

[07] Currently available treatments for hyperoxalurias are inadequate. Strategies for the treatment of primary hyperoxalurias include reducing urinary oxalate with pyridoxine, which is only effective in less than half of patients with PHI, and ineffective in patients with PHII and PHIII (Hoppe *et al.* (2011)). Further, treatments with citrate, orthophosphate, and magnesium to increase the urinary solubility of calcium oxalate, and thus preserve renal function, are not well characterized (Hoppe *et al.* (2011)). Other strategies for the treatment of secondary and enteric hyperoxalurias, which are quite arduous and often ineffective, include reducing the dietary intake of oxalate, oral calcium supplementation, and the use of bile acid sequestrants (Parivar *et al.*, *J. Urol.* 155: 432-40 (1996); Hylander *et al.* (1980); McLeod and Churchill, *J. Urol.* 148: 974-8 (1992)). Generally, dietary restrictions are not entirely effective because patients cannot readily identify the food products to avoid (Parivar *et al.* (1996)). Therefore, there is significant unmet need for effective, reliable, and/or long-term treatment of hyperoxalurias.

### Summary

[08] The present disclosure provides engineered bacterial cells, pharmaceutical compositions thereof, and methods of modulating and treating disorders in which oxalate is detrimental. Specifically, the engineered bacteria disclosed herein have been constructed to comprise genetic circuits composed of, for example, one or more oxalate catabolism genes to treat the disease, as well as other optional circuitry designed to ensure the safety and non-colonization of a subject that is administered the engineered bacteria, such as, for example, auxotrophies, kill switches, and combinations thereof. These engineered bacteria are safe and well tolerated and augment the innate activities of the subject's microbiome to achieve a therapeutic effect.

[08a] In one aspect, the present disclosure refers to the use of a pharmaceutical composition in the manufacture of a medicament for reducing the levels of oxalate in a subject, wherein the pharmaceutical composition comprises a recombinant bacterium comprising one or more gene sequences encoding one or more oxalate catabolism enzymes operably linked to a directly or indirectly first promoter that is not associated with the oxalate catabolism enzyme gene in nature.

[08b] In another aspect, the present disclosure refers to a recombinant bacterium comprising one or more gene sequences encoding one or more oxalate catabolism enzymes operably linked to a directly or indirectly first promoter that is not associated with the oxalate catabolism enzyme gene in nature.

[09] In one example, disclosed herein is a method for reducing the levels of oxalate in a subject, the method comprising administering to the subject a pharmaceutical composition comprising a recombinant bacterium comprising one or more gene sequences encoding one or more oxalate catabolism enzymes operably linked to a directly or indirectly first promoter that is not associated with the oxalate catabolism enzyme gene in nature, thereby reducing the levels of oxalate in the subject.

[010] In one example, the recombinant bacterium has an oxalate consumption activity of  $1\mu\text{mol}/1\times 10^9$  cell. In one example, the recombinant bacterium has an oxalate consumption activity of about 50-600 mg/day, about 100-550 mg/day, about 100-500 mg/day, about 100-400 mg/day, about 100-300 mg/day. In one example, the recombinant bacterium has an oxalate consumption activity of about 150-300 mg/day. In one example, the recombinant bacterium has an oxalate consumption activity of about 50 mg/day, about 100 mg/day, about 150 mg/day, about 200 mg/day, about 210 mg/day, about 211 mg/day, about 225 mg/day, about 250 mg/day, about 275 mg/day, about 300 mg/day, about 325 mg/day, about 350 mg/day, about 350 mg/day, about 375 mg/day, about 400 mg/day, about 425 mg/day, about 450 mg/day, about 475 mg/day, about 500

mg/day, about 525 mg/day, about 550 mg/day, about 575 mg/day, or about 600 mg/day. In one example, the recombinant bacterium has an oxalate consumption activity of about 50 mg/day, about 100 mg/day, about 150 mg/day, about 200 mg/day, about 211 mg/day, about 225 mg/day, about 250 mg/day, about 275 mg/day, about 300 mg/day, about 325 mg/day, about 350 mg/day, about 350 mg/day, about 375 mg/day, about 400 mg/day, about 425 mg/day, about 450 mg/day, about 475 mg/day, about 500 mg/day, about 525 mg/day, about 550 mg/day, about 575 mg/day, or about 600 mg/day under anaerobic conditions. In one example, the recombinant bacterium has an oxalate consumption activity of about 50 mg/day, about 100 mg/day, about 150 mg/day, about 200 mg/day, about 211 mg/day, about 225 mg/day, about 250 mg/day, about 275 mg/day, about 300 mg/day, about 325 mg/day, about 350 mg/day, about 350 mg/day, about 375 mg/day, about 400 mg/day, about 425 mg/day, about 450 mg/day, about 475 mg/day, about 500 mg/day, about 525 mg/day, about 550 mg/day, about 575 mg/day, or about 600 mg/day under anaerobic conditions when administered to the subject three times per day. In one example, the anaerobic conditions are conditions in the intestine and/or colon of the subject.

[011] In one example, the method reduces acute levels of oxalate in the subject by about two fold. In one example, the method reduces acute levels of oxalate in the subject by about three fold. In one example, the method reduces chronic levels of oxalate in the subject by about two fold. In one example, the method reduces chronic levels of oxalate in the subject by about three fold.

[012] In one example, the method reduces acute levels of oxalate in the subject to about 25 mg/day, about 30 mg/day, about 40 mg/day, about 50 mg/day, about 60 mg/day, about 70 mg/day, about 80 mg/day, about 90 mg/day, or about 100 mg/day. In one example, the method reduces chronic levels of oxalate in the subject to about 25 mg/day, about 30 mg/day, about 40 mg/day, about 50 mg/day, about 60 mg/day, about 70 mg/day, about 80 mg/day, about 90 mg/day, or about 100 mg/day.

