 17.5                    | 7.3                  | 2.6                   | 1.7                   |
| Minimum (14 isolates) |                     | 1.5                               | 9.2                     | 1.2                  | 1.8                   | 1.7                   |

|                             |  |     |      |      |      |      |
|-----------------------------|--|-----|------|------|------|------|
| Maximum<br>(14<br>isolates) |  | 4.5 | 42.3 | 27.0 | 17.6 | 21.9 |
| Average<br>(14<br>isolates) |  | 2.8 | 20.9 | 5.4  | 4.0  | 5.4  |

**Example 6. Characterization of individual isolates and an exemplary 4-member consortium (MC-1) in MRS medium show phenotypic diversity and synergy at two relevant pH values**

**[0459]** This and the following Examples describe the characterization of an exemplary microbial consortium (“4-member consortium”) selected according to the above Examples, composed of a *Lactobacillus jensenii* isolate (FBS-003); a *Lactobacillus crispatus* isolate that thrives in the presence of *Lactobacillus jensenii* (FBS-005); and two additional *Lactobacillus crispatus* isolates that thrive independent of the presence of *Lactobacillus jensenii* (FBS-004 and FBS-006), see Fig. 15. The characterization of each bacterial isolate of the consortium on an individual level is also described.

**[0460]** This Example describes the growth and acidification of the isolates and 4-member consortium in medium set to two different pH values – 4.7 and 5.2 – that are both realistic pH values of potential recipients of the 4-member consortium, where the 4-member consortium should thrive.

**[0461]** All bacterial isolates were aseptically retrieved from working cell bank vials stored at -80°C for the inoculation of overnight cultures in 4.5 mL tubes containing 4.2 mL of MRS broth media (VWR international, Denmark) adjusted at pH 5.5. To initiate experimental cultures, live bacteria of overnight cultures were enumerated with an impedance flow cytometer according manufacturer’s instructions using BactoBox (SBT instruments, Herlev, Denmark). Bacteria were grown in microaerophilic conditions using one 1.5 mL Eppendorf tube per time point containing 1.3 ml of MRS broth adjusted at either pH 4.7 or 5.2 accordingly. Multiple tubes were prepared such that one tube per time point could be taken out for sampling without disturbing or aerating the other cultures. All incubations were conducted at a static 37 °C, with three biological replicates for all experiments. Final cell

viability for the absolute quantification of species abundance was measured by plating (CFUs/ml) across time.

**[0462]** Bacterial growth was recorded based on OD<sub>600</sub> absorbance readings. For the measurement of pH, cultures were centrifuged at 8000 rpm for 5 minutes and a pH probe (Microelectrodes, New Hampshire, US or Mettler Toledo, In-lab ultramicro, Denmark) was used at the designated timepoints. A volume of 500µl of supernatant was frozen at -20°C for later use for D- and L-lactic acid production measurements, carried out as per manufacturer's instructions using a D- or L- lactic acid kit (Megazyme, Bray, Ireland). Rates were calculated using the formula below, where r= rate; OD<sub>2</sub>, pH<sub>2</sub>, LAC<sub>2</sub> = final measurement; OD<sub>1</sub>, pH<sub>1</sub>, LAC<sub>1</sub> = initial measurement; T<sub>1</sub>= Initial time and T<sub>2</sub>= Final time.

$$\mathbf{[0463]} \quad r = \frac{(\ln(OD_2, pH_2, LAC_2 / OD_1, pH_1, LAC_1))}{(T_2 - T_1)}$$

**[0464]** Growth rates were determined according to the following equation: Growth rate equals natural logarithm (Ln) of (final OD<sub>600</sub> divided by initial OD<sub>600</sub>) divided by (final time minus initial time). Acidification rates were determined according to the following equation: Acidification rate equals natural logarithm (Ln) of (final pH divided by initial pH) divided by (final time minus initial time). Rates of D-lactic acid production were determined according to the following equation: Rate of D-lactic acid production equals natural logarithm (Ln) of (final concentration of D-lactic acid divided by initial concentration of D-lactic acid) divided by (final time minus initial time). Rates of L-lactic acid production were determined according to the following equation: Rate of L-lactic acid production equals natural logarithm (Ln) of (final concentration of L-lactic acid divided by initial concentration of L-lactic acid) divided by (final time minus initial time).

**[0465]** Pellets of centrifuged samples were stored at -20°C for later DNA extraction using a MolYsis™ complete 5 kit (Molzylm, Bremen, Germany) to aid in removing DNA from non-viable bacterial cells. The specific amplification of unique selected regions for each isolate was performed using the following forward (F) and revers (R) primers; FBS-003 F (5'-GTGTCTAAAGTGCCTGCTGT-3') (SEQ ID NO: 1) and FBS-003 R (5'-TTTCGAGCGACCTGTAACGTC3') (SEQ ID NO: 2), FBS-004 F (5'-TCTTAACCAGTCGTGCATCCC -3') (SEQ ID NO: 3) and FBS-004 R(5'-TTCCCAGCGAGCGTATTGTG-3') (SEQ ID NO: 4), FBS-005 F(5'-CACACTTGACTTCCGAGGCT-3') (SEQ ID NO: 5) and FBS-005 R(5'-ACGATTGGCTGACCTGTTTCT-3') (SEQ ID NO: 6), FBS-006 F (5'-

GCATGTTAGCAACGAAGGCA-3') (SEQ ID NO: 7) and FBS-006 R(5'-GGAGGACGGTTACTATGCAGT-3') (SEQ ID NO: 8) using Luna universal SYBR green qPCR master mix (New England Biolabs, UK). The cycle parameters comprised a 10 min pre-incubation for thermal activation at 95°C, an amplification of 39 cycles comprising a denaturalization step at 95°C for 15 s and an extension/annealing of 30 s at 62°C for FBS-003 primers and 58°C for FBS-004, FBS-005 and FBS-006 primers. Fluorescence was acquired after every cycle.

**[0466]** Under both pH conditions, all isolates and the 4-member consortium exhibited exponential growth for 8 hours. Notably, FBS-006 displayed the slowest growth rate in this period of time, followed by FBS-004, while FBS-003, FBS-005, and the 4-member consortium demonstrated the highest and similar growth rates. These consistent findings were observed across both pH levels tested (**FIGs. 8A-8D**).

**[0467]** The same time points were utilized to measure pH levels, revealing isolate-dependent acidification performance. In cultures with starting pH 4.7, FBS-003 displayed the slowest acidification rate, followed by FBS-004 and FBS-006. In contrast, FBS-005 and the 4-member consortium exhibited the highest acidification rate (**FIGs. 9A-9B**). In cultures with starting pH 5.2, both FBS-003 and FBS-004 demonstrated relatively lower acidification rates compared to FBS-005, FBS-006, and the 4-member consortium, which exhibited similarly higher acidification rates (**FIGs. 9C-9D**).

**[0468]** The production of D-lactic acid and L-lactic acid during glucose fermentation in MRS was assessed in the same samples. During the initial 24 hours of cultures starting at pH 4.7, FBS-003 exhibited the lowest production of both D- and L-lactic acid, followed by FBS-004 and FBS-006. In contrast, FBS-005 and the 4-member consortium displayed superior D- and L-lactic acid production (**FIGs. 10A-10D**). In cultures starting at pH 5.2, FBS-003, FBS-005, and the 4-member consortium showed similar rates of D-lactate production, with FBS-004 and FBS-006 lagging behind (**FIGs. 10E-10F**). However, FBS-003 performed less efficiently than the other isolates and 4-member consortium in terms of L-lactic acid production (**FIGs. 10G-10H**). Proportions of D- versus L-lactic acid production were comparable for FBS-004, FBS-005, FBS-006, and the 4-member consortium at pH 4.7, with D-lactic acid accounting for approximately 75% to 79% of total lactic acid produced after 48 hours. For the FBS-003 isolate, this proportion was approximately 97% (**FIG. 11A**). At starting pH 5.2, a lower proportion of D-lactic acid was produced by these isolates, varying

from 66% to 71% after 48 hours. FBS-003, in particular, accounted for 94% of the total lactic acid produced (**FIG. 11B**).

**[0469]** To understand population dynamics over time, samples were plated for total bacterial count to compare species ratios and pellets used to compare isolate ratios within the 4-member consortium across time. Notably, *L. jensenii* predominated over the three *L. crispatus* species after 8 hours of growth in both starting pH conditions on plates, comprising 70% of the total CFUs/ml and approximately 50% in cultures starting at pH 4.7 and 76% at pH 5.2. However, *L. crispatus* abundance increased to approximately 82% pH 4.7 and 68% at pH 5.2 after 24 hours, and to 55% and 78%, respectively, after 48 hours. The results observed with qPCR showed a similar trend, indicating that FBS-003 comprised 50% of the relative abundance after 8 hours. Subsequently, FBS-005 surpassed all other *L. crispatus* species at 24 and 48 hours, representing about 75% of the relative abundance, while FBS-003 decreased to 20% approximately (**FIGs. 12A-12D**). The results are summarized in **Tables 5-6**.

**[0470]** These results indicate that the isolates have different pH optima for growth and acidification. The combination of these isolates creates a synergistic growth and acidification pattern that allows the 4-member consortium to perform as well as the best single isolate in both cases.

**Table 4.** Phenotypic characterizations (starting pH of 4.7).

| Growth Rates        | FBS-003<br>(DSM 34770) | FBS-004<br>(DSM 34771) | FBS-005<br>(DSM 34772) | FBS-006<br>(DSM 34773) | 4-member<br>Consortium |
|---------------------|------------------------|------------------------|------------------------|------------------------|------------------------|
| Average             | 0.34                   | 0.28                   | 0.38                   | 0.25                   | 0.34                   |
| SE                  | 0.02                   | 0.01                   | 0.03                   | 0.01                   | 0.03                   |
| Acidification rates | FBS-003                | FBS-004                | FBS-005                | FBS-006                | 4-member<br>Consortium |
| Average             | -0.004                 | -0.006                 | -0.008                 | -0.007                 | -0.008                 |
| SE                  | 0.001                  | 0.000                  | 0.000                  | 0.000                  | 0.000                  |
| D-lactic acid rates | FBS-003                | FBS-004                | FBS-005                | FBS-006                | 4-member<br>Consortium |
| Average             | 0.12                   | 0.15                   | 0.17                   | 0.15                   | 0.17                   |
| SE                  | 0.02                   | 0.01                   | 0.02                   | 0.01                   | 0.01                   |

|                     |         |         |         |         |                     |
|---------------------|---------|---------|---------|---------|---------------------|
| L-lactic acid rates | FBS-003 | FBS-004 | FBS-005 | FBS-006 | 4-member Consortium |
| Average             | 0.032   | 0.089   | 0.109   | 0.094   | 0.108               |
| SE                  | 0.007   | 0.004   | 0.004   | 0.021   | 0.004               |

\*Growth rate was calculated in the first 8 hours and acidification, D-, and L-lactic acid rates were calculated in the first 24 hours. Units for all provided rates are hours<sup>-1</sup>.

**Table 5.** Phenotypic characterizations (starting pH of 5.2).

|                     |                        |                        |                        |                        |                     |
|---------------------|------------------------|------------------------|------------------------|------------------------|---------------------|
| Growth Rates        | FBS-003<br>(DSM 34770) | FBS-004<br>(DSM 34771) | FBS-005<br>(DSM 34772) | FBS-006<br>(DSM 34773) | 4-member Consortium |
| Average             | 0.42                   | 0.36                   | 0.4                    | 0.32                   | 0.4                 |
| SE                  | 0.00                   | 0.01                   | 0.00                   | 0.00                   | 0.01                |
| Acidification rates | FBS-003                | FBS-004                | FBS-005                | FBS-006                | 4-member Consortium |
| Average             | -0.008                 | -0.007                 | -0.011                 | -0.011                 | -0.010              |
| SE                  | 0.000                  | 0.000                  | 0.000                  | 0.000                  | 0.000               |
| D-lactic acid rates | FBS-003                | FBS-004                | FBS-005                | FBS-006                | 4-member Consortium |
| Average             | 0.19                   | 0.16                   | 0.19                   | 0.17                   | 0.19                |
| SE                  | 0.00                   | 0.00                   | 0.00                   | 0.00                   | 0.00                |
| L-lactic acid rates | FBS-003                | FBS-004                | FBS-005                | FBS-006                | 4-member Consortium |
| Average             | 0.034                  | 0.103                  | 0.114                  | 0.114                  | 0.110               |
| SE                  | 0.012                  | 0.008                  | 0.007                  | 0.002                  | 0.012               |

**Example 7. Characterization of carbon substrate utilization profiles of individual isolates and an exemplary 4-member consortium (MC-1) in API tests**

[0471] To further characterize the metabolic functionality of the individual isolates and an exemplary microbial consortium (“4-member consortium”), an API-50CH® test was conducted to elucidate and compare what carbon substrates were fermented. All carbon substrates fermented in the API® strips by the individual isolates were also fermented by the



4-member consortium except for D-turanose, which appeared positive for FBS-006 after the 48 h incubation period. (**Table 6**). A designation of “+” in **Table 6** indicates that the individual isolates and/or 4-member consortium were capable of utilizing the listed carbohydrate substrate for growth.

