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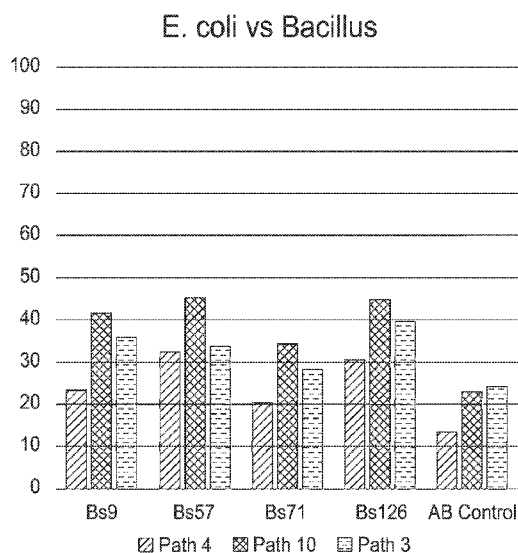


Fig. 1A

(57) Abstract: The invention relates to direct-fed microbials for use in *E. coli* inhibition in animals in combination with enrofloxacin. More particularly, the invention relates to isolated *Bacillus* strains 9, 57, 71, and 126, and strains having all of the identifying characteristics of these strains, for a use comprising the above-mentioned use.



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MICROBIALS AND ANTIBIOTICS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority under 35 U.S.C. § 119(e) to U.S. Provisional Application Serial No. 63/068,678 filed on August 21, 2020, the disclosure of which is
5 expressly incorporated by reference herein.

FIELD OF THE DISCLOSURE

The invention relates to direct-fed microbials for use in *E. coli* inhibition in animals in combination with the antibiotic enrofloxacin. More particularly, the invention
10 relates to isolated *Bacillus* strains 9, 57, 71, and 126 and strains having all of the identifying characteristics of these strains, for a use comprising the above-mentioned use in combination with the antibiotic enrofloxacin.

BACKGROUND AND SUMMARY OF THE INVENTION

15 The present invention relates to direct-fed microbial (DFM) compositions and methods for *E. coli* inhibition in an animal. An animal's gastrointestinal tract is constantly challenged by large numbers of bacteria, viruses, fungi, and protozoa found in feed, bedding, and the environment. The gastrointestinal tract has a sophisticated system to counter these potential pathogens consisting of physical, chemical, and immunological lines of defense.
20 Beneficial bacteria are an important part of this system because they provide animals with bacteria that assist in establishment (or reestablishment) of a normal bacterial profile, they strengthen the animal's immune system, and they help to fight disease.

Antibiotic resistance is an increasingly important issue in human medicine, as well as in animal agriculture. The Food and Drug Administration reported that in 2016 the U.S.
25 swine industry purchased about 6.9 million pounds of medically important antibiotics, which emphasizes the importance of preventing the development of antibiotic resistant organisms in the animal agricultural industry. Methods are being developed in an effort to combat antibiotic resistance, but little has been done to utilize synergistic direct-fed microbials (e.g., probiotics) as tools to combat antibiotic resistance.

30 Applicants have developed direct-fed microbials that result in *E. coli* inhibition in animals in combination with enrofloxacin. The direct-fed microbials and enrofloxacin and

compositions comprising the direct-fed microbials and enrofloxacin described herein offer a commercial benefit by providing *E. coli* inhibition in animals, such as agricultural animals.

Methods are provided for inhibiting *E. coli* in animals. In various embodiments, the animal can be selected from the group consisting of a poultry species, a porcine species, a caprine species, a bovine species, an ovine species, an equine species, and a companion animal. In the embodiment where the animal is a poultry species, the poultry species can be selected from the group consisting of a broiler, a chicken, a layer, a breeder, a turkey, a turkey poult, a gosling, a duckling, a guineakeet, a pullet, a hen, a rooster, a cockerel, and a capon. In the embodiment where the animal is a porcine species, the porcine species can be selected from the group consisting of a grow finish pig, a nursery pig, a sow, and a breeding stock pig.

In one embodiment, a method of feeding an animal is provided. The method comprises the steps of administering to the animal a feed composition or drinking water comprising an effective amount of an additive comprising an isolated *Bacillus* strain selected from the group consisting of *Bacillus* strain 9 (NRRL No. B-67866), a strain having all of the identifying characteristics of *Bacillus* strain 9 (NRRL No. B-67866), *Bacillus* strain 57 (NRRL No. B-67870), a strain having all of the identifying characteristics of *Bacillus* strain 57 (NRRL No. B-67870), *Bacillus* strain 71 (NRRL No. B-67867), a strain having all of the identifying characteristics of *Bacillus* strain 71 (NRRL No. B-67867), *Bacillus* strain 126 (NRRL No. B-67868), a strain having all of the identifying characteristics of *Bacillus* strain 126 (NRRL No. B-67868), and combinations thereof, and administering to the animal an antibiotic, wherein the *Bacillus* strain and the antibiotic cause *E. coli* inhibition in the animal and wherein the antibiotic is enrofloxacin.

In another embodiment, a method of controlling a detrimental effect of *E. coli* is provided. The method comprises the steps of administering to an animal a feed composition or drinking water comprising an effective amount of an additive comprising an isolated *Bacillus* strain selected from the group consisting of *Bacillus* strain 9 (NRRL No. B-67866), a strain having all of the identifying characteristics of *Bacillus* strain 9 (NRRL No. B-67866), *Bacillus* strain 57 (NRRL No. B-67870), a strain having all of the identifying characteristics of *Bacillus* strain 57 (NRRL No. B-67870), *Bacillus* strain 71 (NRRL No. B-67867), a strain having all of the identifying characteristics of *Bacillus* strain 71 (NRRL No. B-67867), *Bacillus* strain 126 (NRRL No. B-67868), a strain having all of the identifying characteristics of *Bacillus* strain 126 (NRRL No. B-67868), and combinations thereof, and administering an antibiotic to the animal, and controlling the detrimental effect of *E. coli* in the animal wherein the antibiotic is enrofloxacin.

In yet another embodiment, a method of feeding an animal is provided. The method comprises the step of administering to the animal a feed composition or drinking water comprising an effective amount of an additive comprising an isolated *Bacillus* strain selected from the group consisting of *Bacillus* strain 9 (NRRL No. B-67866), a strain having all of the identifying characteristics of *Bacillus* strain 9 (NRRL No. B-67866), *Bacillus* strain 57 (NRRL No. B-67870), a strain having all of the identifying characteristics of *Bacillus* strain 57 (NRRL No. B-67870), *Bacillus* strain 71 (NRRL No. B-67867), a strain having all of the identifying characteristics of *Bacillus* strain 71 (NRRL No. B-67867), *Bacillus* strain 126 (NRRL No. B-67868), a strain having all of the identifying characteristics of *Bacillus* strain 126 (NRRL No. B-67868), and combinations thereof, and administering to the animal an antibiotic wherein the antibiotic is enrofloxacin.

The following clauses, and combinations thereof, provide various additional illustrative aspects of the invention described herein. The various embodiments described in any other section of this patent application, including the section titled "DETAILED DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS" and the EXAMPLES are applicable to any of the following embodiments of the invention described in the numbered clauses below.

1. A method of feeding an animal, the method comprising the steps of administering to the animal a feed composition or drinking water comprising an effective amount of an additive comprising an isolated *Bacillus* strain selected from the group consisting of *Bacillus* strain 9 (NRRL No. B-67866), a strain having all of the identifying characteristics of *Bacillus* strain 9 (NRRL No. B-67866), *Bacillus* strain 57 (NRRL No. B-67870), a strain having all of the identifying characteristics of *Bacillus* strain 57 (NRRL No. B-67870), *Bacillus* strain 71 (NRRL No. B-67867), a strain having all of the identifying characteristics of *Bacillus* strain 71 (NRRL No. B-67867), *Bacillus* strain 126 (NRRL No. B-67868), a strain having all of the identifying characteristics of *Bacillus* strain 126 (NRRL No. B-67868), and combinations thereof, and administering to the animal an antibiotic, wherein the *Bacillus* strain and the antibiotic cause *E. coli* inhibition in the animal and wherein the antibiotic is enrofloxacin.

2. The method of clause 1 wherein the animal is selected from the group consisting of a poultry species, a porcine species, a caprine species, a bovine species, an ovine species, an equine species, and a companion animal.

3. The method of any one of clauses 1 to 2 wherein the *E. coli* inhibition prevents *E. coli* disease in the animal.

4. The method of any one of clauses 1 to 2 wherein the *E. coli* inhibition reduces *E. coli* disease in the animal.

5. The method of any one of clauses 1 to 4 wherein the animal is a porcine species and the porcine species is selected from the group consisting of a grow finish pig, a nursery pig, a sow, and a breeding stock pig.

6. The method of any one of clauses 1 to 5 wherein the *Bacillus* strain produces an enzyme selected from the group consisting of an α -galactosidase, a protease, a lipase, an amylase, a xylanase, a cellulase, and combinations thereof.