[013] In one example, the method reduces acute levels of oxalate in the subject by at least about 40% by day 5 after administration. In one example, the method reduces acute levels of oxalate in the subject by at least about 50% by day 5 after administration. In one example, the method reduces acute levels of oxalate in the subject by at least about 60% by day 5 after administration. In one example, the method reduces acute levels of oxalate in the subject by at least about 70% by day 5 after administration. In one example, the method reduces acute levels of oxalate in the subject by at least about 80% by day 5 after administration.

[014] In one example, the method reduces acute levels of oxalate in the subject by at least about 10% by about 24 hours after administration. In one example, the method reduces acute levels of oxalate in the subject by at least about 15% by about 24 hours after administration. In one example, the method reduces acute levels of oxalate in the subject by at least about 20% by about 24 hours after administration.

[015] In one example, the recombinant bacterium is of the genus *Escherichia*. In one example, the recombinant bacterium is of the species *Escherichia coli* strain Nissle.

[016] In one example, the pharmaceutical composition is administered orally. In one example, the subject is fed a meal within one hour of administering the pharmaceutical composition. In one example, the subject is fed a meal concurrently with administering the pharmaceutical composition. In one example, the subject is a human subject.

[017] In one example, disclosed herein is a recombinant bacterium comprising one or more gene sequences encoding one or more oxalate catabolism enzymes operably linked to a directly or indirectly first inducible promoter that is not associated with the oxalate catabolism enzyme gene in nature.

[018] In one example, the recombinant bacterium has an oxalate consumption activity of  $1\mu\text{mol}/1\times 10^9$  cell. In one example, the recombinant bacterium has an oxalate consumption activity of about 150-300 mg/day under anaerobic conditions. In one example, the recombinant bacterium has an oxalate consumption activity of about 200 mg/day under anaerobic conditions. In one example, the recombinant bacterium has an oxalate consumption activity of about 200 mg/day under anaerobic conditions when administered to the subject three times per day. In one example, the anaerobic conditions are conditions in the intestine and/or colon of the subject.

[019] In one embodiment, the one or more gene sequences comprise a *scaaE3* gene, an *frc* gene, and an *oxdC* gene. In one example, the *scaaE3* gene comprises a sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to, comprises, or consists of SEQ ID NO:3. In one example, the *frc* gene comprises a sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to, comprises, or consists of SEQ ID NO:1. In one example, the *scaaE3* gene comprises SEQ ID NO:3. In one example, the *oxdC* gene comprises a sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to, comprises, or consists of SEQ ID NO:2. In one example, the *frc* gene comprises SEQ ID NO:2.

[020] In one example, the recombinant bacterium further comprises a gene encoding an oxalate importer. In one example, the gene encoding the oxalate importer is an *oxlT* gene. In one example, the *oxlT* gene comprises a sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to, comprises, or consists of SEQ ID NO: 11. In one example, the *oxlT* gene comprises SEQ ID NO: 11.

[021] In one example, the recombinant bacterium further comprises an auxotrophy. In one example, the auxotrophy is a *thyA* auxotrophy. In one example, *thyA* has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to, comprises, or consists of SEQ ID NO: 62.

[022] In one example, the recombinant bacterium further comprises a deletion in an endogenous phage. In one example, the endogenous phage comprises a sequence having at least

90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to, comprises, or consists of SEQ ID NO:63. In one example, the endogenous phase comprises a sequence of SEQ ID NO:63.

[023] In one example, the recombinant bacterium does not comprise a gene encoding for antibiotic resistance. In one embodiment, the first promoter is an inducible promoter. In one example, the inducible promoter is induced by low oxygen or anaerobic conditions, temperature, or the hypoxic environment of a tumor. In one example, the inducible promoter is an FNR promoter. In one example, the FNR promoter is a promoter selected from the group consisting of any one of SEQ ID NOs:13-29.

[024] In one example, the recombinant bacterium comprises an *oxlT* gene under the control of an inducible promoter, optionally an FNR promoter; an *scaaE3* gene, an *oxcd* gene, and an *frc* gene under the control of an inducible promoter, optionally an FNR promoter, a *thyA* deletion (or auxotrophy) and a deletion of endogenous phage 3. In one example, the recombinant bacterium comprises HA910::FNR\_*oxlT*, HA12::FNR\_*scaaE3-oxcd-frc*,  $\Delta$ *thyA*,  $\Delta$ phage 3.

[025] In one example, the recombinant bacterium is SYN5752, SYN7169, or SYN8802. In one example, the recombinant bacterium is SYN8802.

[026] In one example, the subject has hyperoxaluria. In one example, the hyperoxaluria is primary hyperoxaluria, dietary hyperoxaluria, or enteric hyperoxaluria. In one example, the subject has short bowel syndrome, chronic pancreatitis, inflammatory bowel disease (IBD), cystic fibrosis, kidney disease, and/or Roux-en-Y gastric bypass.

[027] In one example, the subject has urinary oxalate (Uox) levels of at least 70 mg/day prior to the administering. In one example, the subject exhibits a decrease in Uox levels of at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, or 80% after the administering. In one example, the subject has eGFR < 30 mL/min/1.73 m<sup>2</sup>, requires hemodialysis, or has systemic oxalosis prior to the administering.