**[0472]** Differences in carbohydrate utilization were observed between the individual isolates. Importantly, the exemplary 4-member consortium was capable of utilizing a particular carbon substrate even if not all of the isolates in the 4-member consortium could utilize those substrates. For example, the 4-member consortium was able to utilize glycogen, mannitol, and galactose even though only two of four isolates in the 4-member consortium were able to do so (FBS-004 and FBS-006).

**[0473]** These data provide additional evidence that microbial consortia of the disclosure are capable of successfully propagating within a wide range of environments, which should promote engraftment in a diverse array of subjects, each of whom may have a unique and different vaginal microbiome environment.

**Table 6.** Qualitative identification and comparison of carbohydrate utilization at different times between the individual isolates and 4-member consortium.

|                                 | FBS-003 |      | FBS-004 |      | FBS-005 |      | FBS-006 |      | 4-member Consortium |      |
|---------------------------------|---------|------|---------|------|---------|------|---------|------|---------------------|------|
|                                 | 48 h    | 120h | 48 h    | 120h | 48 h    | 120h | 48 h    | 120h | 48 h                | 120h |
| Blank                           | -       | -    | -       | -    | -       | -    | -       | -    | -                   | -    |
| Glycerol                        | -       | -    | -       | -    | -       | -    | -       | -    | -                   | -    |
| Erythritol                      | -       | -    | -       | -    | -       | -    | -       | -    | -                   | -    |
| D-arabinose                     | -       | -    | -       | -    | -       | -    | -       | -    | -                   | -    |
| L-arabinose                     | -       | -    | -       | -    | -       | -    | -       | -    | -                   | -    |
| D-ribose                        | +/-     | +/-  | -       | -    | +/-     | +    | -       | +/-  | -                   | +/-  |
| D-xylose                        | -       | -    | -       | -    | -       | -    | -       | -    | -                   | -    |
| L-xylose                        | -       | -    | -       | -    | -       | -    | -       | -    | -                   | -    |
| D-adonitol                      | -       | -    | -       | -    | -       | -    | -       | -    | -                   | -    |
| Methyl $\beta$ D-xylopyranoside | -       | -    | -       | -    | -       | -    | -       | -    | -                   | -    |

|                                   |   |   |     |   |   |   |     |   |     |   |
|-----------------------------------|---|---|-----|---|---|---|-----|---|-----|---|
| D-galactose                       | - | - | +   | + | - | + | +   | + | +   | + |
| D-glucose                         | + | + | +   | + | + | + | +   | + | +   | + |
| D-fructose                        | + | + | +   | + | + | + | +   | + | +   | + |
| D-mannose                         | + | + | +   | + | + | + | +   | + | +   | + |
| L-sorbitose                       | - | - | -   | - | - | - | -   | - | -   | - |
| L-rhamnose                        | - | - | -   | - | - | - | -   | - | -   | - |
| Dulcitol                          | - | - | -   | - | - | - | -   | - | -   | - |
| Inositol                          | - | - | -   | - | - | - | -   | - | -   | - |
| D-mannitol                        | - | - | +   | + | - | - | +   | + | +/- | + |
| D-sorbitol                        | - | - | -   | - | - | - | -   | - | -   | - |
| Methyl $\alpha$ D-mannopyranoside | - | - | -   | - | - | - | -   | - | -   | - |
| Methyl $\alpha$ D-glucopyranoside | - | - | -   | - | - | + | -   | + | +   | + |
| N-acetylglucosamine               | - | - | +   | + | + | + | +   | + | +   | + |
| Amygdalin                         | + | + | -   | + | + | + | +/- | + | +   | + |
| Arbutin                           | + | + | +/- | + | + | + | +   | + | +   | + |
| Esculin ferric citrate            | + | + | +   | + | + | + | +   | + | +   | + |
| Salicin                           | + | + | +   | + | + | + | +   | + | +   | + |
| D-cellobiose                      | + | + | +   | + | + | + | +   | + | +   | + |
| D-maltose                         | + | + | +   | + | + | + | +   | + | +   | + |
| D-lactose (bovine origin)         | - | - | +   | + | + | + | +   | + | +   | + |
| D-melibiose                       | - | - | -   | - | - | - | -   | - | -   | - |
| D-saccharose (sucrose)            | + | + | +   | + | + | + | +   | + | +   | + |
| D-trehalose                       | + | + | +   | + | + | + | +   | + | +   | + |
| Inulin                            | - | - | -   | - | - | - | -   | - | -   | - |
| D-melezitose                      | - | - | -   | - | - | - | -   | - | -   | - |

|                           |   |   |   |   |   |   |   |   |   |   |
|---------------------------|---|---|---|---|---|---|---|---|---|---|
| D-raffinose               | - | - | - | - | - | - | - | - | - | - |
| Starch                    | + | + | + | + | + | + | + | + | + | + |
| Glycogen                  | - | - | + | + | - | - | + | + | + | + |
| Xylitol                   | - | - | - | - | - | - | - | - | - | - |
| Gentiobiose               | + | + | - | + | + | + | - | - | + | + |
| D-turanose                | - | - | - | - | - | - | - | + | - | - |
| D-lyxose                  | - | - | - | - | - | - | - | - | - | - |
| D-tagatose                | - | - | - | - | - | - | - | - | - | - |
| D-fucose                  | - | - | - | - | - | - | - | - | - | - |
| L-fucose                  | - | - | - | - | - | - | - | - | - | - |
| D-arabitol                | - | - | - | - | - | - | - | - | - | - |
| L-arabitol                | - | - | - | - | - | - | - | - | - | - |
| Potassium gluconate       | - | - | - | - | - | - | - | - | - | - |
| Potassium 2-ketogluconate | - | - | - | - | - | - | - | - | - | - |
| Potassium 5-ketogluconate | - | - | - | - | - | - | - | - | - | - |

**Example 8. Characterization of individual isolates and the exemplary 4-member consortium (MC-1) show diversity and synergy in vagina-relevant carbon substrates**

[0474] A variety of simple and complex carbohydrates are relevant for engraftment, including those present in cervicovaginal fluid. This includes glycogen, believed to be the primary nutrient supporting the vaginal microbiome. Potential drug substances may contain maltodextrin, a complex carbohydrate capable of also nourishing *Lactobacillus* isolates and also present in the vagina as breakdown product of glycogen. Maltodextrin was tested at levels that may be included in drug product formulations. Glucose, aside from being present in CVS, was used as a control for comparison to their performance in standard MRS medium. Fructose and Mannose are also present in the vaginal epithelium, as part of CVS and ejaculate, and mucin-derived glycoproteins respectively. Mannitol has been observed in CVS samples where lactobacilli are dominant. These carbohydrates were added to a modified

MRS composed of ; Peptone from meat (10g/L, Sigma Aldrich, cat:70175), FNI150 (10ml/L, Lallemand), K<sub>2</sub>HPO<sub>4</sub> (2g/L, Supelco, cat:1.05104), Sodium acetate (5g/L, ThermoScientific, cat:149610010), Triammonium citrate (2g/L, VWR chemicals, cat:03013A), MgSO<sub>4</sub> (0.2g/L, VWR chemicals, cat:7154), MnSO<sub>4</sub> (0.05g/L, VWR chemicals, cat:25303.233), Tween 80 (1.08g/L, Sigma Aldrich, cat:P4780) and (20g/L) of either glucose (Sigma Aldrich, cat:49159), maltodextrin (Glucidex IT6), fructose (Sigma Aldrich, cat:F3510), D-mannose (Sigma Aldrich, cat:102758057) or D-mannitol (Sigma Aldrich, Millipore, cat:102737188). Consortium performance on growth, acidification, D- and L- lactic acid production and population dynamics were assessed as previously described in Example 6. Population dynamics were also measured.

**Table 7.** Steps for determining growth rate:

|        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
|--------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Step 1 | One of the at least two phenotypically distinct <i>Lactobacillus crispatus</i> isolates is retrieved from cell bank storage at -80°C under sterile conditions, inoculated into 4.2 mL of pH 5.5 MRS medium (55.2g/L, VWR, cat:EM.1.10661.0500), and grown in microaerophilic conditions at 37°C for 20 hours.                                                                                                                                                                                                                                                                                                                                                                                      |
| Step 2 | Culture medium is made which consists of Peptone from meat (10g/L, Sigma Aldrich, cat:70175), FNI150 (10ml/L, Lallemand), K <sub>2</sub> HPO <sub>4</sub> (2g/L, Supelco, cat:1.05104), Sodium acetate (5g/L, ThermoScientific, cat:149610010), Triammonium citrate (2g/L, VWR chemicals, cat:03013A), MgSO <sub>4</sub> (0.2g/L, VWR chemicals, cat:7154), MnSO <sub>4</sub> (0.05g/L, VWR chemicals, cat:25303.233), Tween 80 (1.08g/L, Sigma Aldrich, cat:P4780) and (20g/L) of either glucose (Sigma Aldrich, cat:49159), maltodextrin (Glucidex IT6 ), fructose (Sigma Aldrich, cat:F3510), D-mannose (Sigma Aldrich, cat:102758057) or D-mannitol (Sigma Aldrich, Millipore, cat:102737188). |
| Step 3 | The culture medium of Step 2 is adjusted to an initial pH of 4.7 by adding HCl (Merck, cat:1.60238.1003) to the medium.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
| Step 4 | The concentration of the one of the at least two phenotypically distinct <i>Lactobacillus crispatus</i> isolates grown in Step 1 is adjusted to 5 million live cells/mL (measured by BactoBox) in 1.3 mL of the culture medium adjusted to a pH of 4.7 in Step 3.                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| Step 5 | The OD <sub>600</sub> of the inoculated culture medium of Step 4 is measured and then grown in microaerophilic conditions at 37°C for 24 hours.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |

|        |                                                                                                                                                     |
|--------|-----------------------------------------------------------------------------------------------------------------------------------------------------|
| Step 6 | The final OD600 of the inoculated culture medium is measured after the 24 hours of growth.                                                          |
| Step 7 | The growth rate is calculated using the following equation: natural logarithm (Ln) of (final OD600 divided by initial OD600) divided by (24 hours). |

**Table 8.** Steps for determining acidification rate:

|        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
|--------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Step 1 | One of the at least two phenotypically distinct <i>Lactobacillus crispatus</i> isolates is retrieved from cell bank storage at -80°C under sterile conditions, inoculated into 4.2 mL of pH 5.5 MRS medium (55.2g/L, VWR, cat:EM.1.10661.0500), and grown in microaerophilic conditions at 37°C for 20 hours.                                                                                                                                                                                                                                                                                                                                                                                      |
| Step 2 | Culture medium is made which consists of Peptone from meat (10g/L, Sigma Aldrich, cat:70175), FNI150 (10ml/L, Lallemand), K <sub>2</sub> HPO <sub>4</sub> (2g/L, Supelco, cat:1.05104), Sodium acetate (5g/L, ThermoScientific, cat:149610010), Triammonium citrate (2g/L, VWR chemicals, cat:03013A), MgSO <sub>4</sub> (0.2g/L, VWR chemicals, cat:7154), MnSO <sub>4</sub> (0.05g/L, VWR chemicals, cat:25303.233), Tween 80 (1.08g/L, Sigma Aldrich, cat:P4780) and (20g/L) of either glucose (Sigma Aldrich, cat:49159), maltodextrin (Glucidex IT6 ), fructose (Sigma Aldrich, cat:F3510), D-mannose (Sigma Aldrich, cat:102758057) or D-mannitol (Sigma Aldrich, Millipore, cat:102737188). |
| Step 3 | The culture medium of Step 2 is adjusted to an initial pH of 4.7 by adding HCl (Merck, cat:1.60238.1003) to the medium.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
| Step 4 | 5 million live cells/mL (measured by BactoBox) of the one of the at least two phenotypically distinct <i>Lactobacillus crispatus</i> isolates grown in Step 1 are inoculated into 1.3 mL of the culture medium adjusted to a pH of 4.7 in Step 3.                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| Step 5 | The inoculated culture medium of Step 4 is grown in microaerophilic conditions at 37°C for 24 hours.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| Step 6 | The final pH of the culture grown in Step 5 is measured after the 24 hours of growth.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
| Step 7 | The acidification rate is calculated using the following equation: natural logarithm (Ln) of (final pH divided by initial pH) divided by (24 hours).                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |

**[0475]** While most isolates exhibited a similar performance when growing on different carbohydrates, the 4-member consortium compared to the best-performing individual isolates across the board in terms of final OD<sub>600</sub> (**FIG. 16**). This is a surprising finding because

isolates from different environments/donors have not naturally arrived at a steady state/equilibrium and may thus be expected to compete for substrates and other resources, potentially cancelling each other out. FBS-003 cannot grow efficiently on glycogen or mannitol (**Table 6 and FIGs. 16C-D and 16K-L**), but its relative abundance was still relatively high in glycogen and present in mannitol when growing in the 4-member consortium (**FIG. 18**). This suggests that FBS-003 can thrive in glycogen-rich environments perhaps due to the efficient glycogen hydrolysis by other isolates, after which FBS-003 uses the glycogen degradation products for its growth and may benefit from the by-products of mannitol metabolism by other isolates. FBS-003 was one of the best performers in growth on glucose, indicating that it is good at growing on relatively simple carbohydrates. FBS-005 was the top performer on glycogen and, along with FBS-003 and the 4-member consortium, also excelled in growth on glucose. FBS-005 was not performing as well as either FBS-004, FBS-006 or the 4-member consortium in fructose, mannose or mannitol, but its abundance was still very high within the 4-member consortium (**FIG. 18**). This indicates that growth as individual isolates does not necessarily correspond to their growth performance within the 4-member consortium, and that synergy may occur. The 4-member consortium was the best at growing in maltodextrin and its OD<sub>600</sub> and was always similar to the highest OD<sub>600</sub> of the best performing isolates after 48 hours.

