Many vaginal and systemic medications may kill vaginal lactic acid bacteria. Hence, treatment of infections with antibiotics may place women at increased risk for repeated acquisition of the infection or even development of antibiotic resistant infections.
Lactic acid bacteria for intravaginal or oral use have been available for over 50 years in the form of probiotic preparations available in health food stores, and acidophilus milk or yogurt in grocery stores. These products have included vaginal suppositories containing lyophilized *Lactobacillus spp*. These products have been largely non-efficacious in treating infections due to the failure of the products to colonize the vagina with the exogenous *Lactobacilli*, prevent biofilm formation of pathogens or to a lack of antimicrobial activity in vivo. Commercially available *Lactobacillus* has been shown to be unable to prevent biofilm formation of pathogens nor do the commercial *Lactobacillus* have antimicrobial activity against the persistent *G. vaginalis*.

Most commercially available probiotic strains are not systematically selected for their antimicrobial activity but chosen due to their ability to produce acids and to be upscaled (Kwak, Y-K. et al. 2017).

The ability of *G. vaginalis* to form biofilms contributes to the high rates of recurrence that are typical for BV and which unfortunately make repeated antibiotic therapy inevitable.

Hence lactic acid bacteria which are able to prevent, inhibit or reduce the development of biofilm by *G. vaginalis* would be advantageous.

Further there is a need for lactic acid bacteria for the treatment of vaginal infections wherein the lactic acid bacteria maintain the antimicrobial activity, ability to acidify the vagina and prevent biofilm formation of *G. vaginosis* and/or *Candida spp*.

The present invention relates to new isolated bacteria selected for their ability to specifically out compete the pathogenic microorganisms in the vagina and thereby re-establish a healthy microbiota. Further the new isolated bacteria are selected for their targeted antimicrobial activity towards *G. vaginalis* and/ or *Candida spp*.

Summary

Thus, an object of the present disclosure relates to new lactic acid bacteria and their use in topical compositions, suited for the use in gynaecology and urology. Thus, an object of the present disclosure relates to new isolated lactic acid bacteria which are able to either treat

or prevent vaginal or urogenital infection, reduce recurrent infections, reduce STIs, improve fertility and/or reduce premature birth.

In particular, it is an object of the present disclosure to provide new strains that solves the above-mentioned problems of the prior art with the ability to targeted control pathogens and thereby prevent infection.

The present disclosure relates to strains and compositions comprising one or more of the following strains *Lactobacillus crispatus* LB714R deposited under accession number DSM 33732, *Lactobacillus gasseri* LB905R deposited under accession number DSM 34094, *Lactobacillus crispatus* LB912R deposited under accession number DSM 34095, *Lactobacillus jensenii* LB918R deposited under accession number DSM 34096, *Lactobacillus crispatus* LB919R deposited under accession number DSM 34097.

Thus, an example of the present disclosure relates to a composition comprising *Lactobacillus crispatus* LB714R, deposited under the accession number DSM 33732, *Lactobacillus gasseri* LB905R deposited under accession number DSM 34094, *Lactobacillus crispatus* LB912R deposited under accession number DSM 34095, *Lactobacillus jensenii* LB918R deposited under accession number DSM 34096, or *Lactobacillus crispatus* LB919R deposited under accession number DSM 34097. In another aspect, the present disclosure provides a composition comprising a bacterial strain capable of prevention, inhibition, or treatment of biofilm formation of pathogens, wherein the bacterial strain is selected from *Lactobacillus crispatus* LB714R, deposited under the accession number DSM 33732, *Lactobacillus crispatus* LB919R deposited under accession number DSM 34097, *Lactobacillus jensenii* LB918R deposited under accession number DSM 34096, *Lactobacillus crispatus* LB912R deposited under accession number DSM 34095, or *Lactobacillus gasseri* LB905R deposited under accession number DSM 34094.