[028] In one example, the recombinant bacteria is administered at a dose of about  $1 \times 10^{11}$  live recombinant bacteria, about  $2 \times 10^{11}$  live recombinant bacteria, about  $3 \times 10^{11}$  live recombinant bacteria, about  $4 \times 10^{11}$  live recombinant bacteria, about  $5 \times 10^{11}$  live recombinant bacteria, about  $6 \times 10^{11}$  live recombinant bacteria, about  $1 \times 10^{12}$  live recombinant bacteria, or about  $2 \times 10^{12}$  live recombinant bacteria. In one example, the recombinant bacteria is administered at a dose of about  $6 \times 10^{11}$  live recombinant bacteria. In one example, the recombinant bacteria is administered at a dose of about  $3 \times 10^{11}$  live recombinant bacteria. In one example, the recombinant bacteria is administered at a dose of about  $1 \times 10^{11}$  live recombinant bacteria. In one example, the administering is about  $5 \times 10^{11}$  live recombinant bacteria. In one example, the recombinant bacteria is administered at a dose of about  $1 \times 10^{12}$  live recombinant bacteria. In one example, the recombinant bacteria is administered at a dose of about  $2 \times 10^{12}$  live recombinant bacteria. In one example, the administering is about  $5 \times 10^{11}$  live recombinant bacteria with meals three times per day. In one example, the recombinant bacteria is administered at a dose of about  $1 \times 10^{11}$  live

recombinant bacteria to about  $2 \times 10^{12}$  live recombinant bacteria. In one example, the recombinant bacteria is administered at a dose of about  $1 \times 10^{12}$  live recombinant bacteria to about  $2 \times 10^{12}$  live recombinant bacteria. In one example, the recombinant bacteria is administered at a dose of about  $5 \times 10^{11}$  live recombinant bacteria to about  $2 \times 10^{12}$  live recombinant bacteria.

[029] In another example, the administering is oral, with meals, twice per day. In another example, the administering is oral, with meals, three times per day.

[030] In another example, a proton pump inhibitor (PPI) is administered to the subject. In another example, the PPI is esomeprazole. In another example, the administering of the PPI is once a day.

[031] In some examples, the disclosure provides a bacterial cell that has been genetically engineered to comprise one or more genes, gene cassettes, and/or synthetic circuits encoding one or more oxalate catabolism enzyme(s) or oxalate catabolism pathway, and is capable of metabolizing oxalate and/or other metabolites, such as oxalyl-CoA. Thus, the genetically engineered bacterial cells and pharmaceutical compositions comprising the bacterial cells may be used to treat and/or prevent diseases associated with disorders in which oxalate is detrimental, such as primary hyperoxalurias and secondary hyperoxalurias.

[032] In some examples, the disclosure provides a bacterial cell that has been engineered to comprise gene sequence(s) encoding one or more oxalate catabolism enzyme(s). In some examples, the disclosure provides a bacterial cell has been engineered to comprise gene sequence(s) encoding one or more oxalate catabolism enzyme(s) and is capable of reducing the level of oxalate and/or other metabolites, for example, oxalyl-CoA. In some examples, the bacterial cell has been engineered to comprise gene sequence(s) encoding one or more transporter(s) (importer(s)) of oxalate. In some examples, the bacterial cell has been engineered to comprise gene sequence(s) encoding one or more exporter(s) of formate. In some examples, the engineered bacteria comprise gene sequence(s) encoding one or more polypeptide(s) which mediate both the transport (import) of oxalate and the export of formate (*e.g.*, oxalate:formate antiporter(s)). In some examples, the engineered bacteria comprise gene sequence(s) encoding one or more of the following: (i) one or more transporter(s) of oxalate; (ii) one or more exporter(s) of formate; (iii) one or more polypeptide(s) which mediate both the transport (import) of oxalate and the export of formate (*e.g.*, oxalate:formate antiporter(s)); and (iv) any combination thereof. In some examples, the bacterial cell has been engineered to comprise gene sequence(s) encoding one or more oxalate catabolism enzyme(s) and one or more transporter(s) (importer(s)) of oxalate. In some examples, the bacterial cell of the disclosure has been genetically engineered to comprise gene sequence(s) encoding one or more oxalate catabolism enzyme(s) and one or more exporter(s) of formate. In some examples, genetically engineered bacteria comprise gene sequence(s) encoding one or more oxalate catabolism enzyme(s) and one or more polypeptide(s), which mediate both the transport (import) of oxalate and the export of formate (*e.g.*, oxalate:formate antiporter(s)). In some

examples, the bacterial cell has been engineered to comprise gene sequence(s) encoding one or more oxalate catabolism enzyme(s) and gene sequence(s) encoding one or more of the following: (i) one or more transporter(s) of oxalate; (ii) one or more exporter(s) of formate; (iii) one or more polypeptide(s) which mediate both the transport (import) of oxalate and the export of formate (*e.g.*, oxalate:formate antiporter(s)); and (iv) any combination thereof.

[010] In some examples, the gene sequence(s) encoding one or more oxalate catabolism enzyme(s) is operably linked to an inducible promoter. In some examples, the gene sequence(s) encoding one or more oxalate transporter(s) (importer(s)) is operably linked to an inducible promoter. In some examples, the gene sequence(s) encoding one or more exporter(s) of formate is operably linked to an inducible promoter. In some examples, the gene sequence(s) encoding one or more polypeptide(s) which mediate both the transport (import) of oxalate and the export of formate (*e.g.*, oxalate:formate antiporter(s)) is operably linked to an inducible promoter. In some examples, the gene sequence(s) encoding one or more oxalate catabolism enzyme(s) and the gene sequence(s) encoding one or more oxalate transporter(s) (importer(s)) are operably linked to an inducible promoter. In some examples, the gene sequence(s) encoding one or more oxalate catabolism enzyme(s) and the gene sequence(s) encoding one or more exporter(s) of formate are operably linked to an inducible promoter. In some examples, the gene sequence(s) encoding one or more oxalate catabolism enzyme(s) and the gene sequence(s) encoding one or more polypeptide(s) which mediate both the transport (import) of oxalate and the export of formate (*e.g.*, oxalate:formate antiporter(s)) are operably linked to an inducible promoter. In some examples, any one or more of the following gene sequences, if present in the bacterial cell, are operably linked to an inducible promoter: (i) gene sequence(s) encoding one or more oxalate catabolism enzyme(s); (ii) gene sequence(s) encoding one or more oxalate transporter(s); (iii) gene sequence(s) encoding one or more exporter(s) of formate; and (iv) gene sequence(s) encoding one or more polypeptide(s) which mediate both the transport (import) of oxalate and the export of formate (*e.g.*, oxalate:formate antiporter(s)).