**[0476]** The 4-member consortium also demonstrated efficient acidification, particularly in maltodextrin and glycogen in modified MRS, and in MRS, where it ranked as the top or near-top performer compared to the individual isolates (**FIG. 17**). FBS-003 appeared to acidify less well when growing in a modified MRS with the polysaccharides and mannitol, and slightly better with glucose, MRS and best in fructose and mannose. FBS-004 was the second worst acidifier in several cases but for fructose, mannose and mannitol, where it acidified as efficiently or better than the 4-member consortium. FBS-006 was the best or second-best acidifier when growing on the polysaccharides, and the best acidifier in fructose, mannose and mannitol. Abundance of FBS-006 in mannose and mannitol was lower than FBS-005 within the 4-member consortium when growing in mannitol (**FIG. 18**), but the acidification of the 4-member consortium was still lower than FBS-005, indicating that FBS-006 has a high metabolic activity relative to its growth. These results show that lower numbers of bacteria may not necessarily mean a lower contribution to the performance of the 4-member consortium. A similar situation occurred with FBS-003 in fructose. Here, FBS-003 was very abundant within the 4-member consortium, more than FBS-004, FBS-005 and FBS-

006 but its acidification is slower than FBS-004, FBS-006 and the 4-member consortium, indicating a higher metabolic activity of FBS-004 and FBS-006 isolates even in lower numbers. This data indicates that relatively small numbers of FBS-004 and FBS-006 isolates can contribute significantly to optimize performance of the 4-member consortium in environments relevant to the vaginal niche.

[0477] The results indicate that the abundance of each isolate varies depending on the available carbon substrate, suggesting that the population of the 4-member consortium efficiently adapts to optimize both growth and acidification while supporting isolate coexistence. Although isolate populations differ in abundance, each isolate contributes uniquely to the 4-member consortium's overall efficiency, as indicated by the above results.

[0478] The findings suggest that the FBS-003 and FBS-005 combination forms the dominant backbone of the 4-member consortium in terms of abundance (**FIG. 18**). However, despite their lower abundance, FBS-004 and FBS-006 significantly enhance the metabolic efficiency of the 4-member consortium (**FIGs. 16 and 17**). Notably, in the presence of complex carbohydrates such as maltodextrin (at various concentrations), glycogen, and simple sugars such as fructose, mannose, and mannitol, FBS-004 and FBS-006 were more abundant than in standard MRS or modified MRS with glucose (**FIG. 12B and FIG. 18**). This suggests that these isolates play roles in optimizing resource utilization within the 4-member consortium, ensuring efficient operation and enhanced performance that would not be achievable with only the dominant isolates, FBS-003 and FBS-005.

[0479] FBS-004 and FBS-006 showed superior growth in mannitol compared to FBS-003 and FBS-005, despite their lower abundance in this medium (**FIGs. 16K-L and FIG. 18F**).

#### **Example 9. Comparison of the exemplary 4-member consortium (MC-1) with competitor strains**

[0480] This Example describes the comparison of the above-specified exemplary consortium to selected other strains known in the art. An exemplary 4-member consortium (**FIG. 15**) was compared with selected strains known in the art to evaluate performance in parameters important for engraftment including growth (**FIG. 19A-B**) and acidification (**FIG. 19C-D**). Evaluated strains include LBV88 (US 8,846,027 B2), I-3483 (US 2017/0027998 A1), I-3484 (US 2017/0027998 A1), I-3486 (US 2017/0027998 A1), I-3446 (US

2015/0190438 A1), and Lactin-V (CTV-05) (EP4294417). The experimental methods for determining growth, acidification and the calculation of rates were performed as described in Example 6.

[0481] In the first 8 hours of growth, which are considered crucial for rapid colonization of the vaginal niche and therefore engraftment, the 4-member consortium showed the highest growth rate (**FIG. 19B**) and the lowest acidification rate (**FIG. 19D**), while some evaluated strains showed comparative growth and acidification rates at 48 hours (**FIGs. 19A and 19C**). These characteristics are especially important for competing with pathogenic bacteria.

#### **Example 10. Comparing 2-member, 3-member, and 4-member consortia**

[0482] This Example described the comparison of the exemplary 4-member consortium (MC-1) as described in Example 6 with 3-member consortia and 2-member consortia comprising combinations of isolates from the 4-member consortium. The comparison subsets included individual isolates, mixed subsets of the 4-member consortium isolates as 2-member consortia, combining an *L. jensenii* FBS-003 isolate with each of the other *L. crispatus* isolates (FBS-004, FBS-005 or FBS-006) and two 3-member consortia that included the FBS-003 + FBS-005 with either of the other two *L. crispatus* isolates (FBS-004 or FBS-006).

[0483] All experimental procedures were performed as described in Example 6 except that cultures were standardized to either an initial OD<sub>600</sub> of 0.05 or to 5 million CFU/ml using Bactobox.

[0484] To quantify how much of each isolate is present in the different consortia, 2-member consortia were plated using serial dilutions and counting CFU/ml. A distinction between isolates is possible because the *L. jensenii* (FBS-003) isolate has a distinctive morphology when compared to any of the *L. crispatus* isolates. In the case of 3-member and 4-member consortia, the qPCR method described in Example 6 was used, where the DNA extraction was performed using a MoLYsis™ complete 5 kit (Molzylm, Bremen, Germany) to aid in removing DNA from non-viable bacterial cells. The 4-member consortium matched the top-performing individual isolate (FBS-003) in both growth and acidification after 8h (**FIG. 20A and FIG. 21A**) and was the best performer in both cases when compared to 2-member consortia (**FIG. 20B and FIG. 21B**). The FBS-003 and FBS-005 individual isolates had similar growth rates as the 4-member consortium, and no 2-member consortium grew faster than the 4-member consortium (**FIG. 20C**). The FBS-003 + FBS-004 and FBS-003 + FBS-



006 2-member consortia performed similarly to their respective individual isolates, while the FBS-003 + FBS-005 2-member consortium slightly differed from the FBS-005 isolate alone (**FIG. 20C**). A similar trend was observed in acidification, where the 2-member consortia matched *L. crispatus* individual isolate counterparts, except for FBS-003 + FBS-005, which slightly differed from the FBS-005 isolate alone (**FIG. 21C**).

[0485] An exemplary 4-member consortium and 3-member consortia were tested during growth and acidification (**FIG. 22A and FIG. 23A**), with a slightly better performance in both growth and acidification of the 4-member consortium after 8 hours (**FIG. 22B and FIG. 23B**). The FBS-004-containing 3-member consortium showed a slightly faster growth rate than the 3-member consortium including FBS-006 (**FIG. 22B**). Together with the in-human data where these two isolates consistently engraft together, there is an indication that FBS-004 and FBS-006 are not redundant but behave synergistically when growing in the 4-member consortium.

[0486] To investigate how the relative abundance of individual isolates varied within the consortia during growth in MRS (a medium rich in glucose), each isolate's abundance was tracked at designated times for the 2-, 3- and 4-member consortia. The FBS-003+FBS-005 combination appears to act synergistically as this pair was found to coexist during growth as a 2-member consortium, whilst FBS-003 mostly outperformed either FBS-004 or FBS-006 when growing together by 8 hours of growth (**FIG. 24A-C**). A more balanced composition was found in 3-member consortia where the three isolates coexisted in the first 8 hours (**FIG. 25A-B**). A similar result was found in the 4-member consortium at this time point (**FIG. 12B**). The 4-member consortium performed slightly better than the 3-member consortium, suggesting limited continued synergy with additional strains on the simple carbohydrate glucose, but allowing the inclusion of the naturally cooccurring and co-engrafting FBS-004 and FBS-006 that broaden the range of carbon substrates to be used, hence giving the consortium more resilience (see Example 12).

#### **Example 11. Rationale for exemplary 4-member microbial consortium**

[0487] Assembled isolate genomes were annotated with prokka to identify genes involved in alternative carbon utilization and extract the nucleotide sequences. The following genes are included: fructokinase, mannose-6-phosphate isomerase, pullulanase, mannitol-1-

phosphate-5 dehydrogenase. For FBS-003 only fructokinase and mannose-6-phosphate isomerase are present in the genome. **Table 9** summarizes the sequences.

**[0488]** Fructokinase catalyzes the conversion of fructose into a metabolically active state. Mannose-6-phosphate isomerase catalyzes the conversion of mannose-6-phosphate into a metabolically active state. Pullulanase catalyzes the breakdown of glycogen into simpler sugars allowing complex polysaccharides such as glycogen to be used as carbon sources. Mannitol-1-phosphate-5 dehydrogenase catalyzes mannitol-1-phosphate into a metabolically active state.

**[0489]** The FBS003 fructokinase sequence (SEQ ID NO: 9) contains three fewer nucleotides than the other fructokinase sequences (SEQ ID NOs: 11, 15, 19). It is 67.1233% similar to all of the other fructokinase sequences (SEQ ID NOs: 11, 15, 19). All of the other fructokinase sequences (SEQ ID NOs: 11, 15, 19) are 100% identical.

**[0490]** The FBS003 mannose-6-phosphate isomerase sequence (SEQ ID NO: 10) is 63.4476% similar to the FBS004 and FBS006 mannose-6-phosphate isomerase sequences (SEQ ID NOs: 12, 16) which themselves are 100% identical. The FBS003 mannose-6-phosphate isomerase sequence (SEQ ID NO: 10) is 63.5514% similar to the FBS005 mannose-6-phosphate isomerase sequence (SEQ ID NO: 15). The FBS005 mannose-6-phosphate isomerase sequence (SEQ ID NO: 15) is 99.8962% similar to the FBS004 and FBS006 mannose-6-phosphate isomerase sequences (SEQ ID NOs: 12, 16).

**[0491]** The FBS004 pullulanase sequence (SEQ ID NO: 13) contains a 54 nucleotide deletion compared to FBS005 and FBS006 pullulanase sequences (SEQ ID NOs: 17, 25) and is 99.5408% similar to the FBS005 pullulanase sequence (SEQ ID NO: 17) and 99.8649% similar to FBS006 pullulanase sequence (SEQ ID NO: 25). The FBS005 pullulanase sequence (SEQ ID NO: 17) is 99.6805% similar to the FBS006 pullulanase sequence (SEQ ID NO: 21).

**[0492]** The FBS004, FBS005 and FBS006 mannitol-1-phosphate 5-dehydrogenase sequences (SEQ ID NOs: 14, 18, 22) are all 100% identical.

**Table 9.** Summary of sequences.

| SEQ ID NO: | Strain | Gene                          |
|------------|--------|-------------------------------|
| 9          | FBS003 | fructokinase                  |
| 10         | FBS003 | mannose-6-phosphate isomerase |

|    |        |                                      |
|----|--------|--------------------------------------|
| 11 | FBS004 | fructokinase                         |
| 12 | FBS004 | mannose-6-phosphate isomerase        |
| 13 | FBS004 | pullulanase                          |
| 14 | FBS004 | mannitol-1-phosphate 5-dehydrogenase |
| 15 | FBS005 | fructokinase                         |
| 16 | FBS005 | mannose-6-phosphate isomerase        |
| 17 | FBS005 | pullulanase                          |
| 18 | FBS005 | mannitol-1-phosphate 5-dehydrogenase |
| 19 | FBS006 | fructokinase                         |
| 20 | FBS006 | mannose-6-phosphate isomerase        |
| 21 | FBS006 | pullulanase                          |
| 22 | FBS006 | mannitol-1-phosphate 5-dehydrogenase |

#### **Example 12. Rationale for exemplary 4-member microbial consortium**

**[0493]** As demonstrated in the Examples above, the inventors discovered that an exemplary 4-member consortium that utilizes three distinct *L. crispatus* isolates and an *L. jensenii* isolate was capable of thriving in a variety of differing environments relevant to the human vagina (*e.g.*, with respect to pH and carbohydrate substrate). Each of the identified isolates exhibits different characteristics (*e.g.*, with respect to growth, acidification, lactic acid production rates, carbohydrate utilization, *etc.*). In embodiments, the exemplary 4-member consortium improves upon the lowest performer of any particular phenotypic characteristic (see, *e.g.*, the *in vitro* data provided in Example 6).