Another example of the present disclosure relates to a vaginal microbiome transplant comprising *Lactobacillus crispatus*, in particular comprising at least one of the following strains *Lactobacillus crispatus* LB714R, deposited under the accession number DSM 33732, *Lactobacillus gasseri* LB905R deposited under accession number DSM 34094, *Lactobacillus crispatus* LB912R deposited under accession number DSM 34095, *Lactobacillus jensenii* LB918R deposited under accession number DSM 34096, *Lactobacillus crispatus* LB919R deposited under accession number DSM 34097. In yet another aspect, the present disclosure provides a vaginal microbiome transplant comprising a bacterial strain capable of prevention, inhibition, or treatment of biofilm formation of pathogens, wherein the bacterial strain is selected from *Lactobacillus crispatus*, in particular comprising *Lactobacillus crispatus* LB714R, deposited under the

accession number DSM 33732, *Lactobacillus crispatus* LB919R deposited under accession number DSM 34097, *Lactobacillus jensenii* LB918R deposited under accession number DSM 34096, *Lactobacillus crispatus* LB912R deposited under accession number DSM 34095, or *Lactobacillus gasseri* LB905R deposited under accession number DSM 34094.

A further example of the present disclosure relates to a method for preparing a vaginal microbiome transplant comprising the step of obtaining a microbiome sample from a female (such as a human female or an animal female), isolating a fraction from the microbiome sample comprising one or more *Lactobacillus* species, such as one or more *Lactobacillus crispatus* (in particular comprising *Lactobacillus crispatus* LB714R, deposited under the accession number DSM 33732, *Lactobacillus crispatus* LB912R deposited under accession number DSM 34095, or *Lactobacillus crispatus* LB919R deposited under accession number DSM 34097), one or more *Lactobacillus jensenii* (in particular comprising *Lactobacillus jensenii* LB918R deposited under accession number DSM 34096); or one or more *Lactobacillus gasseri* (in particular comprising *Lactobacillus gasseri* LB905R

deposited under accession number DSM 34094), growing the fraction comprising one or more *Lactobacillus* species providing a growth fraction, and formulating the grown fraction into a suspension, spray, gel, cream, lotion, powder, capsule, ointment, oil, solution for lavages, ovules, a vaginal insert, a suppository, lozenge, tablets, microencapsulated product.

A further example of the present disclosure relates to a method for preparing a vaginal microbiome transplant comprising the step of obtaining a microbiome sample from a female (such as a human female or an animal female) with healthy vaginal microbiota, isolate lactic acid bacteria from the microbiome sample (preferably the isolated lactic acid bacteria comprises one or more *Lactobacillus* species, such as one or more *Lactobacillus crispatus,* one or more *Lactobacillus gasseri*, or one or more *Lactobacillus jensenii*; most preferably, the isolated lactic acid bacteria comprises one or more *Lactobacillus crispatus*), selecting isolated bacteria which has antimicrobial activity against a pathogenic microorganism. In one aspect, the present disclosure provides a method for selecting one or more lactic acid bacterial strains for vaginal transplantation comprising the step of selecting at least one first lactic acid bacterial strain characterized by inhibition of *Gardnerella vaginalis* and selecting at least one second strain characterized by inhibition of *Candida albicans*, wherein the inhibition of *Gardnerella vaginalis* and/or *Candida albicans* is provided by the inhibition or reduction in biofilm formation or the capability of preventing, inhibiting, or treating biofilm formation of pathogens.

In an example of the present disclosure the selected isolated bacteria which has antimicrobial activity against a pathogenic microorganism may be allowed to growth; and the selected isolated bacteria may be formulated into a suspension, spray, gel, cream, lotion, powder, capsule, ointment, oil, solution for lavages, ovules, a vaginal insert, a suppository, lozenge, tablets, microencapsulated product. In another aspect, the present disclosure provides an isolated bacterial strain capable of prevention, inhibition, or treatment of biofilm formation of pathogens, wherein the bacterial strain is selected from *Lactobacillus crispatus*, in particular comprising *Lactobacillus crispatus* LB714R, deposited under the accession number DSM 33732, *Lactobacillus crispatus* LB919R deposited under accession number DSM 34097, *Lactobacillus jensenii* LB918R deposited under accession number DSM 34096, *Lactobacillus crispatus* LB912R deposited under accession number DSM 34095, or *Lactobacillus gasseri* LB905R deposited under accession number DSM 34094.