[011] In some examples, the disclosure provides a bacterial cell which has been engineered to comprise gene sequence(s) encoding one or more oxalate catabolism enzyme(s) operably linked to an inducible promoter that is induced under low oxygen and/or anaerobic conditions, *e.g.*, such as those conditions found in the mammalian gut. In some examples, the disclosure provides a bacterial cell which has been engineered to comprise gene sequence(s) encoding one or more oxalate transporter(s) (importer(s)) operably linked to an inducible promoter that is induced under low oxygen and/or anaerobic conditions, *e.g.*, such as those conditions found in the mammalian gut. In some examples, the disclosure provides a bacterial cell which has been engineered to comprise gene sequence(s) encoding one or more exporter(s) of formate operably linked to an inducible promoter that is induced under low oxygen and/or anaerobic conditions, *e.g.*, such as those conditions found in the mammalian gut. In some examples, the disclosure provides a bacterial cell which has been engineered to comprise gene sequence(s) encoding one or more

polypeptide(s) which mediate both the transport (import) of oxalate and the export of formate (*e.g.*, oxalate:formate antiporter(s)) operably linked to an inducible promoter that is induced under low oxygen and/or anaerobic conditions, *e.g.*, such as those conditions found in the mammalian gut. In some examples, the gene sequence(s) encoding one or more oxalate catabolism enzyme(s) and the gene sequence(s) encoding one or more oxalate transporter(s) (importer(s)) are operably linked to an inducible promoter that is induced under low oxygen and/or anaerobic conditions. In some examples, the gene sequence(s) encoding one or more oxalate catabolism enzyme(s) and the gene sequence(s) encoding one or more exporter(s) of formate are operably linked to an inducible promoter that is induced under low oxygen and/or anaerobic conditions. In some examples, the gene sequence(s) encoding one or more oxalate catabolism enzyme(s) and the gene sequence(s) encoding one or more polypeptide(s) which mediate both the transport (import) of oxalate and the export of formate (*e.g.*, oxalate:formate antiporter(s)) are operably linked to an inducible promoter that is induced under low oxygen and/or anaerobic conditions. In some examples, any one or more of the following gene sequences, if present in the bacterial cell, are operably linked to an inducible promoter that is induced under low oxygen and/or anaerobic conditions: (i) gene sequence(s) encoding one or more oxalate catabolism enzyme(s); (ii) gene sequence(s) encoding one or more oxalate transporter(s); (iii) gene sequence(s) encoding one or more exporter(s) of formate; and (iv) gene sequence(s) encoding one or more polypeptide(s) which mediate both the transport (import) of oxalate and the export of formate (*e.g.*, oxalate:formate antiporter(s)).

[012] In one example, the inducible promoter is a *lacI* promoter which can be induced with IPTG. In another example, the inducible promoter is a pBAD promoter which can be induced with arabinose.

[013] In some examples, the disclosure provides a bacterial cell that has been engineered to comprise gene sequence(s) encoding one or more oxalate catabolism enzyme(s) that is operably linked to an inducible promoter that is induced by environmental signals and/or conditions found in the mammalian gut (*e.g.*, induced by metabolites (*e.g.*, oxalate metabolites) or other biomolecules found in the mammalian gut, and/or induced by inflammatory conditions (*e.g.*, reactive nitrogen species and/or reactive oxygen species)). The environmental signals and/or conditions found in the mammalian gut may be signals and conditions found in a healthy mammalian gut or signals and conditions found in a diseased mammalian gut, such as the gut of a subject having hyperoxaluria or other condition in which the level of oxalate and/or an oxalate metabolite is elevated, and/or the gut of a subject having an inflammatory condition, such as irritable bowel disease, an autoimmune disease, and any other condition that results in inflammation in the gut. In some examples, the disclosure provides a bacterial cell which has been engineered to comprise gene sequence(s) encoding one or more oxalate catabolism enzyme(s) operably linked to an inducible promoter that is induced under inflammatory conditions, *e.g.*, such as inflammatory conditions found in a mammalian gut. In some examples, the disclosure provides a bacterial cell which has been

engineered to comprise gene sequence(s) encoding one or more oxalate transporter(s) (importer(s)) operably linked to an inducible promoter that is induced under inflammatory conditions, *e.g.*, such as inflammatory conditions found in a mammalian gut. In some examples, the disclosure provides a bacterial cell which has been engineered to comprise gene sequence(s) encoding one or more exporter(s) of formate operably linked to an inducible promoter that is induced under inflammatory conditions, *e.g.*, such as inflammatory conditions found in a mammalian gut. In some examples, the disclosure provides a bacterial cell which has been engineered to comprise gene sequence(s) encoding one or more polypeptide(s) which mediate both the transport (import) of oxalate and the export of formate (*e.g.*, oxalate:formate antiporter(s)) operably linked to an inducible promoter that is induced under inflammatory conditions, *e.g.*, such as inflammatory conditions found in a mammalian gut. In some examples, the gene sequence(s) encoding one or more oxalate catabolism enzyme(s) and the gene sequence(s) encoding one or more oxalate transporter(s) (importer(s)) are operably linked to an inducible promoter that is induced under inflammatory conditions. In some examples, the gene sequence(s) encoding one or more oxalate catabolism enzyme(s) and the gene sequence(s) encoding one or more exporter(s) of formate are operably linked to an inducible promoter that is induced under inflammatory conditions. In some examples, the gene sequence(s) encoding one or more oxalate catabolism enzyme(s) and the gene sequence(s) encoding one or more polypeptide(s) which mediate both the transport (import) of oxalate and the export of formate (*e.g.*, oxalate:formate antiporter(s)) are operably linked to an inducible promoter that is induced under inflammatory conditions. In some examples, any one or more of the following gene sequences, if present in the bacterial cell, are operably linked to an inducible promoter that is induced under inflammatory conditions: (i) gene sequence(s) encoding one or more oxalate catabolism enzyme(s); (ii) gene sequence(s) encoding one or more oxalate transporter(s); (iii) gene sequence(s) encoding one or more exporter(s) of formate; and (iv) gene sequence(s) encoding one or more polypeptide(s) which mediate both the transport (import) of oxalate and the export of formate (*e.g.*, oxalate:formate antiporter(s)).