**[0494]** Collectively, the three *L. crispatus* isolates cover 81% of the pangenome of the *L. crispatus* library constructed from *L. crispatus* isolates from donors from which SCVMP was used for transplantation into a dysbiotic recipient. The selected isolates were derived from donor material with proven engraftment success in such recipients and represent the two most frequently observed compositions in the donors: multiple *L. crispatus* without any or with only very little *L. jensenii* (FBS-004 and FBS-006), and a single *L. crispatus* (FBS-005) that is together with a single *L. jensenii* (FBS-003). Notably, the three donors from which FBS-003 (donor 1), FBS-004 (donor 7), FBS-005 (donor 5), and FBS-006 (donor 7) were derived provided the SCVMPs with the most successful engraftment profiles. Additionally, FBS-004

and FBS-006 were derived from a common donor (donor 7), which is notable because one of these *L. crispatus* isolates is *pulA*+ (FBS-006) and the other is *pulA*- (FBS-004).

**[0495]** The presence of a diverse/high genetic content does not necessarily correlate with functional gene expression, a specific phenotypic trait, or metabolic capabilities. *In vitro* work on the individual isolates of the exemplary 4-member consortium showed that combining the isolates into the exemplary 4-member consortium results in the 4-member consortium performing best with respect to acidification rate, growth rate, and lactic acid production under most conditions (*e.g.*, when compared to the individual bacteria alone or in combinations of two). The four isolates of the 4-member consortium do not average out the performance of one another. Instead, the isolate that performs best in a particular environment might be the primary driver of performance for the 4-member consortium within that particular environment. The data suggest that the individual isolates of the exemplary 4-member consortium function synergistically to produce the optimal growth profile in a particular environment. This may suggest that these isolates in the context of the exemplary 4-member consortium can help one another in varying environments, can occupy different niches, and can create a more robust composition than single or dual member compositions. This may be a consequence of the selection of isolates from a combination of the two most frequently observed donor microbiome phenotypes and from exhibiting different genotypes and phenotypes that create the synergistic effect. This is relevant both because different recipients of the 4-member consortium will have different vaginal environment properties (such as carbon substrate availability), but also within one person the vaginal environment fluctuates in, *e.g.*, pH and carbon substrate availability over the course of, *e.g.*, menstrual cycle and pregnancy.

**[0496]** Isolate FBS-005 was isolated from a successfully engrafting donor where it was co-existing as a single *L. crispatus* isolate with a single *L. jensenii* isolate similar to FBS-003. This suggests that FBS-003 and FBS-005 can coexist and adapt together within the vaginal niche, forming a stable colonization. FBS-004 and FBS-006 come from a donor without *L. jensenii* but with multiple *L. crispatus* isolates, where FBS-004 and FBS-006 were always seen to engraft together after VMT, indicating they benefit from each other in creating a community. As both donor types were sometimes successful in engrafting and sometimes not, it is plausible that combining the two different donors types' isolates could result in a mixture that engrafts efficiently across a wider variety of recipients. The 4 isolates do not appear to stay in equal amounts over time – in some cases a set of two become dominant, with a

different set of two being optimally suited for a specific environment, and the ratios will fluctuate over time, in a similar way as was observed after VMT (**FIG. 5**).

[0497] The above examples indicate that FBS-003 is good at growing fast using simple carbohydrates such as glucose (**FIGs. 16E-F**) and benefits from the hydrolysis of complex carbohydrates such as glycogen by other members of the 4-member consortium to become highly abundant in the first 8h (**FIGs. 16A-B**). FBS-005 is the top-performing isolate in terms of growth on glycogen (**FIGs. 16C-D**). In one hypothesis, they both work well when a 4-member consortium is implanted in the vaginal cavity, where FBS-003 would compete with dysbiotic species for the niche and the use of simple carbohydrates available (which are also broken down by the dysbiotic species) at the start, and with glycogen being low in dysbiotic environments (Mirmonsef et al., 2014). Then, FBS-005 would begin to efficiently hydrolyze glycogen, which becomes more available as dysbiotic species decrease due to *Lactobacillus jensenii*'s competition, to produce glucose monomers and grow, from which FBS-003 would benefit to keep growing. This hypothesis is supported by the *in vitro* data where FBS-003 shows high abundance in the 4-member consortium in this carbohydrate (**FIG. 18C**) despite its inability to efficiently grow on glycogen (**FIGs. 16C-D**). FBS-003 growth slows down once *L. crispatus* becomes very active, as the pH reaches below *Lactobacillus jensenii*'s optimum. FBS-005 would acidify together with FBS-006, which is not highly abundant in the first 8h, but its rapid acidification (best acidifier of all individual isolates on glycogen – **FIG. 17C-D**) indicates that it could be metabolically highly active relative to its abundance, contributing with FBS-005 to fast acidification (**FIG. 17**), lactate production and inhibiting dysbiotic species. Once growth slows down when a low pH is reached, the isolates need to balance growth and acidification with the constant outflow of cervicovaginal secretions to maintain a stable community. To keep this fine balance across the menstrual cycle with all its carbon substrate fluctuations, the capacities of FBS-004 and 006 to maximally utilize other carbon substrate such as mannose, fructose and mannitol could be highly beneficial.

[0498] The diversity in carbon substrate utilization is not only relevant for entering, adapting and colonizing a dysbiotic environment, but also for enabling resilience, *e.g.*, during pregnancy or over the menstrual cycle, in which carbon substrate availability and composition in mucus is known to fluctuate, *e.g.*, due to changes in glycosylation patterns of mucins under the influence of hormones (Vagios and Mitchell, 2021). The isolates exemplified herein are derived from donors that are selected to be very stable *Lactobacillus*-dominated over the menstrual cycle, which is not the case in all women. Many women

exhibit dysbiosis over their cycle (Gajer et al., 2012; Hugerth et al., 2024), which could be because the lactobacilli they have do not have the right composition to be stable, for example in terms of carbon substrate utilization capacities.

**[0499]** The vaginal cavity has a constant flow-through of mucus produced mostly by the cervix, and epithelial cells in the vaginal wall constantly shed and slough off. This creates a constant washout of the lactobacilli, creating a balance between growth and acidification with the washout, which simultaneously keeps supplying the lactobacilli with new carbon substrates and adhesion sites. Lactobacilli are known to adhere less aggressively than dysbiotic species, and hence it is likely that rapid initial growth and a variety of growth rates on different carbon substrates from there on is more important than cell adhesion for assessing engraftment potential.

**[0500]** Altogether, the four isolates in the 4-member consortium have different pH optima and different preferences for and efficiencies in both growth and acidification on different carbon substrates, which means they can adapt as a consortium to many different environments present in different recipients and, *e.g.*, the fluctuating vaginal environment during dysbiosis resolution and over the menstrual cycle and pregnancy.

**[0501]** The *Lactobacillus* isolates of the 4-member consortium (MC-1) described in the Examples herein have been deposited with DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Inhoffenstr. 7B, D-38124 Braunschweig, on October 4, 2023. The aforementioned deposit was made pursuant to the terms of the Budapest treaty on the international recognition of the deposit of microorganisms for the purposes of patent procedures. Table 10 summarizes the DSM Deposit Number associated with each of the isolates of MC-1.

**Table 10.** Summary of Deposit Numbers associated with each of the isolates of MC-1.

| MC-1               |                               |                                |                                |                                |
|--------------------|-------------------------------|--------------------------------|--------------------------------|--------------------------------|
| Isolate            | FBS-003                       | FBS-004                        | FBS-005                        | FBS-006                        |
| Species            | <i>Lactobacillus jensenii</i> | <i>Lactobacillus crispatus</i> | <i>Lactobacillus crispatus</i> | <i>Lactobacillus crispatus</i> |
| DSM Deposit number | DSM 34770                     | DSM 34771                      | DSM 34772                      | DSM 34773                      |

### ADDITIONAL EMBODIMENTS

1. A microbial consortium comprising a defined population of isolated lactobacilli configured to promote engraftment in a female genitourinary tract of a subject, the defined population comprising:
  - a *Lactobacillus jensenii* isolate;
  - a *Lactobacillus crispatus* isolate that thrives in the presence of *Lactobacillus jensenii*;
  - and
  - at least one additional *Lactobacillus crispatus* isolate that thrives independent of the presence of *Lactobacillus jensenii*.
2. The microbial consortium of embodiment 1, wherein the *Lactobacillus crispatus* isolate that thrives independent of the presence of *Lactobacillus jensenii* is capable of engrafting the female genitourinary tract of the subject for at least a pre-determined period of time.
3. The microbial consortium of embodiment 1 or 2, wherein the defined population of isolated lactobacilli comprises:
  - a relative abundance of 15% to 35% of the total viable cells are *Lactobacillus jensenii* bacteria;
  - a relative abundance of 15% to 35% of the total viable cells are *Lactobacillus crispatus* bacteria that thrive in the presence of *Lactobacillus jensenii*; and
  - a relative abundance of 30% to 70% of the total viable cells are additional *Lactobacillus crispatus* bacteria that thrive independent of the presence of *Lactobacillus jensenii*.
4. The microbial consortium of embodiment 1 or 2, wherein the defined population of isolated lactobacilli comprises:
  - a relative abundance of about 25% of the total viable cells are *Lactobacillus jensenii* bacteria;
  - a relative abundance of about 25% of the total viable cells are *Lactobacillus crispatus* bacteria that thrive in the presence of *Lactobacillus jensenii*; and

a relative abundance of about 50% of the total viable cells are additional *Lactobacillus crispatus* bacteria that thrive independent of the presence of *Lactobacillus jensenii*.

5. The microbial consortium of any one of embodiments 1-3, wherein:
  - the *Lactobacillus jensenii* isolate comprises a plurality of *Lactobacillus jensenii* bacteria;
  - the *Lactobacillus crispatus* isolate that thrives in the presence of *Lactobacillus jensenii* comprises a plurality of *Lactobacillus crispatus* bacteria that thrives in the presence of *Lactobacillus jensenii*; and
  - the at least one additional *Lactobacillus crispatus* isolate that thrives independent of the presence of *Lactobacillus jensenii* comprises a plurality of additional *Lactobacillus crispatus* bacteria that thrives independent of the presence of *Lactobacillus jensenii*.
6. A microbial consortium comprising a defined population of isolated lactobacilli configured to promote engraftment in a female genitourinary tract of a subject, the defined population comprising:
  - a *Lactobacillus jensenii* isolate;
  - a *Lactobacillus crispatus* isolate that thrives in the presence of *Lactobacillus jensenii*;
  - and
  - at least two additional *Lactobacillus crispatus* isolate, wherein the at least two additional *Lactobacillus crispatus* isolates have different growth rates, and wherein the at least two additional *Lactobacillus crispatus* isolates thrive independent of the presence of *Lactobacillus jensenii*.
7. The microbial consortium of embodiment 6, wherein
  - a first of the at least two additional *Lactobacillus crispatus* isolates has a growth rate of 0.27-0.29 hours<sup>-1</sup> and a second of the at least two additional *Lactobacillus crispatus* isolates has a growth rate of 0.24-0.26 hours<sup>-1</sup> over a period of 8 hours in culture (starting pH of 4.7), wherein the growth rate is calculated using the following equation: natural logarithm (Ln) of (final OD600 divided by initial OD600) divided by (final time minus initial time); or



a first of the at least two additional *Lactobacillus crispatus* isolates has a growth rate of 0.35-0.37 hours<sup>-1</sup> and a second of the at least two additional *Lactobacillus crispatus* isolates has a growth rate of 0.31-0.33 hours<sup>-1</sup> over a period of 8 hours in culture (starting pH of 5.2), wherein the growth rate is calculated using the following equation: natural logarithm (Ln) of (final OD600 divided by initial OD600) divided by (final time minus initial time).