7. The method of any one of clauses 1 to 6 further comprising the step of administering to the animal another different bacterial strain selected from the group consisting of another *Bacillus* strain, a lactic acid bacterial strain, and combinations thereof.

8. The method of any one of clauses 1 to 7 wherein the strain administered is *Bacillus* strain 9 (NRRL No. B-67866).

9. The method of any one of clauses 1 to 7 wherein the strain administered is *Bacillus* strain 57 (NRRL No. B-67870).

10. The method of any one of clauses 1 to 7 wherein the strain administered is *Bacillus* strain 71 (NRRL No. B-67867).

11. The method of any one of clauses 1 to 7 wherein the strain administered is *Bacillus* strain 126 (NRRL No. B-67868).

12. The method of any one of clauses 1 to 11 wherein the *Bacillus* strain is administered in the feed composition at a dose of about 1.0×10^3 CFU/gram of the feed composition to about 5.0×10^{12} CFU/gram of the feed composition.

13. The method of any one of clauses 1 to 11 wherein the *Bacillus* strain is administered in the feed composition at a dose of about 1.0×10^3 CFU/gram of the feed composition to about 1.0×10^7 CFU/gram of the feed composition.

14. The method of any one of clauses 1 to 11 wherein the *Bacillus* strain is administered in the feed composition at a dose greater than about 7.0×10^4 CFU/gram of the feed composition.

15. The method of any one of clauses 1 to 14 further comprising the step of administering to the animal an enzyme selected from the group consisting of a galactosidase, a protease, a lipase, an amylase, a hemicellulase, an arabinoxylanase, a xylanase, a cellulase, an NSPase, a phytase, and combinations thereof.

16. The method of any one of clauses 1 to 15 wherein the animal is a sow and the *Bacillus* strain is administered during lactation.

17. The method of any one of clauses 1 to 15 wherein the animal is a sow and the *Bacillus* strain is administered during gestation.

18. The method of any one of clauses 1 to 17 wherein the feed composition is administered daily to the animal.

19. The method of clause 1 wherein the animal is selected from the group consisting of a chicken, a pig, a horse, a pony, a cow, a turkey, a goat, a sheep, a quail, a pheasant, an ostrich, a duck, a fish, a crustacean, and combinations thereof.

20. A method of controlling a detrimental effect of *E. coli*, the method comprising the steps of administering to an animal a feed composition or drinking water comprising an effective amount of an additive comprising an isolated *Bacillus* strain selected from the group consisting of *Bacillus* strain 9 (NRRL No. B-67866), a strain having all of the identifying characteristics of *Bacillus* strain 9 (NRRL No. B-67866), *Bacillus* strain 57 (NRRL No. B-67870), a strain having all of the identifying characteristics of *Bacillus* strain 57 (NRRL No. B-67870), *Bacillus* strain 71 (NRRL No. B-67867), a strain having all of the identifying characteristics of *Bacillus* strain 71 (NRRL No. B-67867), *Bacillus* strain 126 (NRRL No. B-67868), a strain having all of the identifying characteristics of *Bacillus* strain 126 (NRRL No. B-67868), and combinations thereof, and administering an antibiotic to the animal, and controlling the detrimental effect of *E. coli* in the animal wherein the antibiotic is enrofloxacin.

21. The method of clause 20 wherein the animal is selected from the group consisting of a poultry species, a porcine species, a caprine species, a bovine species, an ovine species, an equine species, and a companion animal.

22. The method of any one of clauses 20 to 21 wherein controlling the detrimental effect of the *E. coli* is inhibiting *E. coli* disease in the animal.

23. The method of any one of clauses 20 to 21 wherein controlling the detrimental effect of the *E. coli* is reducing *E. coli* disease in the animal.

24. The method of any one of clauses 20 to 23 wherein the animal is a porcine species and the porcine species is selected from the group consisting of a grow finish pig, a nursery pig, a sow, and a breeding stock pig.

25. The method of any one of clauses 20 to 24 wherein the *Bacillus* strain produces an enzyme selected from the group consisting of an α -galactosidase, a protease, a lipase, an amylase, a xylanase, a cellulase, and combinations thereof.

26. The method of any one of clauses 20 to 25 further comprising the step of administering to the animal another different bacterial strain selected from the group consisting of another *Bacillus* strain, a lactic acid bacterial strain, and combinations thereof.

27. The method of any one of clauses 20 to 26 wherein the strain administered is *Bacillus* strain 9 (NRRL No. B-67866).

28. The method of any one of clauses 20 to 26 wherein the strain administered is *Bacillus* strain 57 (NRRL No. B-67870).

29. The method of any one of clauses 20 to 26 wherein the strain administered is *Bacillus* strain 71 (NRRL No. B-67867).

5 30. The method of any one of clauses 20 to 26 wherein the strain administered is *Bacillus* strain 126 (NRRL No. B-67868).

31. The method of any one of clauses 20 to 30 wherein the *Bacillus* strain is administered in the feed composition at a dose of about 1.0×10^3 CFU/gram of the feed composition to about 5.0×10^{12} CFU/gram of the feed composition.

10 32. The method of any one of clauses 20 to 30 wherein the *Bacillus* strain is administered in the feed composition at a dose of about 1.0×10^3 CFU/gram of the feed composition to about 1.0×10^7 CFU/gram of the feed composition.

33. The method of any one of clauses 20 to 30 wherein the *Bacillus* strain is administered in the feed composition at a dose greater than about 7.0×10^4 CFU/gram of the feed composition.

34. The method of any one of clauses 20 to 33 further comprising the step of administering to the animal an enzyme selected from the group consisting of a galactosidase, a protease, a lipase, an amylase, a hemicellulase, an arabinoxylanase, a xylanase, a cellulase, an NSPase, a phytase, and combinations thereof.

20 35. The method of any one of clauses 20 to 34 wherein the animal is a sow and the *Bacillus* strain is administered during lactation.

36. The method of any one of clauses 20 to 34 wherein the animal is a sow and the *Bacillus* strain is administered during gestation.

25 37. The method of any one of clauses 20 to 36 wherein the feed composition is administered daily to the animal.

38. The method of clause 20 wherein the animal is selected from the group consisting of a chicken, a pig, a horse, a pony, a cow, a turkey, a goat, a sheep, a quail, a pheasant, an ostrich, a duck, a fish, a crustacean, and combinations thereof.

30 39. A method of feeding an animal, the method comprising the step of administering to the animal a feed composition or drinking water comprising an effective amount of an additive comprising an isolated *Bacillus* strain selected from the group consisting of *Bacillus* strain 9 (NRRL No. B-67866), a strain having all of the identifying characteristics of *Bacillus* strain 9 (NRRL No. B-67866), *Bacillus* strain 57 (NRRL No. B-67870), a strain having all of the identifying characteristics of *Bacillus* strain 57 (NRRL No. B-67870), *Bacillus* strain 71 (NRRL No. B-67867), a strain having all of the identifying characteristics of *Bacillus* strain

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71 (NRRL No. B-67867), *Bacillus* strain 126 (NRRL No. B-67868), a strain having all of the identifying characteristics of *Bacillus* strain 126 (NRRL No. B-67868), and combinations thereof, and administering to the animal an antibiotic wherein the antibiotic is enrofloxacin.

40. The method of clause 39 wherein the animal is selected from the group consisting of a poultry species, a porcine species, a caprine species, a bovine species, an ovine species, an equine species, and a companion animal.

41. The method of any one of clauses 39 to 40 wherein the animal is a porcine species and the porcine species is selected from the group consisting of a grow finish pig, a nursery pig, a sow, and a breeding stock pig.

10 42. The method of any one of clauses 39 to 41 wherein the *Bacillus* strain produces an enzyme selected from the group consisting of an α -galactosidase, a protease, a lipase, an amylase, a xylanase, a cellulase, and combinations thereof.

43. The method of any one of clauses 39 to 42 further comprising the step of administering to the animal another different bacterial strain selected from the group consisting of another *Bacillus* strain, a lactic acid bacterial strain, and combinations thereof.

44. The method of any one of clauses 39 to 43 wherein the strain administered is *Bacillus* strain 9 (NRRL No. B-67866).

45. The method of any one of clauses 39 to 43 wherein the strain administered is *Bacillus* strain 57 (NRRL No. B-67870).

20 46. The method of any one of clauses 39 to 43 wherein the strain administered is *Bacillus* strain 71 (NRRL No. B-67867).

47. The method of any one of clauses 39 to 43 wherein the strain administered is *Bacillus* strain 126 (NRRL No. B-67868).

25 48. The method of any one of clauses 39 to 47 wherein the *Bacillus* strain is administered in the feed composition at a dose of about 1.0×10^3 CFU/gram of the feed composition to about 5.0×10^{12} CFU/gram of the feed composition.

49. The method of any one of clauses 39 to 47 wherein the *Bacillus* strain is administered in the feed composition at a dose of about 1.0×10^3 CFU/gram of the feed composition to about 1.0×10^7 CFU/gram of the feed composition.