[014] In some examples, the disclosure provides a bacterial cell that has been engineered to comprise gene sequence(s) encoding one or more polypeptide(s) capable of reducing the level of oxalate and/or other metabolites, for example, oxalyl-CoA, in low-oxygen environments, *e.g.*, the gut. In some examples, the bacterial cell that has been engineered to comprise gene sequence(s) encoding one or more of the following: (i) one or more oxalate catabolism enzyme(s); (ii) one or more oxalate transporter(s); (iii) one or more formate exporter(s); and (iv) one or more oxalate:formate antiporter(s). In some examples, the bacterial cell has been genetically engineered to comprise one or more circuits encoding one or more oxalate catabolism enzyme(s) and is capable of processing and reducing levels of oxalate, and/or oxalyl-CoA *e.g.*, in low-oxygen environments, *e.g.*, the gut. Thus, in some examples, the genetically engineered bacterial cells and pharmaceutical compositions comprising the bacterial cells of the disclosure may be used to

import excess oxalate and/or oxalyl-CoA into the bacterial cell in order to treat and/or prevent conditions associated with disorders in which oxalate is detrimental, such as primary hyperoxalurias and secondary hyperoxalurias. In some examples, the genetically engineered bacterial cells and pharmaceutical compositions comprising the bacterial cells of the disclosure may be used to convert excess oxalate and/or oxalyl-CoA into non-toxic molecules in order to treat and/or prevent conditions associated with disorders in which oxalate is detrimental, such as primary hyperoxalurias and secondary hyperoxalurias.

[015] The present disclosure provides recombinant bacterial cells, pharmaceutical compositions thereof, and methods of modulating and treating disorders in which oxalate is detrimental. The genetically engineered bacterial cells and pharmaceutical compositions comprising the bacterial cells of the disclosure may be used to convert excess oxalate and/or oxalic acid into non-toxic molecules in order to treat and/or prevent conditions associated with disorders in which oxalate is detrimental, such as primary hyperoxalurias and secondary hyperoxalurias. In some examples, a bacterial cell of the disclosure has been engineered to comprise at least one heterologous gene encoding at least one oxalate catabolism enzyme and is capable of processing and reducing levels of oxalate, in low-oxygen environments, *e.g.*, the gut. In some examples, a bacterial cell of the disclosure has been engineered to comprise at least one heterologous gene encoding an importer of oxalate and is capable of reducing levels of oxalate, in low-oxygen environments, *e.g.*, the gut. In some examples, a bacterial cell of the disclosure has been engineered to comprise at least one heterologous gene encoding an exporter of formate and is capable of reducing levels of oxalate, in low-oxygen environments, *e.g.*, the gut. In some examples, a bacterial cell of the disclosure has been engineered to comprise at least one heterologous gene encoding an oxalate:formate antiporter and is capable of reducing levels of oxalate, in low-oxygen environments, *e.g.*, the gut. In some examples, a bacterial cell of the disclosure has been engineered to comprise at least one heterologous gene encoding at least one oxalate catabolism enzyme and is capable of processing and reducing levels of oxalate, in inflammatory environments, such as may be present in the gut. In some examples, a bacterial cell of the disclosure has been engineered to comprise at least one heterologous gene encoding an importer of oxalate and is capable of reducing levels of oxalate, in inflammatory environments, *e.g.*, such as may be present in the gut. In some examples, a bacterial cell of the disclosure has been engineered to comprise at least one heterologous gene encoding an exporter of formate and is capable of reducing levels of oxalate, in inflammatory environments, *e.g.*, such as may be present in the gut. In some examples, a bacterial cell of the disclosure has been engineered to comprise at least one heterologous gene encoding an oxalate:formate antiporter and is capable of reducing levels of oxalate, in inflammatory environments, *e.g.*, such as may be present in the gut.

[016] In some examples, the at least one oxalate catabolism enzyme converts oxalate to formate or formyl CoA. In some examples, the at least one oxalate catabolism enzyme is selected

from an oxalate-CoA ligase, (*e.g.*, ScAAE3 from *S. cerevisiae*), an oxalyl-CoA decarboxylase (Oxc, *e.g.*, from *O. formigenes*), and a formyl-CoA transferase (*e.g.*, Frc, *e.g.*, from *O. formigenes*). In some examples, the at least one heterologous gene encoding at least one oxalate catabolism enzyme is selected from a *frc* gene and an *oxc* gene. In one example, the at least one heterologous gene encoding an oxalate transporter is an *oxlT* gene. In some examples, the at least one heterologous gene encoding at least one oxalate catabolism enzyme is located on a plasmid in the bacterial cell. In some examples, the at least one heterologous gene encoding at least one oxalate catabolism enzyme is located on a chromosome in the bacterial cell. In some examples, the at least one heterologous gene encoding an oxalate transporter is located on a plasmid in the bacterial cell. In some examples, the at least one heterologous gene encoding the oxalate transporter is located on a chromosome in the bacterial cell. In some examples, the at least one heterologous gene encoding a formate exporter is located on a plasmid in the bacterial cell. In some examples, the at least one heterologous gene encoding a formate exporter is located on a chromosome in the bacterial cell. In some examples, the at least one heterologous gene encoding an oxalate:formate antiporter is located on a plasmid in the bacterial cell. In some examples, the at least one heterologous gene encoding an oxalate:formate antiporter is located on a chromosome in the bacterial cell.