8. The microbial consortium of embodiment 6 or 7, wherein at least two of the *Lactobacillus crispatus* isolates differ in their growth rates, glycogen utilization rate, rate of producing lactic acid, acidification rate, and/or time to engraftment in the female genitourinary tract.
9. The microbial consortium of any one of embodiments 6-8, wherein the defined population of isolated lactobacilli comprises:
  - a relative abundance of 15% to 35% of the total viable cells are *Lactobacillus jensenii* bacteria;
  - a relative abundance of 15% to 35% of the total viable cells are *Lactobacillus crispatus* bacteria that thrive in the presence of *Lactobacillus jensenii*; and
  - a relative abundance of 15% to 35% of the total viable cells are additional *Lactobacillus crispatus* bacteria.
10. The microbial consortium of embodiment 6, 7, or 8, wherein the defined population of isolated lactobacilli comprises:
  - a relative abundance of about 25% of the total viable cells are *Lactobacillus jensenii* bacteria;
  - a relative abundance of about 25% of the total viable cells are *Lactobacillus crispatus* bacteria that thrive in the presence of *Lactobacillus jensenii*; and
  - a relative abundance of about 50% of the total viable cells are additional *Lactobacillus crispatus* bacteria.
11. The microbial consortium of any one of embodiments 6-9, wherein:
  - the *Lactobacillus jensenii* isolate comprises a plurality of *Lactobacillus jensenii* bacteria;

the *Lactobacillus crispatus* isolate that thrives in the presence of *Lactobacillus jensenii* comprises a plurality of *Lactobacillus crispatus* bacteria that thrive in the presence of *Lactobacillus jensenii*; and  
each of the at least two additional *Lactobacillus crispatus* isolates comprises a plurality of *Lactobacillus crispatus* bacteria.

12. A microbial consortium comprising a defined population of isolated lactobacilli configured to promote engraftment in a female genitourinary tract of a subject, the defined population comprising:

a *Lactobacillus jensenii* isolate;  
a *Lactobacillus crispatus* isolate that thrives in the presence of *Lactobacillus jensenii*;  
and  
at least two additional *Lactobacillus crispatus* isolates, wherein the at least two additional *Lactobacillus crispatus* isolates have different acidification rates, and wherein the at least two additional *Lactobacillus crispatus* isolates thrive independent of the presence of *Lactobacillus jensenii*.

13. The microbial consortium of embodiment 12, wherein  
a first of the at least two additional *Lactobacillus crispatus* isolates has an acidification rate of about  $-0.006 \text{ hours}^{-1}$  and a second of the at least two additional *Lactobacillus crispatus* isolates has an acidification rate of about  $-0.007 \text{ hours}^{-1}$  over a period of 24 hours in culture (starting pH of 4.7), wherein the acidification rate is calculated using the following equation: natural logarithm (Ln) of (final pH divided by initial pH) divided by (final time minus initial time); or  
a first of the at least two additional *Lactobacillus crispatus* isolates has an acidification rate of  $-0.008$  to  $-0.006 \text{ hours}^{-1}$  and a second of the at least two additional *Lactobacillus crispatus* isolates has an acidification rate of  $-0.012$  to  $-0.010 \text{ hours}^{-1}$  over a period of 24 hours in culture (starting pH of 5.2), wherein the acidification rate is calculated using the following equation: natural logarithm (Ln) of (final pH divided by initial pH) divided by (final time minus initial time).

14. The microbial consortium of embodiment 12 or 13, wherein at least two of the *Lactobacillus crispatus* isolates differ in their growth rates, capability of utilizing glycogen, rate of producing lactic acid, and/or time to engraftment in the female genitourinary tract.
15. The microbial consortium of any one of embodiments 12-14, wherein the defined population of isolated lactobacilli comprises:
- a relative abundance of 15% to 35% of the total viable cells are *Lactobacillus jensenii* bacteria;
  - a relative abundance of 15% to 35% of the total viable cells are *Lactobacillus crispatus* bacteria that thrives in the presence of *Lactobacillus jensenii*;
  - a relative abundance of 30% to 70% of the total viable cells are the at least two additional *Lactobacillus crispatus* bacteria.
16. The microbial consortium of embodiment 6 or 8, wherein the defined population of isolated lactobacilli comprises:
- a relative abundance of about 25% of the total viable cells are *Lactobacillus jensenii* bacteria;
  - a relative abundance of about 25% of the total viable cells are *Lactobacillus crispatus* bacteria that thrives in the presence of *Lactobacillus jensenii*; and
  - a relative abundance of about 50% of the total viable cells are *Lactobacillus crispatus* bacteria.
17. The microbial consortium of any one of embodiments 12-15 wherein:
- the *Lactobacillus jensenii* isolate comprises a plurality of *Lactobacillus jensenii* bacteria;
  - the *Lactobacillus crispatus* isolate that thrives in the presence of *Lactobacillus jensenii* comprises a plurality of *Lactobacillus crispatus* bacteria that thrive in the presence of *Lactobacillus jensenii*; and
  - each of the at least two additional *Lactobacillus crispatus* isolates comprises a plurality of *Lactobacillus crispatus* bacteria.
18. The microbial consortium of any one of the preceding embodiments, wherein the *Lactobacillus crispatus* isolate that thrives in the presence of *Lactobacillus jensenii* is capable

of engrafting the female genitourinary tract of the subject for at least a pre-determined period of time in the presence of *Lactobacillus jensenii*.

19. The microbial consortium of any one of the preceding embodiments, wherein each of the lactobacilli are characterized by a genome having a distinct set of single nucleotide polymorphisms (SNPs), a distinct haplotype signature and/or a distinct set of single nucleotide variants (SNV).

20. The microbial consortium of embodiment 19, wherein the distinct set of SNPs define a SNP profile.

21. The microbial consortium of embodiment 19, wherein each *Lactobacillus* comprises a core genome, wherein the distinct set of SNVs is contained within the core genome.

22. The microbial consortium of embodiment 19 or 21, wherein the genome of a *Lactobacilli* comprises a number of SNVs such that the core genome of the *Lactobacilli*, when aligned with a core genome of a reference *Lactobacilli*, has a percent identity greater than a threshold value, the threshold value defining a distinction between two different *Lactobacilli* isolates.

23. The microbial consortium of embodiment 22, wherein the genome of each *Lactobacillus crispatus* isolate comprises a number of SNVs such that the genome of the *Lactobacillus crispatus* isolate, when aligned with a genome of a reference *Lactobacillus crispatus* isolate, has a percent identity greater than or equal to a threshold value, the threshold value defining a distinction between two different *Lactobacillus crispatus* isolates.

24. The microbial consortium of embodiment 22 or 23, wherein the percent identity is greater than or equal to 99.81%.

25. The microbial consortium of embodiment 33 or 23, wherein the percent identity is greater than or equal to 99.90%.

26. The microbial consortium of embodiment 33 or 23, wherein the percent identity is greater than or equal to 99.96%.
27. The microbial consortium of any one of the preceding embodiments, wherein at least two of the lactobacilli differ in their growth rates.
28. The microbial consortium of embodiment 27, wherein the *Lactobacillus jensenii* and at least one *Lactobacillus crispatus* differ in their growth rates.
29. The microbial consortium of embodiment 27 or 28, wherein at least two *Lactobacillus crispatus* differ in their growth rates.
30. The microbial consortium of any one of embodiments 27-29, wherein the growth rates are growth rates *in vitro* (e.g., in culture) and/or *in vivo*.
31. The microbial consortium of any one of the preceding embodiments, wherein the growth rate of the *Lactobacillus jensenii* is 0.32-0.36 hours<sup>-1</sup> over a period of 8 hours in culture (starting pH of 4.7), wherein the growth rate is calculated using the following equation: natural logarithm (Ln) of (final OD600 divided by initial OD600) divided by (final time minus initial time); or 0.41-0.43 hours<sup>-1</sup> over a period of 8 hours in culture (starting pH of 5.2), wherein the growth rate is calculated using the following equation: natural logarithm (Ln) of (final OD600 divided by initial OD600) divided by (final time minus initial time).
32. The microbial consortium of any one of the preceding embodiments, wherein the growth rate of the *Lactobacillus crispatus* isolate that thrives in the presence of *Lactobacillus jensenii* is 0.35-0.41 hours<sup>-1</sup> over a period of 8 hours in culture (starting pH of 4.7), wherein the growth rate is calculated using the following equation: natural logarithm (Ln) of (final OD600 divided by initial OD600) divided by (final time minus initial time); or 0.39-0.41 hours<sup>-1</sup> over a period of 8 hours in culture (starting pH of 5.2), wherein the growth rate is calculated using the following equation: natural logarithm (Ln) of (final OD600 divided by initial OD600) divided by (final time minus initial time).

33. The microbial consortium of any one of the preceding embodiments, wherein the growth rate of a first additional *Lactobacillus crispatus* isolate is 0.27-0.29 hours<sup>-1</sup> over a period of 8 hours in culture (starting pH of 4.7), wherein the growth rate is calculated using the following equation: natural logarithm (Ln) of (final OD600 divided by initial OD600) divided by (final time minus initial time); or 0.35-0.37 hours<sup>-1</sup> over a period of 8 hours in culture (starting pH of 5.2), wherein the growth rate is calculated using the following equation: natural logarithm (Ln) of (final OD600 divided by initial OD600) divided by (final time minus initial time).

34. The microbial consortium of any one of the preceding embodiments, wherein the growth rate of a second additional *Lactobacillus crispatus* isolate is 0.24-0.26 hours<sup>-1</sup> over a period of 8 hours in culture (starting pH of 4.7), wherein the growth rate is calculated using the following equation: natural logarithm (Ln) of (final OD600 divided by initial OD600) divided by (final time minus initial time); or 0.31-0.33 hours<sup>-1</sup> over a period of 8 hours in culture (starting pH of 5.2), wherein the growth rate is calculated using the following equation: natural logarithm (Ln) of (final OD600 divided by initial OD600) divided by (final time minus initial time).

35. The microbial consortium of any one of the preceding embodiments, wherein at least two of the lactobacilli differ in their acidification rates.

36. The microbial consortium of embodiment 35, wherein the *Lactobacillus jensenii* and at least one *Lactobacillus crispatus* differ in their acidification rates.

37. The microbial consortium of embodiment 35 or 36, wherein at least two *Lactobacillus crispatus* differ in their acidification rates.

38. The microbial consortium of any one of embodiments 35-37, wherein the acidification rates are acidification rates *in vitro* (e.g., in culture) and/or *in vivo*.

39. The microbial consortium of any one of the preceding embodiments, wherein the acidification rate of the *Lactobacillus jensenii* is -0.005 to -0.003 over a period of 24 hours in culture (starting pH of 4.7), wherein the acidification rate is calculated using the following

equation: natural logarithm (Ln) of (final pH divided by initial pH) divided by (final time minus initial time); or -0.009 to -0.007 over a period of 24 hours in culture (starting pH of 5.2), wherein the acidification rate is calculated using the following equation: natural logarithm (Ln) of (final pH divided by initial pH) divided by (final time minus initial time).

40. The microbial consortium of any one of the preceding embodiments, wherein the acidification rate of the *Lactobacillus crispatus* isolate that thrives in the presence of *Lactobacillus jensenii* is -0.009 to -0.007 over a period of 24 hours in culture (starting pH of 4.7), wherein the acidification rate is calculated using the following equation: natural logarithm (Ln) of (final pH divided by initial pH) divided by (final time minus initial time); or -0.012 to -0.010 over a period of 24 hours in culture (starting pH of 5.2), wherein the acidification rate is calculated using the following equation: natural logarithm (Ln) of (final pH divided by initial pH) divided by (final time minus initial time).

41. The microbial consortium of any one of the preceding embodiments, wherein the acidification rate of a first additional *Lactobacillus crispatus* isolate is -0.007 to -0.005 hours<sup>-1</sup> over a period of 24 hours in culture (starting pH of 4.7), wherein the acidification rate is calculated using the following equation: natural logarithm (Ln) of (final pH divided by initial pH) divided by (final time minus initial time); or -0.008 to -0.006 hours<sup>-1</sup> over a period of 24 hours in culture (starting pH of 5.2), wherein the acidification rate is calculated using the following equation: natural logarithm (Ln) of (final pH divided by initial pH) divided by (final time minus initial time).

42. The microbial consortium of any one of the preceding embodiments, wherein the acidification rate of a second additional *Lactobacillus crispatus* isolate is -0.008 to -0.006 hours<sup>-1</sup> over a period of 24 hours in culture (starting pH of 4.7), wherein the acidification rate is calculated using the following equation: natural logarithm (Ln) of (final pH divided by initial pH) divided by (final time minus initial time); or -0.012 to -0.010 hours<sup>-1</sup> over a period of 24 hours in culture (starting pH of 5.2), wherein the acidification rate is calculated using the following equation: natural logarithm (Ln) of (final pH divided by initial pH) divided by (final time minus initial time).