30 50. The method of any one of clauses 39 to 47 wherein the *Bacillus* strain is administered in the feed composition at a dose greater than about 7.0×10^4 CFU/gram of the feed composition.

51. The method of any one of clauses 39 to 50 further comprising the step of administering to the animal an enzyme selected from the group consisting of a galactosidase, a

protease, a lipase, an amylase, a hemicellulase, an arabinoxylanase, a xylanase, a cellulase, an NSPase, a phytase, and combinations thereof.

52. The method of any one of clauses 39 to 51 wherein the animal is a sow and the *Bacillus* strain is administered during lactation.

5 53. The method of any one of clauses 39 to 51 wherein the animal is a sow and the *Bacillus* strain is administered during gestation.

54. The method of any one of clauses 39 to 53 wherein the feed composition is administered daily to the animal.

10 55. The method of clause 39 wherein the animal is selected from the group consisting of a chicken, a pig, a horse, a pony, a cow, a turkey, a goat, a sheep, a quail, a pheasant, an ostrich, a duck, a fish, a crustacean, and combinations thereof.

56. The method of any one of clauses 1 to 55 wherein the *Bacillus* strain has an effect selected from the group consisting of maintaining microbial balance in the gut of the animal, improving animal performance or health, maintaining gut health in the animal, reducing detrimental pathogens in the gut of the animal, odor reduction, reducing detrimental pathogens in the urine or feces of the animal, and preserving the growth of beneficial bacteria in the gut of the animal.

BRIEF DESCRIPTION OF THE DRAWINGS

20 Figs. 1A-B show percent inhibition values from 3 of 15 *E. coli* showing an improved inhibition response when *Bacillus* supernatant and Enrofloxacin are combined. IGS.

Fig. 2 is a photograph displaying RAPD PCR profiles for *Bacillus* strain 9 (NRRL No. B-67866) and *Bacillus* strain 57 (NRRL No. B-67870).

25 Fig. 3 is a photograph displaying RAPD PCR profiles for *Bacillus* strain 71 (NRRL No. B-67867) and *Bacillus* strain 126 (NRRL No. B-67868).

DETAILED DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

Methods are provided for inhibiting *E. coli* in animals. In various embodiments, the animal can be selected from the group consisting of a poultry species, a porcine species, a caprine species, a bovine species, an ovine species, an equine species, and a companion animal. In the embodiment where the animal is a poultry species, the poultry species can be selected from the group consisting of a broiler, a chicken, a layer, a breeder, a turkey, a turkey poult, a gosling, a duckling, a guineakeet, a pullet, a hen, a rooster, a cockerel, and a capon. In the embodiment where the animal is a porcine species, the porcine species can be selected from the group consisting of a grow finish pig, a nursery pig, a sow, and a breeding stock pig.

In one embodiment, a method of feeding an animal is provided. The method comprises the steps of administering to the animal a feed composition or drinking water comprising an effective amount of an additive comprising an isolated *Bacillus* strain selected from the group consisting of *Bacillus* strain 9 (NRRL No. B-67866), a strain having all of the identifying characteristics of *Bacillus* strain 9 (NRRL No. B-67866), *Bacillus* strain 57 (NRRL No. B-67870), a strain having all of the identifying characteristics of *Bacillus* strain 57 (NRRL No. B-67870), *Bacillus* strain 71 (NRRL No. B-67867), a strain having all of the identifying characteristics of *Bacillus* strain 71 (NRRL No. B-67867), *Bacillus* strain 126 (NRRL No. B-67868), a strain having all of the identifying characteristics of *Bacillus* strain 126 (NRRL No. B-67868), and combinations thereof, and administering to the animal an antibiotic, wherein the *Bacillus* strain and the antibiotic cause *E. coli* inhibition in the animal and wherein the antibiotic is enrofloxacin.

In another embodiment, a method of controlling a detrimental effect of *E. coli* is provided. The method comprises the steps of administering to an animal a feed composition or drinking water comprising an effective amount of an additive comprising an isolated *Bacillus* strain selected from the group consisting of *Bacillus* strain 9 (NRRL No. B-67866), a strain having all of the identifying characteristics of *Bacillus* strain 9 (NRRL No. B-67866), *Bacillus* strain 57 (NRRL No. B-67870), a strain having all of the identifying characteristics of *Bacillus* strain 57 (NRRL No. B-67870), *Bacillus* strain 71 (NRRL No. B-67867), a strain having all of the identifying characteristics of *Bacillus* strain 71 (NRRL No. B-67867), *Bacillus* strain 126 (NRRL No. B-67868), a strain having all of the identifying characteristics of *Bacillus* strain 126 (NRRL No. B-67868), and combinations thereof, and administering an antibiotic to the animal wherein the antibiotic is enrofloxacin, and controlling the detrimental effect of *E. coli* in the animal.

In yet another embodiment, a method of feeding an animal is provided. The method comprises the step of administering to the animal a feed composition or drinking water comprising an effective amount of an additive comprising an isolated *Bacillus* strain selected from the group consisting of *Bacillus* strain 9 (NRRL No. B-67866), a strain having all of the identifying characteristics of *Bacillus* strain 9 (NRRL No. B-67866), *Bacillus* strain 57 (NRRL No. B-67870), a strain having all of the identifying characteristics of *Bacillus* strain 57 (NRRL No. B-67870), *Bacillus* strain 71 (NRRL No. B-67867), a strain having all of the identifying characteristics of *Bacillus* strain 71 (NRRL No. B-67867), *Bacillus* strain 126 (NRRL No. B-67868), a strain having all of the identifying characteristics of *Bacillus* strain 126 (NRRL No. B-67868), and combinations thereof, and administering an antibiotic to the animal wherein the antibiotic is enrofloxacin.

The following clauses, and combinations thereof, provide various additional illustrative aspects of the invention described herein. The various embodiments described in this section titled "DETAILED DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS" are applicable to any of the following embodiments of the invention described in the numbered
5 clauses below.

1. A method of feeding an animal, the method comprising the steps of administering to the animal a feed composition or drinking water comprising an effective amount of an additive comprising an isolated *Bacillus* strain selected from the group consisting of *Bacillus* strain 9 (NRRL No. B-67866), a strain having all of the identifying characteristics of
10 *Bacillus* strain 9 (NRRL No. B-67866), *Bacillus* strain 57 (NRRL No. B-67870), a strain having all of the identifying characteristics of *Bacillus* strain 57 (NRRL No. B-67870), *Bacillus* strain 71 (NRRL No. B-67867), a strain having all of the identifying characteristics of *Bacillus* strain 71 (NRRL No. B-67867), *Bacillus* strain 126 (NRRL No. B-67868), a strain having all of the identifying characteristics of *Bacillus* strain 126 (NRRL No. B-67868), and combinations
15 thereof, and administering to the animal an antibiotic, wherein the *Bacillus* strain and the antibiotic cause *E. coli* inhibition in the animal and wherein the antibiotic is enrofloxacin.

2. The method of clause 1 wherein the animal is selected from the group consisting of a poultry species, a porcine species, a caprine species, a bovine species, an ovine species, an equine species, and a companion animal.

20 3. The method of any one of clauses 1 to 2 wherein the *E. coli* inhibition prevents *E. coli* disease in the animal.

4. The method of any one of clauses 1 to 2 wherein the *E. coli* inhibition reduces *E. coli* disease in the animal.

5. The method of any one of clauses 1 to 4 wherein the animal is a porcine
25 species and the porcine species is selected from the group consisting of a grow finish pig, a nursery pig, a sow, and a breeding stock pig.

6. The method of any one of clauses 1 to 5 wherein the *Bacillus* strain produces an enzyme selected from the group consisting of an α -galactosidase, a protease, a lipase, an amylase, a xylanase, a cellulase, and combinations thereof.

30 7. The method of any one of clauses 1 to 6 further comprising the step of administering to the animal another different bacterial strain selected from the group consisting of another *Bacillus* strain, a lactic acid bacterial strain, and combinations thereof.

8. The method of any one of clauses 1 to 7 wherein the strain administered is *Bacillus* strain 9 (NRRL No. B-67866).

9. The method of any one of clauses 1 to 7 wherein the strain administered is *Bacillus* strain 57 (NRRL No. B-67870).

10. The method of any one of clauses 1 to 7 wherein the strain administered is *Bacillus* strain 71 (NRRL No. B-67867).

5 11. The method of any one of clauses 1 to 7 wherein the strain administered is *Bacillus* strain 126 (NRRL No. B-67868).

12. The method of any one of clauses 1 to 11 wherein the *Bacillus* strain is administered in the feed composition at a dose of about 1.0×10^3 CFU/gram of the feed composition to about 5.0×10^{12} CFU/gram of the feed composition.

10 13. The method of any one of clauses 1 to 11 wherein the *Bacillus* strain is administered in the feed composition at a dose of about 1.0×10^3 CFU/gram of the feed composition to about 1.0×10^7 CFU/gram of the feed composition.