[017] In some examples, the engineered bacterial cell is a probiotic bacterial cell. In some examples, the engineered bacterial cell is a member of a genus selected from the group consisting of *Bacteroides*, *Bifidobacterium*, *Clostridium*, *Escherichia*, *Lactobacillus* and *Lactococcus*. In some examples, the engineered bacterial cell is of the genus *Escherichia*. In some examples, the recombinant bacterial cell is of the species *Escherichia coli* strain Nissle.

[018] In some examples, the engineered bacterial cell is an auxotroph in a gene that is complemented when the engineered bacterial cell is present in a mammalian gut. In some examples, the mammalian gut is a human gut. In some examples, the engineered bacterial cell is an auxotroph in diaminopimelic acid or an enzyme in the thymine biosynthetic pathway. In some examples, the engineered bacterial cell further comprises a heterologous gene encoding a substance that is toxic to the bacterial cell that is operably linked to an inducible promoter, wherein the inducible promoter is directly or indirectly induced by an environmental condition not naturally present in the mammalian gut.

[019] In another example, the disclosure provides a pharmaceutical composition comprising a recombinant bacterial cell comprising at least one heterologous gene encoding at least one oxalate catabolism enzyme operably linked to a first inducible promoter and a pharmaceutically acceptable carrier. In another example, the disclosure provides a pharmaceutical composition comprising a recombinant bacterial cell comprising at least one heterologous gene encoding at least one oxalate catabolism enzyme operably linked to a first inducible promoter, at least one heterologous gene encoding an oxalate transporter operably linked to a second inducible promoter, which may be the

same or different promoter from the first inducible promoter, and a pharmaceutically acceptable carrier. In another example, the disclosure provides a pharmaceutical composition comprising a recombinant bacterial cell comprising at least one heterologous gene encoding at least one oxalate catabolism enzyme operably linked to a first inducible promoter, at least one heterologous gene encoding a formate exporter operably linked to a second inducible promoter, which may be the same or different promoter from the first inducible promoter, and a pharmaceutically acceptable carrier. In another example, the disclosure provides a pharmaceutical composition comprising a recombinant bacterial cell comprising at least one heterologous gene encoding at least one oxalate catabolism enzyme operably linked to a first inducible promoter, at least one heterologous gene encoding an oxalate:formate antiporter operably linked to a second inducible promoter, which may be the same or different promoter from the first inducible promoter, and a pharmaceutically acceptable carrier. In any of these examples, the first promoter and the second promoter may be separate copies of the same promoter. In some examples, the first inducible promoter, the second inducible promoter, or the first inducible promoter and the second inducible promoter, are each directly induced by environmental conditions. In some examples, the first inducible promoter, the second inducible promoter, or the first inducible promoter and the second inducible promoter, are each indirectly induced by environmental conditions. In some examples, the first inducible promoter, the second inducible promoter, or the first inducible promoter and the second inducible promoter, are each directly or indirectly induced by low-oxygen or anaerobic conditions. In some examples, the first inducible promoter, the second inducible promoter, or the first inducible promoter and the second inducible promoter, are each directly or indirectly induced by inflammatory conditions. In some examples, the first inducible promoter, the second inducible promoter, or the first inducible promoter and the second inducible promoter, are each an FNR responsive promoter. In some examples, the first inducible promoter, the second inducible promoter, or the first inducible promoter and the second inducible promoter, are each an RNSresponsive promoter. In some examples, the first inducible promoter, the second inducible promoter, or the first inducible promoter and the second inducible promoter, are each an ROS responsive promoter. In another example, the disclosure provides a method for treating a disease or disorder in which oxalate is detrimental in a subject, the method comprising administering a an engineered bacterial cell or a pharmaceutical composition comprising an engineered bacterial cell to the subject, wherein the engineered bacterial cell comprises gene sequence encoding one or more oxalate catabolism enzyme(s). In another example, the disclosure provides a method for treating a disease or disorder in which oxalate is detrimental in a subject, the method comprising administering an engineered bacterial cell or a pharmaceutical composition comprising an engineered bacterial cell to the subject, wherein the engineered bacterial cell comprises gene sequence encoding one or more

oxalate transporter(s). In another example, the disclosure provides a method for treating a disease or disorder in which oxalate is detrimental in a subject, the method comprising administering an engineered bacterial cell or a pharmaceutical composition comprising an engineered bacterial cell to the subject, wherein the engineered bacterial cell comprises gene sequence encoding one or more formate exporter(s). In another example, the disclosure provides a method for treating a disease or disorder in which oxalate is detrimental in a subject, the method comprising administering an engineered bacterial cell or a pharmaceutical composition comprising an engineered bacterial cell to the subject, wherein the engineered bacterial cell comprises gene sequence encoding one or more oxalate:formate antiporter(s). In another example, the disclosure provides a method for treating a disease or disorder in which oxalate is detrimental in a subject, the method comprising administering an engineered bacterial cell or a pharmaceutical composition comprising an engineered bacterial cell to the subject, wherein the engineered bacterial cell comprises gene sequence encoding one or more of the following: (i) oxalate catabolism enzyme(s); (ii) one or more oxalate transporter(s); (iii) one or more formate exporter(s); and (iv) one or more oxalate:formate antiporter(s).