43. The microbial consortium of any one of the preceding embodiments, wherein at least two of the lactobacilli differ in their capability of utilizing glycogen.
44. The microbial consortium of embodiment 43, wherein the *Lactobacillus jensenii* and at least one *Lactobacillus crispatus* differ in their capability of utilizing glycogen.
45. The microbial consortium of embodiment 43 or 44, wherein at least two *Lactobacillus crispatus* differ in their capability of utilizing glycogen.
46. The microbial consortium of any one of embodiments 43-45, wherein the capability of utilizing glycogen is a capability of utilizing glycogen *in vitro* (e.g., in culture) and/or *in vivo*.
47. The microbial consortium of any one of the preceding embodiments, wherein at least two of the lactobacilli differ in their rate of producing lactic acid.
48. The microbial consortium of embodiment 47, wherein the *Lactobacillus jensenii* and at least one *Lactobacillus crispatus* differ in their rate of producing lactic acid.
49. The microbial consortium of embodiment 47 or 48, wherein at least two *Lactobacillus crispatus* differ in their rate of producing lactic acid.
50. The microbial consortium of any one of embodiment 47-49, wherein the rate of producing lactic acid is a rate of producing lactic acid *in vitro* (e.g., in culture) and/or *in vivo*.
51. The microbial consortium of any one of the preceding embodiments, wherein at least two of the lactobacilli differ in their time to engraftment in the female genitourinary tract.
52. The microbial consortium of embodiment 51, wherein the *Lactobacillus jensenii* and at least one *Lactobacillus crispatus* differ in their time to engraftment in the female genitourinary tract.



53. The microbial consortium of embodiment 51 or 52, wherein at least two *Lactobacillus crispatus* differ in their time to engraftment in the female genitourinary tract.
54. The microbial consortium of any one of embodiment 51-53, wherein the time to engraftment in the female genitourinary tract is determined *in vivo*.
55. The microbial consortium of any one of the preceding embodiments, wherein at least one of the lactobacilli isolates is capable of engrafting the female genitourinary tract of the subject within 24 hours after administration to the female genitourinary tract of the subject.
56. The microbial consortium of any one of the preceding embodiments, wherein at least one of the lactobacilli isolates is capable of persisting within the female genitourinary tract of the subject for at least 4 days after administration to the female genitourinary tract of the subject.
57. The microbial consortium of embodiment 56, wherein 2, 3 or 4 of the lactobacilli isolates are capable of persisting within the female genitourinary tract of the subject for at least 4, 5, 6, or 7 days after administration to the female genitourinary tract of the subject, optionally wherein 2, 3 or 4 of the lactobacilli isolates are capable of persisting within the female genitourinary tract of the subject for at least 1 menstrual cycle after administration to the female genitourinary tract of the subject.
58. The microbial consortium of any one of the preceding embodiments, wherein the lactobacilli of the consortium:
- are capable of competing with the resident microbiota within the female genitourinary tract of the subject, optionally, without at least partial removal the resident microbiota, *e.g.*, by administration to the genitourinary tract of an antimicrobial agent, such as, *e.g.*, an antibiotic; or
  - are capable of engrafting in the presence of the resident microbiota.

59. The microbial consortium of any one of the preceding embodiments, wherein each of the lactobacilli of the consortium were obtained from a healthy (*e.g.*, *Lactobacillus*-dominated) human donor.
60. The microbial consortium of any one of the preceding embodiments, wherein the lactobacilli comprised in the defined population were obtained from at least two different healthy (*e.g.*, *Lactobacillus*-dominated) human donors, optionally wherein the lactobacilli comprised in the defined population were obtained from three different healthy (*e.g.*, *Lactobacillus*-dominated) human donors.
61. The microbial consortium of any one of the preceding embodiments, wherein the *Lactobacillus jensenii* isolate and the *Lactobacillus crispatus* isolate that thrives in the presence of *Lactobacillus jensenii* were obtained from different healthy (*e.g.*, *Lactobacillus*-dominated) human donors.
62. The microbial consortium of any one of embodiments 6-61, wherein the at least two additional *Lactobacillus crispatus* isolates were obtained from the same healthy (*e.g.*, *Lactobacillus*-dominated) human donor.
63. The microbial consortium of any one of embodiments 6-61, wherein the at least two *Lactobacillus crispatus* isolates thrive independent of the presence of *Lactobacillus jensenii* each of the lactobacilli of the consortium are individually propagated, optionally using a large-scale culture method.
64. A microbial consortium comprising a defined population of isolated lactobacilli configured to promote engraftment in a female genitourinary tract of a subject, the defined population comprising:
- a *Lactobacillus jensenii* isolate; and
  - two or three *Lactobacillus crispatus* isolates, wherein at least two of the two or three *Lactobacillus crispatus* isolates have different growth rates, acidification rates, and/or rates of lactic acid production.

65. A pharmaceutical composition comprising the microbial consortium of any one of embodiments 1-64 and a pharmaceutically acceptable buffer, diluent or excipients.
66. A method of increasing the relative abundance of lactobacilli in the female genitourinary tract of a subject comprising administering the microbial consortium of any one of embodiments 1-65 to the genitourinary tract of the subject.
67. The method of embodiment 66, wherein the female genitourinary tract of the subject comprises a bacterial population having a relative abundance of *Lactobacillus* that is less than 90% (e.g., less than 80%, 70%, 60%, 50%, 40%, 30%, or 20%) of the total bacterial population prior to administration of the microbial consortium.
68. The method of embodiment 66 or 67, wherein the female genitourinary tract of the subject comprises a bacterial population having a relative abundance of *Lactobacillus* that is greater than 70% (e.g., greater than 80%, 85%, 90%, 95%, or 98%) of the total bacterial population following administration of the microbial consortium.
69. The method of embodiment 66 or 67, wherein the relative abundance of *Lactobacillus* in the bacterial population of the female genitourinary tract of the subject is increased by at least 5 percentage points (e.g., at least 10, 15, 20, 25, 30, 40, 50, or 60 percentage points) following administration of the microbial consortium.
70. The microbial consortium of any one of embodiments 1-64 for use in therapy.
71. The microbial consortium of any one of embodiments 1-64 for use in a method of treating dysbiosis of the female genitourinary tract of a subject, said method comprising administering the microbial consortium to the genitourinary tract of the subject, optionally wherein the subject is asymptomatic.
72. The microbial consortium of any one of embodiments 1-64 for use in a method of treating inflammation of the female genitourinary tract of a subject, said method comprising administering the microbial consortium to the genitourinary tract of the subject, optionally wherein the subject is asymptomatic.

73. The microbial consortium for use of embodiment 71 or 72, wherein the female genitourinary tract of the subject comprises a bacterial population having a relative abundance of *Lactobacillus* that is less than 90% (*e.g.*, less than 80%, 70%, 60%, 50%, 40%, 30%, or 20%) of the total bacterial population prior to administration of the microbial consortium.
74. The microbial consortium for use of any one of embodiments 71-73, wherein:  
the female genitourinary tract of the subject comprises a bacterial population having a relative abundance of *Lactobacillus* that is greater than 70% (*e.g.*, greater than 80%, 85%, 90%, 95%, or 98%) of the total bacterial population following administration of the microbial consortium; or  
the relative abundance of *Lactobacillus* in the bacterial population of the female genitourinary tract of the subject is increased by at least 5 percentage points (*e.g.*, at least 10, 15, 20, 25, 30, 40, 50, or 60 percentage points) following administration of the microbial consortium.
75. A method of assembling a microbial consortium comprising two or more isolates belonging to one or more bacterial species, the method comprising:  
obtaining genetic sequencing data obtained from a purported sample of each of the two or more isolates;  
obtaining genetic sequencing data obtained from a reference sample of each of the isolates;  
using at least one computer processor to:  
align at least a core portion of the genetic sequencing data from the purported sample to the genetic sequencing data from the reference sample;  
calculate, using the aligned genetic sequencing data, a percent identity value describing a percent of the aligned genetic sequencing data that is identical; and  
determine that the purported sample and the reference sample comprise the same set of bacteria by determining that the percent identity value is greater than or equal to a threshold value defining a distinction between different isolates of the same bacterial species; and  
assembling the microbial consortium using the purported sample.

76. The method of embodiment 75, further comprising determining the threshold value by:
- obtaining genetic sequencing data obtained from two or more samples of each of the two or more isolates;
  - aligning, using at least one computer processor, at least a core portion of the obtained genetic sequencing data for pairs of the two or more isolates;
  - calculating, using the at least one computer processor, a percent identity between each of the aligned pairs; and
  - determining the threshold value by determining, using the at least one computer processor, a largest value of the calculated percent identities.
77. The method of embodiment 75 or 76, wherein the bacterial species comprises a species of *Lactobacilli*.
78. The method of any preceding embodiment, wherein the bacterial species comprises *Lactobacillus crispatus*.
79. The method of any preceding embodiment, wherein determining that the purported sample and the reference sample comprise the same set of bacteria comprises determining that the percent identity is greater than or equal to a threshold value of 99.81%.
80. The method of any preceding embodiment, wherein determining that the purported sample and the reference sample comprise the same set of bacteria comprises determining that the percent identity is greater than or equal to a threshold value of 99.90%.
81. The method of any preceding embodiment, wherein determining that the purported sample and the reference sample comprise the same set of bacteria comprises determining that the percent identity is greater than or equal to a threshold value of 99.96%.
82. A microbial consortium comprising:
- (i) a *Lactobacillus jensenii* isolate and
  - (ii) at least two phenotypically distinct *Lactobacillus crispatus* isolates,

wherein

the *Lactobacillus jensenii* isolate originates from one donor;

a first of the at least two *Lactobacillus crispatus* isolates originates from a second donor; and

a second of the at least two *Lactobacillus crispatus* isolates originates from a third donor.

83. A microbial consortium comprising:

(i) a *Lactobacillus jensenii* isolate and

(ii) at least three phenotypically distinct *Lactobacillus crispatus* isolates,

wherein

the *Lactobacillus jensenii* isolate originates from one donor;

a first of the at least three *Lactobacillus crispatus* isolates originates from a second donor; and

a second and a third of the at least three *Lactobacillus crispatus* isolates originate from a third donor.

84. A microbial consortium comprising a defined population of isolated lactobacilli configured to promote engraftment in a female genitourinary tract of a subject, the defined population comprising:

(i) a *Lactobacillus jensenii* isolate and

(ii) at least one *Lactobacillus crispatus* isolate.

85. In another embodiment, the specification provides a microbial consortium, comprising:

(i) a *Lactobacillus jensenii* isolate and

(ii) at least two phenotypically distinct *Lactobacillus crispatus* isolates,

wherein each of the at least two phenotypically distinct *Lactobacillus crispatus* isolates independently have different growth rates and different acidification rates during logarithmic growth phase on media comprising either mannitol or glucose as carbon substrates.

86. In yet another embodiment, the disclosure provides a microbial consortium, comprising:

- (i) a *Lactobacillus jensenii* isolate and
- (ii) at least two phenotypically distinct *Lactobacillus crispatus* isolates, wherein a first *Lactobacillus crispatus* isolate has a higher growth rate during logarithmic growth phase on glucose than a second *Lactobacillus crispatus* isolate, but approximately the same growth rate on mannitol, and wherein the first *Lactobacillus crispatus* isolate has a higher acidification rate during logarithmic growth phase on glucose than the second *Lactobacillus crispatus* isolate, but a lower acidification rate during logarithmic growth on mannitol.

87. In another embodiment, the disclosure provides a microbial consortium, comprising:

- (i) a *Lactobacillus jensenii* isolate and
- (ii) at least three phenotypically distinct *Lactobacillus crispatus* isolates, wherein the acidification rate during logarithmic growth phase on glucose of a first of the least three phenotypically distinct *Lactobacillus crispatus* isolates is in the range of  $-0.00475$  to  $-0.00544$  hours<sup>-1</sup>, a second of the least three phenotypically distinct *Lactobacillus crispatus* isolates is in the range of  $-0.00572$  to  $-0.00727$  hours<sup>-1</sup>, and a third of the least three phenotypically distinct *Lactobacillus crispatus* isolates is in the range of  $-0.00489$  to  $-0.00566$  hours<sup>-1</sup>, wherein the acidification rate is determined for each isolate according to the steps of Table 8.

88. In still another embodiment, the disclosure provides a microbial consortium, comprising:

- (i) a *Lactobacillus jensenii* isolate and
- (ii) at least three phenotypically distinct *Lactobacillus crispatus* isolates, wherein the acidification rate during logarithmic growth phase on mannitol of a first of the least three phenotypically distinct *Lactobacillus crispatus* isolates is in the range of  $-0.00502$  to  $-0.00536$  hours<sup>-1</sup>, a second of the least three phenotypically distinct *Lactobacillus crispatus* isolates is in the range of  $-0.00152$  to  $-0.00154$  hours<sup>-1</sup>, and a third of the least three phenotypically distinct *Lactobacillus crispatus* isolates is in the range of  $-0.00571$  to  $-0.0062$  hours<sup>-1</sup>,

wherein the acidification rate is determined for each isolate according to the steps of Table 8.

89. In other embodiments, the disclosure provides a microbial consortium, comprising:

(i) a *Lactobacillus jensenii* isolate and

(ii) at least three phenotypically distinct *Lactobacillus crispatus* isolates,

wherein the growth rate during logarithmic growth phase on glucose of a first of the least three phenotypically distinct *Lactobacillus crispatus* isolates is in the range of 0.13516 to 0.174545 hours<sup>-1</sup>, a second of the least three phenotypically distinct *Lactobacillus crispatus* isolates is in the range of 0.188003 to 0.201931 hours<sup>-1</sup>, and a third of the least three phenotypically distinct *Lactobacillus crispatus* isolates is in the range of 0.161753 to 0.177467 hours<sup>-1</sup>,

wherein the growth rate is determined for each isolate according to the steps of Table 7.