14. The method of any one of clauses 1 to 11 wherein the *Bacillus* strain is administered in the feed composition at a dose greater than about 7.0×10^4 CFU/gram of the feed composition.

15 15. The method of any one of clauses 1 to 14 further comprising the step of administering to the animal an enzyme selected from the group consisting of a galactosidase, a protease, a lipase, an amylase, a hemicellulase, an arabinoxylanase, a xylanase, a cellulase, an NSPase, a phytase, and combinations thereof.

20 16. The method of any one of clauses 1 to 15 wherein the animal is a sow and the *Bacillus* strain is administered during lactation.

17. The method of any one of clauses 1 to 15 wherein the animal is a sow and the *Bacillus* strain is administered during gestation.

25 18. The method of any one of clauses 1 to 17 wherein the feed composition is administered daily to the animal.

19. The method of clause 1 wherein the animal is selected from the group consisting of a chicken, a pig, a horse, a pony, a cow, a turkey, a goat, a sheep, a quail, a pheasant, an ostrich, a duck, a fish, a crustacean, and combinations thereof.

30 20. A method of controlling a detrimental effect of *E. coli*, the method comprising the steps of administering to an animal a feed composition or drinking water comprising an effective amount of an additive comprising an isolated *Bacillus* strain selected from the group consisting of *Bacillus* strain 9 (NRRL No. B-67866), a strain having all of the identifying characteristics of *Bacillus* strain 9 (NRRL No. B-67866), *Bacillus* strain 57 (NRRL No. B-67870), a strain having all of the identifying characteristics of *Bacillus* strain 57 (NRRL No. B-67870), *Bacillus* strain 71 (NRRL No. B-67867), a strain having all of the identifying

characteristics of *Bacillus* strain 71 (NRRL No. B-67867), *Bacillus* strain 126 (NRRL No. B-67868), a strain having all of the identifying characteristics of *Bacillus* strain 126 (NRRL No. B-67868), and combinations thereof, and administering an antibiotic to the animal, and controlling the detrimental effect of *E. coli* in the animal wherein the antibiotic is enrofloxacin.

5 21. The method of clause 20 wherein the animal is selected from the group consisting of a poultry species, a porcine species, a caprine species, a bovine species, an ovine species, an equine species, and a companion animal.

 22. The method of any one of clauses 20 to 21 wherein controlling the detrimental effect of the *E. coli* is inhibiting *E. coli* disease in the animal.

10 23. The method of any one of clauses 20 to 21 wherein controlling the detrimental effect of the *E. coli* is reducing *E. coli* disease in the animal.

 24. The method of any one of clauses 20 to 23 wherein the animal is a porcine species and the porcine species is selected from the group consisting of a grow finish pig, a nursery pig, a sow, and a breeding stock pig.

15 25. The method of any one of clauses 20 to 24 wherein the *Bacillus* strain produces an enzyme selected from the group consisting of an α -galactosidase, a protease, a lipase, an amylase, a xylanase, a cellulase, and combinations thereof.

 26. The method of any one of clauses 20 to 25 further comprising the step of administering to the animal another different bacterial strain selected from the group consisting
20 of another *Bacillus* strain, a lactic acid bacterial strain, and combinations thereof.

 27. The method of any one of clauses 20 to 26 wherein the strain administered is *Bacillus* strain 9 (NRRL No. B-67866).

 28. The method of any one of clauses 20 to 26 wherein the strain administered is *Bacillus* strain 57 (NRRL No. B-67870).

25 29. The method of any one of clauses 20 to 26 wherein the strain administered is *Bacillus* strain 71 (NRRL No. B-67867).

 30. The method of any one of clauses 20 to 26 wherein the strain administered is *Bacillus* strain 126 (NRRL No. B-67868).

30 31. The method of any one of clauses 20 to 30 wherein the *Bacillus* strain is administered in the feed composition at a dose of about 1.0×10^3 CFU/gram of the feed composition to about 5.0×10^{12} CFU/gram of the feed composition.

 32. The method of any one of clauses 20 to 30 wherein the *Bacillus* strain is administered in the feed composition at a dose of about 1.0×10^3 CFU/gram of the feed composition to about 1.0×10^7 CFU/gram of the feed composition.

33. The method of any one of clauses 20 to 30 wherein the *Bacillus* strain is administered in the feed composition at a dose greater than about 7.0×10^4 CFU/gram of the feed composition.

34. The method of any one of clauses 20 to 33 further comprising the step of administering to the animal an enzyme selected from the group consisting of a galactosidase, a protease, a lipase, an amylase, a hemicellulase, an arabinoxylanase, a xylanase, a cellulase, an NSPase, a phytase, and combinations thereof.

35. The method of any one of clauses 20 to 34 wherein the animal is a sow and the *Bacillus* strain is administered during lactation.

36. The method of any one of clauses 20 to 34 wherein the animal is a sow and the *Bacillus* strain is administered during gestation.

37. The method of any one of clauses 20 to 36 wherein the feed composition is administered daily to the animal.

38. The method of clause 20 wherein the animal is selected from the group consisting of a chicken, a pig, a horse, a pony, a cow, a turkey, a goat, a sheep, a quail, a pheasant, an ostrich, a duck, a fish, a crustacean, and combinations thereof.

39. A method of feeding an animal, the method comprising the step of administering to the animal a feed composition or drinking water comprising an effective amount of an additive comprising an isolated *Bacillus* strain selected from the group consisting of *Bacillus* strain 9 (NRRL No. B-67866), a strain having all of the identifying characteristics of *Bacillus* strain 9 (NRRL No. B-67866), *Bacillus* strain 57 (NRRL No. B-67870), a strain having all of the identifying characteristics of *Bacillus* strain 57 (NRRL No. B-67870), *Bacillus* strain 71 (NRRL No. B-67867), a strain having all of the identifying characteristics of *Bacillus* strain 71 (NRRL No. B-67867), *Bacillus* strain 126 (NRRL No. B-67868), a strain having all of the identifying characteristics of *Bacillus* strain 126 (NRRL No. B-67868), and combinations thereof, and administering to the animal an antibiotic wherein the antibiotic is enrofloxacin.

40. The method of clause 39 wherein the animal is selected from the group consisting of a poultry species, a porcine species, a caprine species, a bovine species, an ovine species, an equine species, and a companion animal.

41. The method of any one of clauses 39 to 40 wherein the animal is a porcine species and the porcine species is selected from the group consisting of a grow finish pig, a nursery pig, a sow, and a breeding stock pig.

42. The method of any one of clauses 39 to 41 wherein the *Bacillus* strain produces an enzyme selected from the group consisting of an α -galactosidase, a protease, a lipase, an amylase, a xylanase, a cellulase, and combinations thereof.

43. The method of any one of clauses 39 to 42 further comprising the step of administering to the animal another different bacterial strain selected from the group consisting of another *Bacillus* strain, a lactic acid bacterial strain, and combinations thereof.

44. The method of any one of clauses 39 to 43 wherein the strain
5 administered is *Bacillus* strain 9 (NRRL No. B-67866).

45. The method of any one of clauses 39 to 43 wherein the strain administered is *Bacillus* strain 57 (NRRL No. B-67870).

46. The method of any one of clauses 39 to 43 wherein the strain administered is *Bacillus* strain 71 (NRRL No. B-67867).

10 47. The method of any one of clauses 39 to 43 wherein the strain administered is *Bacillus* strain 126 (NRRL No. B-67868).

48. The method of any one of clauses 39 to 47 wherein the *Bacillus* strain is administered in the feed composition at a dose of about 1.0×10^3 CFU/gram of the feed composition to about 5.0×10^{12} CFU/gram of the feed composition.

15 49. The method of any one of clauses 39 to 47 wherein the *Bacillus* strain is administered in the feed composition at a dose of about 1.0×10^3 CFU/gram of the feed composition to about 1.0×10^7 CFU/gram of the feed composition.

50. The method of any one of clauses 39 to 47 wherein the *Bacillus* strain is administered in the feed composition at a dose greater than about 7.0×10^4 CFU/gram of the
20 feed composition.

51. The method of any one of clauses 39 to 50 further comprising the step of administering to the animal an enzyme selected from the group consisting of a galactosidase, a protease, a lipase, an amylase, a hemicellulase, an arabinoxylanase, a xylanase, a cellulase, an NSPase, a phytase, and combinations thereof.

25 52. The method of any one of clauses 39 to 51 wherein the animal is a sow and the *Bacillus* strain is administered during lactation.

53. The method of any one of clauses 39 to 51 wherein the animal is a sow and the *Bacillus* strain is administered during gestation.

54. The method of any one of clauses 39 to 53 wherein the feed composition
30 is administered daily to the animal.

55. The method of clause 39 wherein the animal is selected from the group consisting of a chicken, a pig, a horse, a pony, a cow, a turkey, a goat, a sheep, a quail, a pheasant, an ostrich, a duck, a fish, a crustacean, and combinations thereof.