[020] In another example, the disclosure provides a method for treating a disease or disorder in which oxalate is detrimental in a subject, the method comprising administering an engineered bacterial cell or a pharmaceutical composition comprising an engineered bacterial cell to the subject, wherein the engineered bacterial cell expresses at least one heterologous gene encoding at least one oxalate catabolism enzyme in response to an exogenous environmental condition in the subject, thereby treating the disease or disorder in which oxalate is detrimental in the subject. In some examples, the engineered bacterial cell further expresses one or more of the following: (i) at least one heterologous gene encoding an importer of oxalate; (ii) at least one heterologous gene encoding an exporter of formate; and/or (iii) at least one heterologous gene encoding an oxalate:formate antiporter. In one example, the disclosure provides a method for treating a disorder in which oxalate is detrimental in a subject, the method comprising administering an engineered bacterial cell or a pharmaceutical composition of the disclosure to the subject, thereby treating the disorder in which oxalate is detrimental in the subject. In another example, the disclosure provides a method for decreasing a level of oxalate in plasma of a subject, the method comprising administering an engineered bacterial cell or a pharmaceutical composition of the disclosure to the subject, thereby decreasing the level of oxalate in the plasma of the subject. In another example, the disclosure provides a method for decreasing a level of oxalate in urine of a subject, the method comprising administering an engineered bacterial cell or a pharmaceutical composition of the disclosure to the subject, thereby decreasing the level of oxalate in the urine of the subject. In one example, the level of oxalate is decreased in plasma of the subject after administering the engineered bacterial cell or pharmaceutical composition to the subject. In another example, the level of oxalate is reduced in urine of the subject after administering the engineered bacterial cell or pharmaceutical composition to the subject. In one example, the engineered bacterial cell or pharmaceutical composition is administered

orally. In another example, the method further comprises isolating a plasma sample from the subject or a urine sample from the subject after administering the engineered bacterial cell or pharmaceutical composition to the subject, and determining the level of oxalate in the plasma sample from the subject or the urine sample from the subject. In another example, the method further comprises comparing the level of oxalate in the plasma sample from the subject or the urine sample from the subject to a control level of oxalate. In one example, the control level of oxalate is the level of oxalate in the plasma of the subject or in the urine of the subject before administration of the engineered bacterial cell or pharmaceutical composition.

[021] In one example, the disorder in which oxalate is detrimental is a hyperoxaluria. In one example, the hyperoxaluria is primary hyperoxaluria type I. In another example, the hyperoxaluria is primary hyperoxaluria type II. In another example, the hyperoxaluria is primary hyperoxaluria type III. In one example, the hyperoxaluria is enteric hyperoxaluria. In another example, the hyperoxaluria is dietary hyperoxaluria. In another example, the hyperoxaluria is idiopathic hyperoxaluria.

[022] In one example, the subject is fed a meal within one hour of administering the pharmaceutical composition. In another example, the subject is fed a meal concurrently with administering the pharmaceutical composition.

#### **Brief Description of the Drawings**

[022] **FIG. 1** depicts a graph showing the results of an *in vitro* oxalate degradation assay using an engineered *E. coli* Nissle strain as compared to a wild type *E. coli* Nissle strain.

[023] **FIG. 2A** depicts a bar graph showing the results of an *in vivo* oxalate consumption experiment by measuring acute <sup>13</sup>C-oxalate urinary recovery when using an engineered *E. coli* Nissle strain (Engineered EcN) as compared to a wild type *E. coli* Nissle strain (EcN). **FIG. 2B** depicts a bar graph showing the results of an *in vivo* oxalate consumption experiment by measuring chronic urinary oxalate recovery when using an engineered *E. coli* Nissle strain (Engineered EcN) as compared to a wild type *E. coli* Nissle strain (EcN).

[024] **FIG. 3** is a figure summarizing the disease pathogenesis of enteric hyperoxaluria.

[025] **FIG. 4A** depicts the components of strain SYN8802, and **FIG. 4B** depicts a graph showing the results of an *in vitro* oxalate degradation assay using SYN8802 as compared to a wild type *E. coli* Nissle strain. **FIG. 4C** depicts a graph showing the results of an *in vitro* oxalate degradation and formate production assay using SYN8802 as compared to a wild type *E. coli* Nissle strain.

[026] **FIG. 5A** depicts oxalate consumption with SYN-HOX (SYN5752) when SYN-HOX was activated in simulated stomach and colon fluid. **FIG. 5B** depicts that an engineered *E. coli* Nissle strain (Engineered EcN), SYN5752 consumed oxalate in mice in the gut. SYN5752 is an integrated strain with antibiotic resistance. SYN7169 is an integrated strain with antibiotic resistance,

## CLAIMS

1. Use of a pharmaceutical composition in the manufacture of a medicament for reducing the levels of oxalate in a subject, wherein the pharmaceutical composition comprises a recombinant bacterium comprising one or more gene sequences encoding one or more oxalate catabolism enzymes operably linked to a directly or indirectly first promoter that is not associated with the oxalate catabolism enzyme gene in nature.
2. The use of claim 1, wherein the recombinant bacterium has an oxalate consumption activity of at least about  $1\mu\text{mol}/1\times 10^9$  cell.
3. The use of claim 1 or claim 2, wherein the recombinant bacterium has an oxalate consumption activity of 50 to 600 mg/day under anaerobic conditions.
4. The use of claim 3, wherein the recombinant bacterium has an oxalate consumption activity of about 211 mg/day under anaerobic conditions.
5. The use of any one of the previous claims, wherein the recombinant bacterium has an oxalate consumption activity of about 211 mg/day under anaerobic conditions when it is to be administered to the subject three times per day.
6. The use of any one of claims 3-5, wherein the anaerobic conditions are conditions in the intestine and/or colon of the subject.
7. The use of any one of the previous claims, wherein the medicament reduces acute levels of oxalate in the subject by about two fold.
8. The use of any one of claims 1-6, wherein the medicament reduces acute levels of oxalate in the subject by about three fold.
9. The use of any one of claims 1-6, wherein the medicament reduces chronic levels of oxalate in the subject by about two fold.
10. The use of any one of claims 1-6, wherein the medicament reduces chronic levels of oxalate in the subject by about three fold.
11. The use of any one of claims 1-6, wherein the medicament reduces acute levels of oxalate in the subject to about 25 mg/day, about 30 mg/day, about 40 mg/day, about 50 mg/day, about 60 mg/day, about 70 mg/day, about 80 mg/day, about 90 mg/day, or about 100 mg/day.