90. In still other embodiments, the disclosure provides a microbial consortium, comprising:

(i) a *Lactobacillus jensenii* isolate and

(ii) at least three phenotypically distinct *Lactobacillus crispatus* isolates,

wherein the growth rate during logarithmic growth phase on mannitol of a first of the least three phenotypically distinct *Lactobacillus crispatus* isolates is in the range of 0.159734 to 0.1701789 hours<sup>-1</sup>, a second of the least three phenotypically distinct *Lactobacillus crispatus* isolates is in the range of 0.161946 to 0.169543 hours<sup>-1</sup>, and a third of the least three phenotypically distinct *Lactobacillus crispatus* isolates is in the range of 0.157928 to 0.1639561 hours<sup>-1</sup>,

wherein the growth rate is determined for each isolate according to the steps of Table 7.

91. A microbial consortium, comprising:

(i) a *Lactobacillus jensenii* isolate and

(ii) at least one *Lactobacillus crispatus* isolate.

92. A microbial consortium, comprising:

(i) a *Lactobacillus jensenii* isolate and



- (ii) at least one *Lactobacillus crispatus* isolate, wherein the microbial consortium does not contain any detectable levels of other *Lactobacillus* species.
93. A microbial consortium, comprising:
- (i) a *Lactobacillus jensenii* isolate and
  - (ii) at least two phylogenetically distinct *Lactobacillus crispatus* isolates.
94. A microbial consortium, comprising:
- (i) a *Lactobacillus jensenii* isolate and
  - (ii) at least two phylogenetically distinct *Lactobacillus crispatus* isolates, wherein the microbial consortium does not contain detectable levels of any other *Lactobacillus* species.
95. A microbial consortium, comprising:
- (i) a *Lactobacillus jensenii* isolate and
  - (ii) at least three phylogenetically distinct *Lactobacillus crispatus* isolates.
96. A microbial consortium, comprising:
- (i) a *Lactobacillus jensenii* isolate and
  - (ii) at least three phylogenetically distinct *Lactobacillus crispatus* isolates, wherein the microbial consortium does not contain detectable levels of any other *Lactobacillus* species.
97. A microbial consortium comprising:
- (i) a *Lactobacillus jensenii* isolate and
  - (ii) at least two phenotypically distinct *Lactobacillus crispatus* isolates, wherein, the consortium does not contain detectable levels of *Lactobacillus acidophilus*; *Lactobacillus rhamnosus*; *Lactobacillus gasseri*; and/or *Lactobacillus iners*.
98. The microbial consortium of any of the preceding embodiments wherein the *Lactobacillus* isolates are selected from:
- (a) a *Lactobacillus jensenii* isolate obtained from a bacterial sample deposited as DSM Deposit Number 34770;

(b) a *Lactobacillus crispatus* isolate obtained from a bacterial sample deposited as DSM Deposit Number 34772;

(c) a *Lactobacillus crispatus* isolate obtained from a bacterial sample deposited as DSM Deposit Number 34771, and

(d) a *Lactobacillus crispatus* isolate obtained from a bacterial sample deposited as DSM Deposit Number 34773.

**EQUIVALENTS, SCOPE, AND INCORPORATION BY REFERENCE**

**[0502]** Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. The scope of the present invention is not intended to be limited to the above description, but rather is as set forth in the appended claims.

**[0503]** In the claims articles such as “a,” “an,” and “the” may mean one or more than one unless indicated to the contrary or otherwise evident from the context. Claims or descriptions that include “or” between one or more members of a group are considered satisfied if one, more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process unless indicated to the contrary or otherwise evident from the context. The invention includes embodiments in which exactly one member of the group is present in, employed in, or otherwise relevant to a given product or process. The invention also includes embodiments in which more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process.

**[0504]** Furthermore, it is to be understood that the invention encompasses all variations, combinations, and permutations in which one or more limitations, elements, clauses, descriptive terms, *etc.*, from one or more of the claims or from relevant portions of the description is introduced into another claim. For example, any claim that is dependent on another claim can be modified to include one or more limitations found in any other claim that is dependent on the same base claim. Furthermore, where the claims recite a composition, it is to be understood that methods of using the composition for any of the purposes disclosed herein are included, and methods of making the composition according to any of the methods of making disclosed herein or other methods known in the art are included, unless otherwise indicated or unless it would be evident to one of ordinary skill in the art that a contradiction or inconsistency would arise.

**[0505]** Where elements are presented as lists, *e.g.*, in Markush group format, it is to be understood that each subgroup of the elements is also disclosed, and any element(s) can be removed from the group. It should be understood that, in general, where the invention, or aspects of the invention, is/are referred to as comprising particular elements, features, steps, *etc.*, certain embodiments of the invention or aspects of the invention consist, or consist essentially of, such elements, features, steps, *etc.* For purposes of simplicity those embodiments have not been specifically set forth *in haec verba* herein. Thus, for each embodiment of the invention that comprises one or more elements, features, steps, *etc.*, the

invention also provides embodiments that consist or consist essentially of those elements, features, steps, *etc.*

**[0506]** Where ranges are given, endpoints are included. Furthermore, it is to be understood that unless otherwise indicated or otherwise evident from the context and/or the understanding of one of ordinary skill in the art, values that are expressed as ranges can assume any specific value within the stated ranges in different embodiments of the invention, to the tenth of the unit of the lower limit of the range, unless the context clearly dictates otherwise. It is also to be understood that unless otherwise indicated or otherwise evident from the context and/or the understanding of one of ordinary skill in the art, values expressed as ranges can assume any subrange within the given range, wherein the endpoints of the subrange are expressed to the same degree of accuracy as the tenth of the unit of the lower limit of the range.

**[0507]** In addition, it is to be understood that any particular embodiment of the present invention may be explicitly excluded from any one or more of the claims. Where ranges are given, any value within the range may explicitly be excluded from any one or more of the claims. Any embodiment, element, feature, application, or aspect of the compositions and/or methods of the invention, can be excluded from any one or more claims. For purposes of brevity, all of the embodiments in which one or more elements, features, purposes, or aspects is excluded are not set forth explicitly herein.

**[0508]** All patents, patent applications, and publications identified are expressly incorporated herein by reference for the purpose of describing and disclosing, for example, the methodologies described in such publications that might be used in connection with the present invention.

What is claimed is:

1. A microbial consortium comprising a defined population of isolated lactobacilli, the defined population comprising:
  - (a) a *Lactobacillus jensenii* isolate obtained from a bacterial sample deposited as DSM Deposit Number 34770;
  - (b) a *Lactobacillus crispatus* isolate obtained from a bacterial sample deposited as DSM Deposit Number 34772;
  - (c) a *Lactobacillus crispatus* isolate obtained from a bacterial sample deposited as DSM Deposit Number 34771, and
  - (d) a *Lactobacillus crispatus* isolate obtained from a bacterial sample deposited as DSM Deposit Number 34773.
2. A microbial consortium comprising a defined population of isolated lactobacilli, the defined population comprising:
  - (a) a *Lactobacillus jensenii* isolate having a genome that, when aligned with the genome of a bacterial sample deposited as DSM 34770, has a percent identity greater than or equal to a threshold value;
  - (b) a *Lactobacillus crispatus* isolate having a genome that, when aligned with the genome of a bacterial sample deposited as DSM 34771, has a percent identity greater than or equal to a threshold value;
  - (c) a *Lactobacillus crispatus* isolate having a genome that, when aligned with the genome of a bacterial sample deposited as DSM 34772, has a percent identity greater than or equal to a threshold value; and

(d) a *Lactobacillus crispatus* isolate having a genome that, when aligned with the genome of a bacterial sample deposited as DSM 34773, has a percent identity greater than or equal to a threshold value.

3. The microbial consortium of claim 2, wherein the threshold value for (a), (b), (c), and/or (d) is 99.81%.

4. The microbial consortium of claim 2, wherein the threshold value for (a), (b), (c), and/or (d) is 99.90%.

5. The microbial consortium of claim 2, wherein the threshold value for (a), (b), (c), and/or (d) is 99.96%.

6. The microbial consortium of any one of the preceding claims, wherein the isolate of (a) is derived or propagated from a bacterial sample deposited as DSM 34770.

7. The microbial consortium of any one of the preceding claims, wherein the isolate of (b) is derived or propagated from a bacterial sample deposited as DSM 34771.

8. The microbial consortium of any one of the preceding claims, wherein the isolate of (c) is derived or propagated from a bacterial sample deposited as DSM 34772.

9. The microbial consortium of any one of the preceding claims, wherein the isolate of (d) is derived or propagated from a bacterial sample deposited as DSM 34773.

10. The microbial consortium of any one of the preceding claims, wherein the defined population is configured to promote engraftment in a female genitourinary tract of a subject.

11. The microbial consortium of claim 10, wherein at least one of the lactobacilli are capable of engrafting the female genitourinary tract of the subject for at least a pre-determined period of time.

12. The microbial consortium of any one of the preceding claims, wherein the defined population of isolated lactobacilli comprises:

a relative abundance of 15% to 35% of the total viable cells are bacteria of the *Lactobacillus jensenii* isolate of (a);

a relative abundance of 15% to 35% of the total viable cells are bacteria of the *Lactobacillus crispatus* isolate of (b);

a relative abundance of 15% to 35% of the total viable cells are bacteria of the *Lactobacillus crispatus* isolate of (c); and

a relative abundance of 15% to 35% of the total viable cells are bacteria of the *Lactobacillus crispatus* isolate of (d).

13. The microbial consortium of any one of the preceding claims, wherein the defined population of isolated lactobacilli comprises:

a relative abundance of about 25% of the total viable cells are bacteria of the *Lactobacillus jensenii* isolate of (a);

a relative abundance of about 25% of the total viable cells are bacteria of the *Lactobacillus crispatus* isolate of (b);

a relative abundance of about 25% of the total viable cells are bacteria of the *Lactobacillus crispatus* isolate of (c); and

a relative abundance of about 25% of the total viable cells are bacteria of the *Lactobacillus crispatus* isolate of (d).

14. The microbial consortium of any one of the preceding claims, wherein:

the *Lactobacillus jensenii* isolate of (a) comprise a plurality of *Lactobacillus jensenii* bacteria;

the *Lactobacillus crispatus* isolate of (b) comprise a plurality of *Lactobacillus crispatus* bacteria;

the *Lactobacillus crispatus* isolate of (c) comprise a plurality of *Lactobacillus crispatus* bacteria; and

the *Lactobacillus crispatus* isolate of (d) comprise a plurality of *Lactobacillus crispatus* bacteria.

15. The microbial consortium of any one of the preceding claims, wherein the *Lactobacillus crispatus* isolate of (b) thrive in the presence of *Lactobacillus jensenii* bacteria.

16. The microbial consortium of any one of the preceding claims, wherein the *Lactobacillus crispatus* isolate of (c) and/or (d) thrive independent of the presence of *Lactobacillus jensenii* bacteria.

17. The microbial consortium of any one of the preceding claims, wherein at least two of the lactobacilli differ in their growth rates.

18. The microbial consortium of claim 17, wherein the *Lactobacillus jensenii* and at least one *Lactobacillus crispatus* differ in their growth rates.

19. The microbial consortium of claim 17 or 18, wherein at least two *Lactobacillus crispatus* differ in their growth rates.

20. The microbial consortium of any one of claims 17-19, wherein the growth rates are growth rates *in vitro* (e.g., in culture) and/or *in vivo*.



21. The microbial consortium of any one of the preceding claims, wherein the growth rate of the *Lactobacillus jensenii* is 0.32-0.36 hours<sup>-1</sup> at starting pH of 4.7 or 0.41-0.43 hours<sup>-1</sup> at starting pH of 5.2.
22. The microbial consortium of any one of the preceding claims, wherein the growth rate of the *Lactobacillus crispatus* isolate of (b) is 0.35-0.41 hours<sup>-1</sup> at starting pH of 4.7 or 0.39-0.41 hours<sup>-1</sup> at starting pH of 5.2.
23. The microbial consortium of any one of the preceding claims, wherein the growth rate of the *Lactobacillus crispatus* isolate of (c) is 0.27-0.29 hours<sup>-1</sup> at a starting pH of 4.7 or 0.35-0.37 hours<sup>-1</sup> at a starting pH of 5.2.
24. The microbial consortium of any one of the preceding claims, wherein the growth rate of the *Lactobacillus crispatus* isolate of (d) is 0.24-0.26 hours<sup>-1</sup> at a starting pH of 4.7 or 0.31-0.33 hours<sup>-1</sup> at a starting pH of 5.2.
25. The microbial consortium of any one of the preceding claims, wherein at least two of the lactobacilli differ in their acidification rates.
26. The microbial consortium of claim 25, wherein the *Lactobacillus jensenii* and at least one *Lactobacillus crispatus* differ in their acidification rates.
27. The microbial consortium of claim 25 or 26, wherein at least two *Lactobacillus crispatus* differ in their acidification rates.
28. The microbial consortium of any one of claims 25-27, wherein the acidification rates are acidification rates *in vitro* (e.g., in culture) and/or *in vivo*.