Yet an example of the present disclosure relates to a method for treating or preventing dysfunctional microbiota in a female, the method comprising the following steps:

1. Isolate lactic acid bacteria from the vagina of a female (such as a human female) with healthy vaginal microbiota;

2. Select isolated bacteria which has antimicrobial activity against a pathogenic microorganism;

3. Prepare a composition comprising one or more of the selected isolated bacteria; and

4. Transplant the composition to the vagina of female with a dysfunctional vaginal microbiota.

A further example of the present disclosure relates to an isolated bacterial strain selected from Lactobacillaceae, in particular at least one of the following strains *Lactobacillus*

crispatus LB714R, deposited under the accession number DSM 33732, *Lactobacillus gasseri* LB905R deposited under accession number DSM 34094, *Lactobacillus crispatus* LB912R deposited under accession number DSM 34095, *Lactobacillus jensenii* LB918R deposited under accession number DSM 34096, or *Lactobacillus crispatus* LB919R deposited under accession number DSM 34097.

Yet an example of the present disclosure relates to a composition comprising one or more bacterial strains selected from the species *Lactobacillus crispatus, Lactobacillus gasseri* or, *Lactobacillus jensenii* for use as a medicament. In another aspect, the present disclosure provides a composition comprising one or more bacterial strains capable of prevention, inhibition, or treatment of biofilm formation of pathogens, wherein the bacterial strain is selected from the species *Lactobacillus crispatus, Lactobacillus gasseri* or, *Lactobacillus jensenii* for use as a medicament. In yet another aspect, the present disclosure provides a composition comprising *Lactobacillus crispatus* LB714R, deposited under the accession number DSM 33732 for use as a medicament.

Still an example of the present disclosure relates to a composition comprising a bacterial strain selected from one or more strain selected from *Lactobacillus crispatus, Lactobacillus gasseri,* or *Lactobacillus jensenii* for use in the prevention and/or treatment of a vaginal infection in a human or in an animal. In another aspect, the present disclosure provides a composition comprising a bacterial strain capable of prevention, inhibition, or treatment of biofilm formation of pathogens, wherein the bacterial strain is selected from one or more *Lactobacillus crispatus*, one or more *Lactobacillus gasseri*, or one or more *Lactobacillus jensenii* for use in the prevention and/or treatment of a vaginal infection in a human or in an animal. In yet another aspect, the present disclosure provides a composition comprising *Lactobacillus crispatus* LB714R, deposited under the accession number DSM 33732 for use in the prevention and/or treatment of a vaginal infection in a human or in an animal.

Another example of the present disclosure relates to a composition comprising a bacterial strain selected from one or more *Lactobacillus crispatus, one or more Lactobacillus gasseri,* or *one or more Lactobacillus jensenii* for use in the improvement of fertility of a human or an animal.

Yet an example of the present disclosure relates to a composition comprising a bacterial strain selected from one or more strain selected from *Lactobacillus crispatus, Lactobacillus gasseri,* or *Lactobacillus jensenii* for use in reducing the risk of premature birth of a human or an animal. In one aspect, the present disclosure provides a composition comprising a bacterial strain capable of prevention, inhibition, or treatment of

biofilm formation of pathogens, wherein the bacterial strain is selected from one or more *Lactobacillus crispatus*, one or more *Lactobacillus gasseri*, or one or more *Lactobacillus jensenii* for use in the improvement of fertility of a human or an animal and/or reducing the risk of premature birth of a human or an animal. In another aspect, the present disclosure provides a composition comprising *Lactobacillus crispatus* LB714R, deposited under the accession number DSM 33732 for use in the improvement of fertility of a human or an animal and/or reducing the risk of premature birth of a human or an animal.

A further example of the present disclosure relates to a composition comprising one or more vaginal isolate in particular, *Lactobacillus crispatus* LB714R, deposited under the accession number DSM 33732, *Lactobacillus gasseri* LB905R deposited under accession number DSM 34094, *Lactobacillus crispatus* LB912R deposited under accession number DSM 34095, *Lactobacillus jensenii* LB918R deposited under accession number DSM 34096 and/ or *Lactobacillus crispatus* LB919R deposited under accession number DSM 34097 for use in the prevention, inhibition, or treatment of biofilm formation. In one aspect, the present disclosure provides a composition comprising a bacterial strain selected from one or more *Lactobacillus crispatus*, one or more *Lactobacillus gasseri*, or one or more *Lactobacillus jensenii* for use in the prevention, inhibition, or treatment of biofilm formation. In one aspect, the present disclosure provides a composition comprising one or more *Lactobacillus crispatus* (in particular, *Lactobacillus crispatus* LB714R, deposited under the accession number DSM 33732), one or more *Lactobacillus jensenii* (in particular comprising *Lactobacillus jensenii* LB918R deposited under accession number DSM 34096), or one or more *Lactobacillus gasseri* (in particular comprising *Lactobacillus gasseri* LB905R deposited under accession number DSM 34094) for use in the prevention, inhibition, or treatment of biofilm formation.