12. The use of any one of claims 1-6, wherein the medicament reduces chronic levels of oxalate in the subject to about 25 mg/day, about 30 mg/day, about 40 mg/day, about 50 mg/day, about 60 mg/day, about 70 mg/day, about 80 mg/day, about 90 mg/day, or about 100 mg/day.
13. The use of any one of the previous claims, wherein the recombinant bacterium is of the genus *Escherichia*.
14. The use of claim 13, wherein the recombinant bacterium is of the species *Escherichia coli* strain Nissle.
15. The use of any one of the previous claims, wherein the pharmaceutical composition is to be administered orally.
16. The use of any one of the previous claims, wherein the subject is to be fed a meal within one hour of administering the pharmaceutical composition.
17. The use of any one of claims 1-15, wherein the subject is to be fed a meal concurrently with administering the pharmaceutical composition.
18. The use of any one of the previous claims, wherein the subject is a human subject.
19. The use of any one of the previous claims, wherein the one or more gene sequences comprise a *scaaE3* gene, an *frc* gene, and an *oxdC* gene.
20. The use of claim 19, wherein the *scaaE3* gene comprises a sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to, comprises, or consists of SEQ ID NO:3.
21. The use of claim 19 or claim 20, wherein the *frc* gene comprises a sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to, comprises, or consists of SEQ ID NO:1.
22. The use of any one of claims 19-21, wherein the *oxdC* gene comprises a sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to, comprises, or consists of SEQ ID NO:2.
23. The use of any one of the previous claims, wherein the recombinant bacterium further comprises a gene encoding an oxalate importer.

24. The use of claim 23, wherein the gene encoding the oxalate importer is an *oxlT* gene.
25. The use of claim 24, wherein the *oxlT* gene comprises a sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to, comprises, or consists of SEQ ID NO: 11.
26. The use of any one of the previous claims, wherein the recombinant bacterium further comprises an auxotrophy.
27. The use of claim 26, wherein the auxotrophy is a *thyA* auxotrophy.
28. The use of any one of the previous claims, wherein the recombinant bacterium further comprises a deletion in an endogenous phage.
29. The use of claim 26, wherein the endogenous phage comprises a sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to, comprises, or consists of SEQ ID NO: 63.
30. The use of any one of the previous claims, wherein the recombinant bacterium does not comprise a gene encoding for antibiotic resistance.
31. The use of any one of the previous claims, wherein the first promoter is an inducible promoter, optionally when the inducible promoter is a FNR promoter, optionally wherein the FNR promoter comprises a sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to, comprises, or consists of any one of SEQ ID NOs:13-29.
32. The use of any one of claims 1-17, wherein the recombinant bacterium is SYN5752, SYN7169, or SYN8802.
33. The use of any one of the previous claims, wherein the subject has hyperoxaluria.
34. The use of claim 33, wherein the hyperoxaluria is primary hyperoxaluria, dietary hyperoxaluria, or enteric hyperoxaluria.
35. The use of claim 33 or claim 34, wherein the subject has short bowel syndrome, chronic pancreatitis, inflammatory bowel disease (IBD), cystic fibrosis, kidney disease, and/or Roux-en-Y gastric bypass.

36. The use of any one of the previous claims, wherein the subject has urinary oxalate (Uox) levels of at least 70 mg/day prior to the administering.
37. The use of any one of the previous claims, wherein the subject exhibits a decrease in Uox levels of at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, or 80% after the administering.
38. The use of any one of the previous claims, wherein the subject has eGFR < 30 mL/min/1.73 m<sup>2</sup>, requires hemodialysis, or has systemic oxalosis prior to the administering.
39. The use of any one of the previous claims, wherein the recombinant bacteria is to be administered at a dose of about  $1 \times 10^{11}$  live recombinant bacteria, about  $3 \times 10^{11}$  live recombinant bacteria, about  $6 \times 10^{11}$  live recombinant bacteria, about  $1 \times 10^{12}$  live recombinant bacteria, or about  $2 \times 10^{12}$  live recombinant bacteria.
40. The use of any one of claims 1-38, wherein the recombinant bacteria is to be administered at a dose of about  $6 \times 10^{11}$  live recombinant bacteria.
41. The use of any one of claims 1-38, wherein the recombinant bacteria is to be administered at a dose of about  $2 \times 10^{12}$  live recombinant bacteria.
42. The use of any one of claims 1-38, wherein the recombinant bacteria is to be administered at a dose of about  $1 \times 10^{12}$  live recombinant bacteria.
43. The use of any one of claims 1-38, wherein the recombinant bacteria is to be administered at a dose of about  $1 \times 10^{11}$  live recombinant bacteria.
44. The use of any one of claims 1-38, wherein the recombinant bacteria is to be administered at a dose of about  $3 \times 10^{11}$  live recombinant bacteria.
45. The use of any one of the previous claims, wherein the administration is about  $5 \times 10^{11}$  live recombinant bacteria with meals three times per day.
46. The use of any one of the previous claims, further comprises that a proton pump inhibitor (PPI) is to be administered to the subject.
47. The use of claim 46, wherein the PPI is esomeprazole.

48. The use of claim 46 or 47, wherein the administration of the PPI is once a day.
49. A recombinant bacterium comprising one or more gene sequences encoding one or more oxalate catabolism enzymes operably linked to a directly or indirectly first promoter that is not associated with the oxalate catabolism enzyme gene in nature.
50. The recombinant bacterium of claim 49, wherein the recombinant bacterium has an oxalate consumption activity of at least about  $1\mu\text{mol}/1\times 10^9$  cell.
51. The recombinant bacterium of claim 49 or claim 50, wherein the recombinant bacterium has an oxalate consumption activity of about 50-600 mg/day under anaerobic conditions.
52. The recombinant bacterium of any one of claims 45-50, wherein the recombinant bacterium is SYN8802, SYN7169, or SYN5752.
53. The recombinant bacterium of claim 52, wherein the recombinant bacterium is SYN8802.