56. The method of any one of clauses 1 to 55 wherein the *Bacillus* strain has
35 an effect selected from the group consisting of maintaining microbial balance in the gut of the

animal, improving animal performance or health, maintaining gut health in the animal, reducing detrimental pathogens in the gut of the animal, odor reduction, reducing detrimental pathogens in the urine or feces of the animal, and preserving the growth of beneficial bacteria in the gut of the animal.

5 In various embodiments, the animal to which a feed additive, a feed composition, or drinking water, and an antibiotic, wherein the antibiotic is enrofloxacin, as described herein is administered can be selected from the group consisting of a poultry species, a porcine species, a caprine species, a bovine species, an ovine species, an equine species, and a companion animal. In the embodiment where the animal is a companion animal, the
10 companion animal can be, for example, a canine species or a feline species. In the embodiment where the animal is a porcine species, the porcine species can be selected from the group consisting of a grow finish pig, a nursery pig, a sow, and a breeding stock pig. In various exemplary embodiments, the animal can be selected from the group consisting of a chicken (e.g., a broiler or a layer), a pig, a horse, a pony, a cow, a turkey, a goat, a sheep, a quail, a
15 pheasant, an ostrich, a duck, a fish (e.g., a tilapia, a catfish, a flounder, or a salmon), a crustacean (e.g., a shrimp or a crab), and combinations thereof. In another embodiment, the feed additive, feed composition, or drinking water, and the antibiotic wherein the antibiotic is enrofloxacin, as described herein, can be administered to an animal and the animal is a human.

 In various embodiments of the methods or compositions described herein, the
20 *Bacillus* strain and the antibiotic, wherein the antibiotic is enrofloxacin, can have an effect selected from the group consisting of maintaining microbial balance in the gut of the animal, preventing or reducing *E. coli* disease in the animal, improving animal performance or health, maintaining gut health in the animal, reducing detrimental pathogens in the gut of the animal, odor reduction, reducing detrimental pathogens in the urine or feces of the animal, and
25 preserving the growth of beneficial bacteria in the gut of the animal. In any of the embodiments described herein, the *Bacillus* strain can be a *Bacillus subtilis* strain or a *Bacillus licheniformis* strain.

 In any embodiments described herein, the *Bacillus* strains can be administered alone or in any combination, with enrofloxacin, or can be in the form of any composition
30 described herein. In one embodiment, the strain or strains are administered with enrofloxacin in the same or separate compositions. The *Bacillus* strains described herein, with enrofloxacin, can also be used in combination with other different microbial strains, including other *Bacillus* strains or *Lactobacillus* strains.

 In one embodiment of the invention, an effective amount of the *Bacillus* strain
35 can be administered to inhibit *E. coli* in the animal. As used herein, "inhibit *E. coli*" can mean

reducing *E. coli* disease, preventing *E. coli* disease, maintaining the normal microbial balance in the animal, reducing the number of detrimental *E. coli* organisms in the animal, reducing the activity of *E. coli* in the animal, or reducing the symptoms of *E. coli* disease in the animal, or combinations thereof. By “effective amount” is meant an amount of the *Bacillus* strain (e.g.,
5 strain 9, 57, 71, and 126) capable of *E. coli* inhibition or capable of controlling a detrimental effect of *E. coli*, as described below, by any mechanism.

In embodiments described herein wherein the compositions of the present invention comprising *Bacillus* strains 9, 57, 71, and/or 126, or strains having their identifying characteristics, with enrofloxacin are administered to an animal, the compositions are preferably
10 administered to animals orally in a feed composition or in drinking water, but any other effective method of administration known to those skilled in the art may be utilized such as in a paste, a gel, a liquid drench, a top dress, a powder, a liquid, a pellet, or a capsule. In one illustrative embodiment, the *Bacillus* strains 9, 57, 71, and/or 126, or strains having their identifying characteristics, and enrofloxacin, are provided in the form of an additive for addition
15 to the drinking water of an animal, or enrofloxacin is added to the drinking water separately.

In another illustrative embodiment, the *Bacillus* strains 9, 57, 71, and/or 126, or strains having their identifying characteristics, and enrofloxacin, are provided in the form of a feed additive for addition to a feed composition, or the enrofloxacin is administered separately. The feed composition may contain *Bacillus* strain 9, 57, 71, and/or 126, or strains having their
20 identifying characteristics, in a mixture with an animal feed blend, including any art-recognized animal feed blend or any animal feed blend described herein. As used herein, “feed composition” or “animal feed composition” means a feed composition comprising *Bacillus* strain 9, 57, 71, and/or 126, or strains having their identifying characteristics, in a mixture with an animal feed blend, and, optionally any other components that could be used in a feed
25 composition, including other bacterial strains, such as other different *Bacillus* strains or *Lactobacillus* strains. In one embodiment, the feed composition may be in the form of a ground meal.

Any animal feed blend, including those known in the art and those described herein, may be used in accordance with the methods and compositions described in this patent
30 application, such as rapeseed meal, cottonseed meal, soybean meal, cornmeal, barley, wheat, silage, and haylage. In various embodiments, the animal feed blend can be supplemented with *Bacillus* strain 9, 57, 71, and/or 126, or strains having their identifying characteristics, but other ingredients may optionally be added to the animal feed blend, including other different bacterial strains, such as other *Bacillus* strains or *Lactobacillus* strains.

In various illustrative embodiments, optional ingredients of the animal feed blend include sugars and complex carbohydrates such as both water-soluble and water-insoluble monosaccharides, disaccharides, and polysaccharides. Other optional ingredients include dried distillers grain solubles, fat (e.g., crude fat), phosphorous, sodium bicarbonate, limestone, salt, phytate, calcium, sodium, sulfur, magnesium, potassium, copper, iron, manganese, zinc, ash, fish oil, an oil derived from fish meal, raw seed (e.g., flaxseed), an antioxidant, and starch. In another embodiment, minerals may be added in the form of a mineral premix.

Optional amino acid ingredients that may be added to the animal feed blend are arginine, histidine, isoleucine, leucine, lysine, cysteine, methionine, phenylalanine, threonine, tryptophan, valine, tyrosine ethyl HCl, alanine, aspartic acid, sodium glutamate, glycine, proline, serine, cysteine ethyl HCl, and analogs, and salts thereof. Vitamins that may be optionally added are thiamine HCl, riboflavin, pyridoxine HCl, niacin, niacinamide, inositol, choline chloride, calcium pantothenate, biotin, folic acid, ascorbic acid, and vitamins A, B, K, D, E, and the like. In another embodiment, vitamins may be added in the form of a vitamin premix. In yet another embodiment, protein ingredients may be added to the animal feed blend and include protein obtained from meat meal, bone meal, or fish meal, liquid or powdered egg, fish solubles, crude protein, and the like.

In the method embodiments described herein, the antibiotic used is enrofloxacin. In various embodiments, the dosage of the antibiotic can be from about 0.1 mg/pound of feed to about 1000 mg/pound of feed, about 0.1 mg/pound of feed to about 900 mg/pound of feed, about 0.1 mg/pound of feed to about 800 mg/pound of feed, about 0.1 mg/pound of feed to about 700 mg/pound of feed, about 0.1 mg/pound of feed to about 600 mg/pound of feed, about 0.1 mg/pound of feed to about 500 mg/pound of feed, about 0.1 mg/pound of feed to about 400 mg/pound of feed, about 0.1 mg/pound of feed to about 300 mg/pound of feed, about 0.1 mg/pound of feed to about 200 mg/pound of feed, about 0.1 mg/pound of feed to about 100 mg/pound of feed, about 0.1 mg/pound of feed to about 90 mg/pound of feed, about 0.1 mg/pound of feed to about 80 mg/pound of feed, about 0.1 mg/pound of feed to about 70 mg/pound of feed, about 0.1 mg/pound of feed to about 60 mg/pound of feed, about 0.1 mg/pound of feed to about 50 mg/pound of feed, about 0.1 mg/pound of feed to about 40 mg/pound of feed, about 0.1 mg/pound of feed to about 30 mg/pound of feed, about 0.1 mg/pound of feed to about 20 mg/pound of feed, about 0.1 mg/pound of feed to about 10 mg/pound of feed, about 0.1 mg/pound of feed to about 9 mg/pound of feed, about 0.1 mg/pound of feed to about 8 mg/pound of feed, about 0.1 mg/pound of feed to about 7 mg/pound of feed, about 0.1 mg/pound of feed to about 6 mg/pound of feed, about 0.1 mg/pound of feed to about 5 mg/pound of feed, about 0.1 mg/pound of feed to about 4