Still an aspect of the present disclosure relates to a medical device comprising the composition according to the present disclosure, or a vaginal microbiome transplant according to the present disclosure, or an isolated bacterial strain according to the present disclosure.

Yet an example of the present disclosure relates to a food ingredient, a feed ingredient, and/or a personal hygiene product comprising the composition according to the present disclosure.

Brief description of the figures

Figure 1 shows the co-aggregation of *G. vaginalis* with LB714R isolated from vagina of woman in late pregnancy, and

Figure 2 shows a preferred preparation of a composition or a vaginal microbiota transplant according to the present invention, including identification of suitable lactic acid bacteria capable of inhibiting *Gardnerella vaginalis*, such as one or more lactic acid bacteria, e.g. a *Lactobacillus* species, including one or more *Lactobacillus crispatus, Lactobacillus gasseri or Lactobacillus jensenii* (in particular, *Lactobacillus crispatus* LB714R, deposited under the accession number DSM 33732, *Lactobacillus gasseri* LB905R deposited under accession number DSM 34094, *Lactobacillus crispatus* LB912R deposited under accession number DSM 34095, *Lactobacillus jensenii* LB918R deposited under accession number DSM 34096 or *Lactobacillus crispatus* LB919R deposited under accession number DSM 34097). The method includes transplantation of vaginal microbiota which involves a method for isolation and identification of strains for prevention or treatment of vaginal infections or urogenital infections. In particular the method includes (i) collection of lactic acid bacteria from women having a healthy vaginal microbiota; (ii) isolate individual strains from the healthy vaginal microbiota; (iii) select isolated strains based on their antimicrobial activity against vaginal pathogenic microorganisms; (iv) grow the selected stains as monocultures and formulate in a composition suitable for vaginal transplant; (v) optionally enhance composition by mixing more than one strain into the composition; and (vi) transplant composition to a woman with a dysfunctional vaginal microbiota,

Figure 3 shows co-aggregation scores of *Lactobacillus crispatus* LB714R, deposited under the accession number DSM 33732, *Lactobacillus gasseri* LB905R deposited under accession number DSM 34094, *Lactobacillus crispatus* LB912R deposited under accession number DSM 34095, *Lactobacillus jensenii* LB918R deposited under accession number DSM 34096 and *Lactobacillus crispatus* LB919R deposited under accession number DSM 34097 according to the present invention illustrated by the light gray columns determined according to Cisar, J. O. et al. (1979).

Figure 4 shows microscope images of the ability of *Lactobacillus crispatus* LB714R, deposited under the accession number DSM 33732, to co-aggregate with G. vaginalis using

Claims

1. A composition comprising a bacterial strain capable of prevention, inhibition, or treatment of biofilm formation of pathogens, wherein the bacterial strain is selected from *Lactobacillus crispatus* LB714R, deposited under the accession number DSM 33732, *Lactobacillus crispatus* LB919R deposited under accession number DSM 34097, *Lactobacillus jensenii* LB918R deposited under accession number DSM 34096, *Lactobacillus crispatus* LB912R deposited under accession number DSM 34095, or *Lactobacillus gasseri* LB905R deposited under accession number DSM 34094.

2. A vaginal microbiome transplant comprising a bacterial strain capable of prevention, inhibition, or treatment of biofilm formation of pathogens, wherein the bacterial strain is selected from *Lactobacillus crispatus*, in particular comprising *Lactobacillus crispatus* LB714R, deposited under the accession number DSM 33732, *Lactobacillus crispatus* LB919R deposited under accession number DSM 34097, *Lactobacillus jensenii* LB918R deposited under accession number DSM 34096, *Lactobacillus crispatus* LB912R deposited under accession number DSM 34095, or *Lactobacillus gasseri* LB905R deposited under accession number DSM 34094.