29. The microbial consortium of any one of the preceding claims, wherein the acidification rate of the *Lactobacillus jensenii* is -0.005 to -0.003 at a starting pH of 4.7 or -0.009 to -0.007 at a starting pH of 5.2.
30. The microbial consortium of any one of the preceding claims, wherein the acidification rate of the *Lactobacillus crispatus* isolate of (b) is -0.009 to -0.007 at a starting pH of 4.7 or -0.012 to -0.010 at a starting pH of 5.2.
31. The microbial consortium of any one of the preceding claims, wherein the acidification rate of the *Lactobacillus crispatus* isolate of (c) is -0.007 to -0.005 hours<sup>-1</sup> at a starting pH of 4.7 or -0.008 to -0.006 hours<sup>-1</sup> at a starting pH of 5.2.
32. The microbial consortium of any one of the preceding claims, wherein the acidification rate of the *Lactobacillus crispatus* isolate of (d) is -0.008 to -0.006 hours<sup>-1</sup> at a starting pH of 4.7 or -0.012 to -0.010 hours<sup>-1</sup> at a starting pH of 5.2.
33. The microbial consortium of any one of the preceding claims, wherein at least two of the lactobacilli differ in their capability of degrading glycogen.
34. The microbial consortium of claim 33, wherein the *Lactobacillus jensenii* and at least one *Lactobacillus crispatus* differ in their capability of degrading glycogen.
35. The microbial consortium of claim 33 or 34, wherein at least two *Lactobacillus crispatus* differ in their capability of degrading glycogen.
36. The microbial consortium of any one of claims 33-35, wherein the capability of degrading glycogen is a capability of degrading glycogen *in vitro* (e.g., in culture) and/or *in vivo*.

37. The microbial consortium of any one of the preceding claims, wherein at least two of the lactobacilli differ in their rate of producing lactate.
38. The microbial consortium of claim 37, wherein the *Lactobacillus jensenii* and at least one *Lactobacillus crispatus* differ in their rate of producing lactate.
39. The microbial consortium of claim 37 or 38, wherein at least two *Lactobacillus crispatus* differ in their rate of producing lactate.
40. The microbial consortium of any one of claims 37-39, wherein the rate of producing lactate is a rate of producing lactate *in vitro* (e.g., in culture) and/or *in vivo*.
41. The microbial consortium of any one of the preceding claims, wherein at least two of the lactobacilli differ in their rate of engraftment in the female genitourinary tract.
42. The microbial consortium of claim 41, wherein the *Lactobacillus jensenii* and at least one *Lactobacillus crispatus* differ in their rate of engraftment in the female genitourinary tract.
43. The microbial consortium of claim 41 or 42, wherein at least two *Lactobacillus crispatus* differ in their rate of engraftment in the female genitourinary tract.
44. The microbial consortium of any one of claims 41-43, wherein the rate of engraftment in the female genitourinary tract is determined *in vivo*.
45. The microbial consortium of any one of the preceding claims, wherein at least one of the lactobacilli bacteria is capable of engrafting the female genitourinary tract of the subject within 24 hours after administration to the female genitourinary tract of the subject.

46. The microbial consortium of any one of the preceding claims, wherein at least one of the lactobacilli bacteria is capable of persisting within the female genitourinary tract of the subject for at least 4 days after administration to the female genitourinary tract of the subject.

47. The microbial consortium of claim 46, wherein 2, 3 or 4 of the lactobacilli bacteria is capable of persisting within the female genitourinary tract of the subject for at least 4 days after administration to the female genitourinary tract of the subject.

48. The microbial consortium of any one of the preceding claims, wherein the lactobacilli of the consortium are capable of competing with the resident microbiota within the female genitourinary tract of the subject.

49. The microbial consortium of any one of the preceding claims, wherein each of the lactobacilli of the consortium were obtained from a healthy human donor.

50. The microbial consortium of any one of the preceding claims, wherein the lactobacilli comprised in the defined population were obtained from at least two different healthy human donors.

51. The microbial consortium of any one of the preceding claims, wherein the lactobacilli comprised in the defined population were obtained from three different healthy human donors.

52. The microbial consortium of any one of the preceding claims, wherein the *Lactobacillus jensenii* isolate and the *Lactobacillus crispatus* isolate that thrives in the presence of the *Lactobacillus jensenii* bacteria were obtained from different healthy human donors.

53. The microbial consortium of any one of the preceding claims, wherein the *Lactobacillus crispatus* isolate of (c) and the *Lactobacillus crispatus* isolate of (d) were obtained from the same healthy human donor.
54. A method of increasing the relative abundance of lactobacilli in the female genitourinary tract of a subject comprising administering the microbial consortium of any one of claims 1-53 to the genitourinary tract of the subject.
55. The method of claim 54, wherein the female genitourinary tract of the subject comprises a bacterial population having a relative abundance of *Lactobacillus* that is less than 90% (*e.g.*, less than 80%, 70%, 60%, 50%, 40%, 30%, or 20%) of the total bacterial population prior to administration of the microbial consortium.
56. The method of claim 54 or 55, wherein the female genitourinary tract of the subject comprises a bacterial population having a relative abundance of *Lactobacillus* that is greater than 70% (*e.g.*, greater than 80%, 85%, 90%, 95%, or 98%) of the total bacterial population following administration of the microbial consortium.
57. The method of claim 54 or 55, wherein the relative abundance of *Lactobacillus* in the bacterial population of the female genitourinary tract of the subject is increased by at least 5 percentage points (*e.g.*, at least 10, 15, 20, 25, 30, 40, 50, or 60 percentage points) following administration of the microbial consortium.
58. The microbial consortium of any one of claims 1-53 for use in therapy.
59. The microbial consortium of any one of claims 1-53 for use in a method of treating dysbiosis of the female genitourinary tract of a subject, said method comprising administering

the microbial consortium to the genitourinary tract of the subject, optionally wherein the subject is asymptomatic.

60. The microbial consortium for use of claim 59, wherein the female genitourinary tract of the subject comprises a bacterial population having a relative abundance of *Lactobacillus* that is less than 90% (e.g., less than 80%, 70%, 60%, 50%, 40%, 30%, or 20%) of the total bacterial population prior to administration of the microbial consortium.

61. The microbial consortium for use of claim 59 or 60, wherein the female genitourinary tract of the subject comprises a bacterial population having a relative abundance of *Lactobacillus* that is greater than 70% (e.g., greater than 80%, 85%, 90%, 95%, or 98%) of the total bacterial population following administration of the microbial consortium.

62. The microbial consortium for use of claim 59 or 60, wherein the relative abundance of *Lactobacillus* in the bacterial population of the female genitourinary tract of the subject is increased by at least 5 percentage points (e.g., at least 10, 15, 20, 25, 30, 40, 50, or 60 percentage points) following administration of the microbial consortium.

63. A composition comprising *Lactobacillus jensenii* isolate obtained from a bacterial sample deposited as DSM Deposit Number 34770.

64. A composition comprising *Lactobacillus crispatus* isolate obtained from a bacterial sample deposited as DSM Deposit Number 34772.

65. A composition comprising *Lactobacillus crispatus* isolate obtained from a bacterial sample deposited as DSM Deposit Number 34771.

66. A composition comprising *Lactobacillus crispatus* isolate obtained from a bacterial sample deposited as DSM Deposit Number 34773.
67. A composition comprising two or more *Lactobacillus* bacteria selected from the group consisting of:
- (a) a *Lactobacillus jensenii* isolate obtained from a bacterial sample deposited as DSM Deposit Number 34770;
  - (b) a *Lactobacillus crispatus* isolate obtained from a bacterial sample deposited as DSM Deposit Number 34772;
  - (c) a *Lactobacillus crispatus* isolate obtained from a bacterial sample deposited as DSM Deposit Number 34771, and
  - (d) a *Lactobacillus crispatus* isolate obtained from a bacterial sample deposited as DSM Deposit Number 34773.
68. A composition comprising *Lactobacillus jensenii* isolate having a genome that, when aligned with the genome of a bacterial sample deposited as DSM 34770, has a percent identity greater than or equal to a threshold value.
69. A composition comprising *Lactobacillus crispatus* isolate having a genome that, when aligned with the genome of a bacterial sample deposited as DSM 34771, has a percent identity greater than or equal to a threshold value.
70. A composition comprising *Lactobacillus crispatus* isolate having a genome that, when aligned with the genome of a bacterial sample deposited as DSM 34772, has a percent identity greater than or equal to a threshold value.

71. A composition comprising *Lactobacillus crispatus* isolate having a genome that, when aligned with the genome of a bacterial sample deposited as DSM 34773, has a percent identity greater than or equal to a threshold value.
72. A composition comprising two or more *Lactobacillus* bacteria selected from the group consisting of:
- (a) *Lactobacillus jensenii* isolate having a genome that, when aligned with the genome of a bacterial sample deposited as DSM 34770, has a percent identity greater than or equal to a threshold value;
  - (b) *Lactobacillus crispatus* isolate having a genome that, when aligned with the genome of a bacterial sample deposited as DSM 34771, has a percent identity greater than or equal to a threshold value;
  - (c) *Lactobacillus crispatus* isolate having a genome that, when aligned with the genome of a bacterial sample deposited as DSM 34772, has a percent identity greater than or equal to a threshold value; and
  - (d) *Lactobacillus crispatus* isolate having a genome that, when aligned with the genome of a bacterial sample deposited as DSM 34773, has a percent identity greater than or equal to a threshold value.
73. The composition of any one of claims 68-72, wherein the threshold value is 99.81%, 99.90%, or 99.96%.
74. A pharmaceutical composition comprising the microbial consortium of any one of claims 1-53 or the composition of any one of claims 63-73 and a pharmaceutically acceptable buffer, diluent or excipients.
75. A method of increasing the relative abundance of lactobacilli in the female genitourinary tract of a subject comprising administering the microbial consortium of any one of claims 1-53,



the composition of any one of claims 63-73, or the pharmaceutical composition of claim 74 to the genitourinary tract of the subject.

76. The method of claim 75, wherein the female genitourinary tract of the subject comprises a bacterial population having a relative abundance of *Lactobacillus* that is less than 90% (e.g., less than 80%, 70%, 60%, 50%, 40%, 30%, or 20%) of the total bacterial population prior to administration of the microbial consortium.

77. The method of claims 75 or 76, wherein the female genitourinary tract of the subject comprises a bacterial population having a relative abundance of *Lactobacillus* that is greater than 70% (e.g., greater than 80%, 85%, 90%, 95%, or 98%) of the total bacterial population following administration of the microbial consortium.

78. The method of any one of claims 75-77, wherein the relative abundance of *Lactobacillus* in the bacterial population of the female genitourinary tract of the subject is increased by at least 5 percentage points (e.g., at least 10, 15, 20, 25, 30, 40, 50, or 60 percentage points) following administration of the microbial consortium.

79. The microbial consortium of any one of claims 1-53, the composition of any one of claims 63-73, or the pharmaceutical composition of claim 74 for use in therapy.

80. The microbial consortium of any one of claims 1-53, the composition of any one of claims 63-73, or the pharmaceutical composition of claim 74 for use in a method of treating dysbiosis of the female genitourinary tract of a subject, said method comprising administering the microbial consortium to the genitourinary tract of the subject, optionally wherein the subject is asymptomatic.

81. The microbial consortium of any one of claims 1-53, the composition of any one of claims 63-73, or the pharmaceutical composition of claim 74 for use in a method of treating inflammation of the female genitourinary tract of a subject, said method comprising administering the microbial consortium to the genitourinary tract of the subject, optionally wherein the subject is asymptomatic.

82. The microbial consortium, the composition, or the pharmaceutical composition for use of claims 80 or 81, wherein the female genitourinary tract of the subject comprises a bacterial population having a relative abundance of *Lactobacillus* that is less than 90% (*e.g.*, less than 80%, 70%, 60%, 50%, 40%, 30%, or 20%) of the total bacterial population prior to administration of the microbial consortium.

83. The microbial consortium, the composition, or the pharmaceutical composition for use of any one of claims 80-82, wherein:

the female genitourinary tract of the subject comprises a bacterial population having a relative abundance of *Lactobacillus* that is greater than 70% (*e.g.*, greater than 80%, 85%, 90%, 95%, or 98%) of the total bacterial population following administration of the microbial consortium; or

the relative abundance of *Lactobacillus* in the bacterial population of the female genitourinary tract of the subject is increased by at least 5 percentage points (*e.g.*, at least 10, 15, 20, 25, 30, 40, 50, or 60 percentage points) following administration of the microbial consortium.