mg/pound of feed, about 0.1 mg/pound of feed to about 3 mg/pound of feed, about 0.1 mg/pound of feed to about 2 mg/pound of feed, about 0.1 mg/pound of feed to about 1 mg/pound of feed, about 0.1 mg/pound of feed to about 0.5 mg/pound of feed, about 0.1 mg/pound of feed to about 0.3 mg/pound of feed, about 0.5 mg/pound of feed to about 1 mg/pound of feed, about 1 mg/ml to about 1000 mg/ml of drinking water, about 1 mg/ml to about 900 mg/ml of drinking water, about 1 mg/ml to about 800 mg/ml of drinking water, about 1 mg/ml to about 700 mg/ml of drinking water, about 1 mg/ml to about 600 mg/ml of drinking water, about 1 mg/ml to about 500 mg/ml of drinking water, about 1 mg/ml to about 400 mg/ml of drinking water, about 1 mg/ml to about 300 mg/ml of drinking water, about 1 mg/ml to about 200 mg/ml of drinking water, about 1 mg/ml to about 100 mg/ml of drinking water, about 50 mg/ml to about 100 mg/ml of drinking water, about 50 mg/ml to about 90 mg/ml of drinking water, about 50 mg/ml to about 80 mg/ml of drinking water, about 50 mg/ml to about 70 mg/ml of drinking water, about 50 mg/ml to about 60 mg/ml of drinking water, about 1000 ppm, about 900 ppm, about 800 ppm, about 700 ppm, about 600 ppm, about 500 ppm, about 400 ppm, about 300 ppm, about 200 ppm, about 100 ppm, about 90 ppm, about 80 ppm, about 70 ppm, about 60 ppm, about 50 ppm, about 40 ppm, about 30 ppm, about 20 ppm, or about 10 ppm.

In another illustrative embodiment, one or more enzymes may be added to the animal feed blend or to the feed composition or feed additive or the bacterial strain. In various embodiments, the enzymes that may be added include a galactosidase, a phytase, a protease, a lipase, an amylase, a hemicellulase, an arabinoxylanase, a xylanase, a cellulase, an NSPase, combinations thereof, and any other enzyme that improves the effectiveness of the feed composition or feed additive for *E. coli* inhibition or controlling a detrimental effect of *E. coli*. In yet another embodiment, yeast, fungi (e.g., *Aspergillus* or *Trichoderma*), or micronutrients may be added to the animal feed, feed composition, or feed additive, or the bacterial strain. Any of the ingredients described above that are suitable for addition to an additive for the drinking water of the animal may be added as a component of the additive for the drinking water of the animal as described herein.

In various illustrative embodiments, the *Bacillus* strain (e.g., *Bacillus* strain 9, 57, 71, and/or 126, or strains having their identifying characteristics), or any other different bacterial strains added in addition to *Bacillus* strain 9, 57, 71, and/or 126, or strains having their identifying characteristics, can be administered in the feed composition at a dose of about 1.0×10^3 CFU/gram of the feed composition to about 5.0×10^{12} CFU/gram of the feed composition or at a dose of about 1.0×10^3 CFU/gram of the feed composition to about 1.0×10^7 CFU/gram of the feed composition. In other embodiments, the *Bacillus* strain (e.g., *Bacillus* strain 2, 3, 4,

5, 9, 57, 71, and/or 126, or strains having their identifying characteristics) is administered in the feed composition at a dose greater than about 1.0×10^3 CFU/gram of the feed composition, at a dose greater than about 1.1×10^3 CFU/gram of the feed composition, at a dose greater than about 1.25×10^3 CFU/gram of the feed composition, at a dose greater than about 1.5×10^3 CFU/gram of the feed composition, at a dose greater than about 1.75×10^3 CFU/gram of the feed composition, at a dose greater than about 1.0×10^4 CFU/gram of the feed composition, at a dose greater than about 2.0×10^4 CFU/gram of the feed composition, at a dose greater than about 3.0×10^4 CFU/gram of the feed composition, at a dose greater than about 4.0×10^4 CFU/gram of the feed composition, at a dose greater than about 5.0×10^4 CFU/gram of the feed composition, at a dose greater than about 6.0×10^4 CFU/gram of the feed composition, at a dose greater than about 7.0×10^4 CFU/gram of the feed composition, at a dose greater than about 8.0×10^4 CFU/gram of the feed composition, at a dose greater than about 1.0×10^5 CFU/gram of the feed composition, at a dose greater than about 1.0×10^6 CFU/gram of the feed composition, at a dose greater than about 1.0×10^7 CFU/gram of the feed composition, at a dose greater than about 1.0×10^8 CFU/gram of the feed composition, at a dose greater than about 1.0×10^9 CFU/gram of the feed composition, at a dose greater than about 1.0×10^{10} CFU/gram of the feed composition, at a dose greater than about 1.0×10^{11} CFU/gram of the feed composition, or at a dose greater than about 1.0×10^{12} CFU/gram of the feed composition. In another embodiment, the *Bacillus* strain (e.g., *Bacillus* strain 9, 57, 71, and/or 126, or strains having their identifying characteristics), or any other different bacterial strains added in addition to *Bacillus* strain 9, 57, 71, and/or 126, or strains having their identifying characteristics, can be administered in the feed composition at a dose of about 1.0×10^2 CFU/gram of the feed composition to about 5.0×10^{12} CFU/gram of the feed composition or at a dose of about 1.0×10^2 CFU/gram of the feed composition to about 1.0×10^7 CFU/gram of the feed composition, or at a dose greater than about 1.0×10^2 CFU/gram of the feed composition. In another embodiment, any of the dosages described herein can be in CFU/ml of drinking water in embodiments where the strains are administered in the drinking water of the animal.

In various embodiments, the *Bacillus* strain (e.g., *Bacillus* strain 9, 57, 71, and/or 126, or strains having their identifying characteristics) for use in accordance with the methods or compositions described herein can be selected from the group consisting of *Bacillus* strain 9 (NRRL No. B-67866), a strain having all of the identifying characteristics of *Bacillus* strain 9 (NRRL No. B-67866), *Bacillus* strain 57 (NRRL No. B-67870), a strain having all of the identifying characteristics of *Bacillus* strain 57 (NRRL No. B-67870), *Bacillus* strain 71 (NRRL No. B-67867), a strain having all of the identifying characteristics of *Bacillus* strain 71 (NRRL No. B-67867), *Bacillus* strain 126 (NRRL No. B-67868), a strain having all of the identifying

characteristics of *Bacillus* strain 126 (NRRL No. B-67868), and combinations thereof. *Bacillus* strains 9, 57, 71, and 126 were deposited on October 10, 2019 at the Agricultural Research Service Culture Collection (NRRL), National Center for Agricultural Utilization Research, Agricultural Research Service, USDA, 1815 North University Street, Peoria, Illinois 61604-3999, and were given accession numbers B-67866, B-67870, B-67867, and B-67868, respectively. The deposits were made under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. NRRL No. B-67866, NRRL No. B-67870, NRRL No., B-67867, and NRRL No. B-67868, are equivalent to *Bacillus* strains 9, 57, 71, and 126, respectively, as referred to in the application. The deposit certificates refer to the strains as MDG9, MDG57, MDG71, and MDG126, respectively. These four strains are *Bacillus subtilis* strains.

In one aspect, any of these strains can be administered alone or in combination in the form of a feed composition (e.g., a complete feed comprising an animal feed blend) or drinking water for an animal. In one embodiment, multiple strains are administered in combination in a single composition. In another embodiment, multiple strains are administered in combination in separate compositions. In yet another embodiment, any of these strains, or combinations thereof, is administered in combination with enrofloxacin, as described herein in the same or separate compositions.

In another embodiment, one or more of the *Bacillus* strains described in the preceding paragraphs (e.g., *Bacillus* strain 9, 57, 71, and/or 126, or strains having their identifying characteristics) can be administered to the animal along with another different bacterial strain selected from the group consisting of another *Bacillus* strain, a lactic acid bacterial strain, and combinations thereof. In yet another embodiment, one or more of the *Bacillus* strains described in the preceding paragraphs (e.g., *Bacillus* strain 9, 57, 71, and/or 126, or strains having their identifying characteristics) can be administered to the animal along with any other bacterial strain effective to inhibit or control detrimental effects of *E. coli* in the animal.

As used herein “a strain having all of the identifying characteristics of” *Bacillus* strain 9, 57, 71, or 126, can be a mutant strain having all of the identifying characteristics of *Bacillus* strain 9, 57, 71, or 126 (e.g., a DNA fingerprint based on DNA analysis that corresponds to the DNA fingerprint of *Bacillus* strain 9, 57, 71, or 126, enzyme activities that correspond to *Bacillus* strain 9, 57, 71, or 126, antimicrobial activity that corresponds to *Bacillus* strain 9, 57, 71, or 126, antibiotic sensitivity and tolerance profiles that correspond to *Bacillus* strain 9, 57, 71, or 126, or combinations of these identifying characteristics). In alternate embodiments, the mutation can be a natural mutation, or a genetically engineered

mutation. In another embodiment, “a strain having all of the identifying characteristics of” *Bacillus* strain 9, 57, 71, or 126, can be a strain, for example, produced by isolating one or more plasmids from *Bacillus* strain 9, 57, 71, or 126, and introducing the one or more plasmids into another bacterium, such as another *Bacillus* strain, as long as the one or more plasmids contain DNA that provides the identifying characteristics of *Bacillus* strain 9, 57, 71, or 126 (e.g., a DNA fingerprint based on DNA analysis that corresponds to the DNA fingerprint of *Bacillus* strain 9, 57, 71, or 126).