3. The vaginal microbiome transplant according to claim 2, wherein the vaginal microbiome transplant further comprises a lactic acid bacterial strain selected from one or more *Pediococcus* strains (such as *Pediococcus pentosaceus* LB606R*,* deposited under the deposit accession number DSM 33730); and/or the one or more *Lactiplantibacillus* strains, (such as *Lactiplantibacillus plantarum* LB679R, deposited under the deposit accession number DSM 33731).

4. The vaginal microbiome transplant according to anyone of claims 2-3, wherein the vaginal microbiome transplant is formulated as a suspension, spray, gel, cream, lotion, powder, capsule, oil, solution for lavages, ovules, a vaginal insert, a suppository, lozenge, tablets, microencapsulated product or in form of a food supplement or a food product.

5. An isolated bacterial strain capable of prevention, inhibition, or treatment of biofilm formation of pathogens, wherein the bacterial strain is selected from *Lactobacillus crispatus*, in particular comprising *Lactobacillus crispatus* LB714R, deposited under the accession number DSM 33732, *Lactobacillus crispatus* LB919R deposited under accession number DSM 34097, *Lactobacillus jensenii* LB918R deposited under accession number DSM 34096, *Lactobacillus crispatus* LB912R deposited under accession number DSM 34095, or *Lactobacillus gasseri* LB905R deposited under accession number DSM 34094.

6. A composition comprising one or more bacterial strains capable of prevention, inhibition, or treatment of biofilm formation of pathogens, wherein the bacterial strain is selected from the species *Lactobacillus crispatus, Lactobacillus gasseri* or, *Lactobacillus jensenii* for use as a medicament.

7. A composition comprising a bacterial strain capable of prevention, inhibition, or treatment of biofilm formation of pathogens, wherein the bacterial strain is selected from one or more *Lactobacillus crispatus*, one or more *Lactobacillus gasseri*, or one or more *Lactobacillus jensenii* for use in the prevention and/or treatment of a vaginal infection in a human or in an animal.

8. A composition comprising a bacterial strain capable of prevention, inhibition, or treatment of biofilm formation of pathogens, wherein the bacterial strain is selected from one or more *Lactobacillus crispatus*, one or more *Lactobacillus gasseri*, or one or more *Lactobacillus jensenii* for use in the improvement of fertility of a human or an animal and/or reducing the risk of premature birth of a human or an animal.

9. A composition comprising a bacterial strain selected from one or more *Lactobacillus crispatus*, one or more *Lactobacillus gasseri*, or one or more *Lactobacillus jensenii* for use in the prevention, inhibition, or treatment of biofilm formation.

10. A composition comprising one or more *Lactobacillus crispatus* (in particular, *Lactobacillus crispatus* LB714R, deposited under the accession number DSM 33732), one or more *Lactobacillus jensenii* (in particular comprising *Lactobacillus jensenii* LB918R deposited under accession number DSM 34096), or one or more *Lactobacillus gasseri* (in particular comprising *Lactobacillus gasseri* LB905R deposited under accession number DSM 34094) for use in the prevention, inhibition, or treatment of biofilm formation.

11. A composition comprising *Lactobacillus crispatus* LB714R, deposited under the accession number DSM 33732 for use as a medicament.

12. A composition comprising *Lactobacillus crispatus* LB714R, deposited under the accession number DSM 33732 for use in the prevention and/or treatment of a vaginal infection in a human or in an animal.

13. A composition comprising *Lactobacillus crispatus* LB714R, deposited under the accession number DSM 33732 for use in the improvement of fertility of a human or an animal and/or reducing the risk of premature birth of a human or an animal.

14. A medical device comprising the composition according to anyone of claims 1 or 6-13, or a vaginal microbiome transplant according to claim 2-4, or an isolated bacterial strain according to claim 5.

15. A method for selecting one or more lactic acid bacterial strains for vaginal transplantation comprising the step of selecting at least one first lactic acid bacterial strain characterized by inhibition of *Gardnerella vaginalis* and selecting at least one second strain characterized by inhibition of *Candida albicans*, wherein the inhibition of *Gardnerella vaginalis* and/or *Candida albicans* is provided by the inhibition or reduction in biofilm formation or the capability of preventing, inhibiting, or treating biofilm formation of pathogens.