The feed composition or drinking water comprising *Bacillus* strain 9, 57, 71, or 126, or strains with their identifying characteristics, and enrofloxacin, may be administered to the animal for any time period that is effective to inhibit *E. coli* or control a detrimental effect of *E. coli*, or combinations thereof. For example, in one embodiment the feed composition or drinking water may be provided to the animal daily. In an alternate embodiment, the feed composition or drinking water may be administered to the animal during lactation and/or during gestation. The time periods for administration of the feed composition or drinking water described above are non-limiting examples and it should be appreciated that any time period or administration schedule determined to be effective to inhibit *E. coli* or control a detrimental effect of *E. coli*, or combinations thereof, may be used.

In embodiments involving “controlling a detrimental effect of *E. coli*” controlling a detrimental effect can mean reducing *E. coli* disease, preventing *E. coli* disease, maintaining the normal microbial balance in the animal, reducing the number of detrimental *E. coli* in the animal, reducing the activity of *E. coli* in the animal, or reducing the symptoms of *E. coli* disease in the animal, or combinations thereof. By “effective amount” is meant an amount of the *Bacillus* strain (e.g., *Bacillus* strain 9, 57, 71, and/or 126) capable of controlling a detrimental effect of *E. coli* as described herein, by any mechanism.

In one embodiment, the feed additive for addition to an animal feed blend to produce a complete feed composition can be mixed with the animal feed blend, for example, with an automated micro-nutrient delivery system, or, for example, by hand-weighing and addition to achieve any of the doses of *Bacillus* strain 9, 57, 71, and/or 126, or strains with their identifying characteristics, described herein, for administration to the animal in the form of a complete feed composition. The mixing can also be done by any other suitable method known in the art for combining direct-fed microbials with an animal feed blend to obtain a uniform mixture. In various embodiments, the mixing can be done for any suitable time period (e.g., about 1 to about 4 minutes). In the embodiment where *Bacillus* strain 9, 57, 71, and/or 126, or strains with their identifying characteristics, are in the form of an additive for the drinking water of the animal, the *Bacillus* strain 9, 57, 71, and/or 126, or strains with their identifying

characteristics, can be in the form of, for example, a powder, a liquid, or pellets, and can be mixed with the drinking water using any suitable method known in the art to achieve any of the doses of *Bacillus* strain 9, 57, 71, and/or 126, or strains with their identifying characteristics, described herein, for administration to the animal in the drinking water of the animal. *Bacillus* strain 9, 57, 71, and/or 126, or strains with their identifying characteristics, can also be fed directly to the animal orally (i.e., by oral insertion) in the form of a powder, a freeze-dried composition, a gel, a top-dressing, a liquid, a capsule, a paste, a liquid drench, or a pellet. Any of these embodiments can also be applied to mixing and administration of enrofloxacin as described herein.

In additional embodiments of the invention, compositions are provided comprising *Bacillus* strain 9 (NRRL No. B-67866), a strain having all of the identifying characteristics of *Bacillus* strain 9 (NRRL No. B-67866), *Bacillus* strain 57 (NRRL No. B-67870), a strain having all of the identifying characteristics of *Bacillus* strain 57 (NRRL No. B-67870), *Bacillus* strain 71 (NRRL No. B-67867), a strain having all of the identifying characteristics of *Bacillus* strain 71 (NRRL No. B-67867), *Bacillus* strain 126 (NRRL No. B-67868), a strain having all of the identifying characteristics of *Bacillus* strain 126 (NRRL No. B-67868), and combinations thereof.

In one embodiment, a commercial package is provided comprising an isolated *Bacillus* strain selected from the group consisting of *Bacillus* strain 9 (NRRL No. B-67866), a strain having all of the identifying characteristics of *Bacillus* strain 9 (NRRL No. B-67866), *Bacillus* strain 57 (NRRL No. B-67870), a strain having all of the identifying characteristics of *Bacillus* strain 57 (NRRL No. B-67870), *Bacillus* strain 71 (NRRL No. B-67867), a strain having all of the identifying characteristics of *Bacillus* strain 71 (NRRL No. B-67867), *Bacillus* strain 126 (NRRL No. B-67868), a strain having all of the identifying characteristics of *Bacillus* strain 126 (NRRL No. B-67868), and combinations thereof.

In another embodiment, a feed additive for an animal feed is provided comprising an isolated *Bacillus* strain selected from the group consisting of *Bacillus* strain 9 (NRRL No. B-67866), a strain having all of the identifying characteristics of *Bacillus* strain 9 (NRRL No. B-67866), *Bacillus* strain 57 (NRRL No. B-67870), a strain having all of the identifying characteristics of *Bacillus* strain 57 (NRRL No. B-67870), *Bacillus* strain 71 (NRRL No. B-67867), a strain having all of the identifying characteristics of *Bacillus* strain 71 (NRRL No. B-67867), *Bacillus* strain 126 (NRRL No. B-67868), a strain having all of the identifying characteristics of *Bacillus* strain 126 (NRRL No. B-67868), and combinations thereof.

In yet another embodiment, an additive for the drinking water of an animal is provided comprising an isolated *Bacillus* strain 9 (NRRL No. B-67866), a strain having all of

the identifying characteristics of *Bacillus* strain 9 (NRRL No. B-67866), *Bacillus* strain 57 (NRRL No. B-67870), a strain having all of the identifying characteristics of *Bacillus* strain 57 (NRRL No. B-67870), *Bacillus* strain 71 (NRRL No. B-67867), a strain having all of the identifying characteristics of *Bacillus* strain 71 (NRRL No. B-67867), *Bacillus* strain 126 (NRRL No. B-67868), a strain having all of the identifying characteristics of *Bacillus* strain 126 (NRRL No. B-67868), and combinations thereof.

In yet another illustrative aspect of the invention, an animal feed composition is provided comprising an isolated *Bacillus* strain selected from the group consisting of *Bacillus* strain 9 (NRRL No. B-67866), a strain having all of the identifying characteristics of *Bacillus* strain 9 (NRRL No. B-67866), *Bacillus* strain 57 (NRRL No. B-67870), a strain having all of the identifying characteristics of *Bacillus* strain 57 (NRRL No. B-67870), *Bacillus* strain 71 (NRRL No. B-67867), a strain having all of the identifying characteristics of *Bacillus* strain 71 (NRRL No. B-67867), *Bacillus* strain 126 (NRRL No. B-67868), a strain having all of the identifying characteristics of *Bacillus* strain 126 (NRRL No. B-67868), and combinations thereof.

In one illustrative aspect, the strains for addition to the commercial package, feed additive, additive for the drinking water of the animal, or the feed composition can be in the form of a concentrate (e.g., about 1×10^8 to about 5×10^9 CFU/g) or a superconcentrate (e.g., about 1×10^{10} to about 5×10^{12} CFU/g). In another embodiment, the strains for addition to the commercial package, feed additive, feed composition, or additive for the drinking water of the animal can be in a dry form (e.g., a powder), a pelleted form, a liquid form, a liquid drench, a powder, a freeze-dried composition, in the form of a top-dressing, a paste, a capsule, or in the form of a gel, or any other suitable form.

In another illustrative embodiment, the strain or strains in the commercial package, the feed additive, additive for the drinking water of the animal, or feed composition can further comprise a carrier for the *Bacillus* strain or strains. The carrier can be selected from the group consisting of a bran, rice hulls, a salt, mineral oil, a dextrin (e.g., maltodextrin), whey, sugar, sucrose, limestone, yeast culture, dried starch, sodium silico aluminate, silicon dioxide, polypropylene glycol, polysorbate 80, vegetable oil, and combinations thereof. The carrier is exogenously added to the bacterial strain (i.e., not naturally present or not present in nature with the bacterial strain). In another embodiment, the feed additive, additive for the drinking water of the animal, or feed composition can further comprise a binder such as clay, yeast cell wall components, aluminum silicate, glucan, or other known binders. In another embodiment, the commercial package, feed additive, additive for the drinking water of the animal, or feed composition can further comprise inorganic/organic binders, essential oils, and/or organic acids.

The binder is exogenously added to the bacterial strain (i.e., not naturally present or not present in nature with the bacterial strain).

In yet other embodiments, the commercial package, feed additive, additive for the drinking water of the animal, or feed composition comprising *Bacillus* strain 9 (NRRL No. B-67866), a strain having all of the identifying characteristics of *Bacillus* strain 9 (NRRL No. B-67866), *Bacillus* strain 57 (NRRL No. B-67870), a strain having all of the identifying characteristics of *Bacillus* strain 57 (NRRL No. B-67870), *Bacillus* strain 71 (NRRL No. B-67867), a strain having all of the identifying characteristics of *Bacillus* strain 71 (NRRL No. B-67867), *Bacillus* strain 126 (NRRL No. B-67868), a strain having all of the identifying characteristics of *Bacillus* strain 126 (NRRL No. B-67868), and combinations thereof, is in a container for commercial use.

In various embodiments the container can be, for example, a bag (e.g., a 20-pound bag, a 50-pound bag, a 2-ounce bag, a 1-pound bag, or a 1-kilogram bag), a pouch, a drum, a bottle, or a box. In illustrative aspects, the container for the commercial package, feed additive, additive for the drinking water of the animal, or feed composition comprising *Bacillus* strain 9 (NRRL No. B-67866), a strain having all of the identifying characteristics of *Bacillus* strain 9 (NRRL No. B-67866), *Bacillus* strain 57 (NRRL No. B-67870), a strain having all of the identifying characteristics of *Bacillus* strain 57 (NRRL No. B-67870), *Bacillus* strain 71 (NRRL No. B-67867), a strain having all of the identifying characteristics of *Bacillus* strain 71 (NRRL No. B-67867), *Bacillus* strain 126 (NRRL No. B-67868), a strain having all of the identifying characteristics of *Bacillus* strain 126 (NRRL No. B-67868), and combinations thereof, can comprise plastic, metal, foil, paper, fiber, or cardboard (e.g., a plastic pail, a paper bag, a foil bag, a fiber drum, etc.). The commercial package, feed additive, additive for the drinking water of the animal, or feed composition can further comprise instructions for use of one or more of the *Bacillus* strains.

In one aspect, the commercial package, feed additive, additive for the drinking water of the animal, or feed composition described herein can further comprise an exogenously added nutrient component (i.e., a nutrient component not present with the bacterial strain in nature) selected from the group consisting of a vitamin, an antibiotic, an enzyme, a water-soluble or water-insoluble monosaccharide, disaccharide, or polysaccharide, a fat, phosphorous, sodium bicarbonate, limestone, calcium, sodium, sulfur, magnesium, potassium, copper, iron, manganese, zinc, fish oil, raw seed, an antioxidant, and a starch.

In one embodiment, the exogenously added nutrient component is an enzyme and the enzyme is selected from the group consisting of a galactosidase, a protease, a lipase, an

amylase, a hemicellulase, an arabinoxylanase, a xylanase, a cellulase, an NSPase, a phytase, and combinations thereof.

In one embodiment, any of the compositions described herein can be a dietary nutrient composition (e.g., a probiotic composition).

5 In embodiments of the methods and compositions described herein, synergism occurs between the *Bacillus* strains and enrofloxacin.

The following examples are for illustrative purposes only. The examples are non-limiting, and are not intended to limit the invention in any way.

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EXAMPLE 1

Bacillus Testing on Baytril (15) resistant *E. coli*

Utilizing a broth AST method, four *Bacillus* strains were tested against 15 Baytril-resistant *E. coli*. All four *Bacillus* strains were observed to be more effective at
15 inhibiting the growth of these resistant *E. coli* than Mecadox™ was capable of alone.

Bacillus strain 9 (NRRL No. B-67866), *Bacillus* strain 57 (NRRL No. B-67870),
Bacillus strain 71 (NRRL No. B-67867), and *Bacillus* strain 126 (NRRL No. B-67868),
consistently improved and effectively inhibited (>50% inhibition) antibiotic resistant pathogens
when the same concentration of antibiotic was administered in conjunction with *Bacillus*
20 supernatant. Of the 15 Enrofloxacin resistant *E. coli*: when combined with antibiotics *Bacillus*
strains 9 and 71 effectively inhibited 14/15 isolates. When combined with antibiotics *Bacillus*
strains 57 and 126 effectively inhibited 15/15 isolates.

The goal of this project was to identify *Bacillus* strains that exhibit strong
inhibition potential of target pathogens in combination with antibiotics. This synergy can be
25 defined as a resulting higher percent inhibition of antibiotic-resistant pathogens when *Bacillus*
supernatants are combined with antibiotics than when the supernatant and antibiotic are tested
separately against the same isolates. Four *Bacillus* strains yielded consistent strong inhibition
activity against Baytril-resistant *E. coli* and were tested. These four *Bacillus* strains are (BS 9,
57, 71, and 126), identified as strains (NRRL No. B-67866), (NRRL No. B-67870), (NRRL No.
30 B-67867), (NRRL No. B-67868), respectively.

EXAMPLE 2

RAPD-PCR DNA Profiles

The Randomly Amplified Polymorphic DNA PCR method (hereafter referred to as RAPD-PCR) was used to identify genetic variability of each strain. Preparation of the DNA to be used in the RAPD-PCR reaction was done by using the QIAGEN® Tissue and Blood single column kit (QIAGEN®, Venlo, The Netherlands). Figures 2 and 3 illustrate RAPD-PCR results for strains 9, 57, 71, and 126, with the unlabeled lanes being a molecular weight ladder. The results show that all strains are unique from each other.

WHAT IS CLAIMED IS:

1. A method of feeding an animal, the method comprising the step of administering to the animal a feed composition or drinking water comprising an effective
5 amount of an additive comprising an isolated *Bacillus* strain selected from the group consisting of *Bacillus* strain 9 (NRRL No. B-67866), a strain having all of the identifying characteristics of *Bacillus* strain 9 (NRRL No. B-67866), *Bacillus* strain 57 (NRRL No. B-67870), a strain having all of the identifying characteristics of *Bacillus* strain 57 (NRRL No. B-67870), *Bacillus* strain 71 (NRRL No. B-67867), a strain having all of the identifying characteristics of *Bacillus* strain
10 71 (NRRL No. B-67867), *Bacillus* strain 126 (NRRL No. B-67868), a strain having all of the identifying characteristics of *Bacillus* strain 126 (NRRL No. B-67868), and combinations thereof, and administering to the animal an antibiotic wherein the antibiotic is enrofloxacin.
2. The method of claim 1 wherein the animal is selected from the group consisting of a poultry species, a porcine species, a caprine species, a bovine species, an ovine
15 species, an equine species, and a companion animal.
3. The method of claim 1 wherein the animal is a porcine species and the porcine species is selected from the group consisting of a grow finish pig, a nursery pig, a sow, and a breeding stock pig.
4. The method of claim 1 wherein the *Bacillus* strain produces an enzyme
20 selected from the group consisting of an α -galactosidase, a protease, a lipase, an amylase, a xylanase, a cellulase, and combinations thereof.
5. The method of claim 1 further comprising the step of administering to the animal another different bacterial strain selected from the group consisting of another *Bacillus* strain, a lactic acid bacterial strain, and combinations thereof.
- 25 6. The method of claim 1 wherein the strain administered is *Bacillus* strain 9 (NRRL No. B-67866).
7. The method of claim 1 wherein the strain administered is *Bacillus* strain 57 (NRRL No. B-67870).
8. The method of claim 1 wherein the strain administered is *Bacillus* strain
30 71 (NRRL No. B-67867).
9. The method of claim 1 wherein the strain administered is *Bacillus* strain 126 (NRRL No. B-67868).
10. The method of claim 1 wherein the *Bacillus* strain is administered in the feed composition at a dose of about 1.0×10^3 CFU/gram of the feed composition to about $5.0 \times$
35 10^{12} CFU/gram of the feed composition.

11. The method of claim 1 wherein the *Bacillus* strain is administered in the feed composition at a dose of about 1.0×10^3 CFU/gram of the feed composition to about 1.0×10^7 CFU/gram of the feed composition.

12. The method of claim 1 wherein the *Bacillus* strain is administered in the feed composition at a dose greater than about 7.0×10^4 CFU/gram of the feed composition.

13. The method of claim 1 further comprising the step of administering to the animal an enzyme selected from the group consisting of a galactosidase, a protease, a lipase, an amylase, a hemicellulase, an arabinoxylanase, a xylanase, a cellulase, an NSPase, a phytase, and combinations thereof.

14. The method of claim 1 wherein the animal is a sow and the *Bacillus* strain is administered during lactation.

15. The method of claim 1 wherein the animal is a sow and the *Bacillus* strain is administered during gestation.

16. The method of claim 1 wherein the feed composition is administered daily to the animal.

17. The method of claim 1 wherein the animal is selected from the group consisting of a chicken, a pig, a horse, a pony, a cow, a turkey, a goat, a sheep, a quail, a pheasant, an ostrich, a duck, a fish, a crustacean, and combinations thereof.

18. The method of claim 1 wherein the *Bacillus* strain has an effect selected from the group consisting of maintaining microbial balance in the gut of the animal, improving animal performance or health, maintaining gut health in the animal, reducing detrimental pathogens in the gut of the animal, odor reduction, reducing detrimental pathogens in the urine or feces of the animal, and preserving the growth of beneficial bacteria in the gut of the animal.

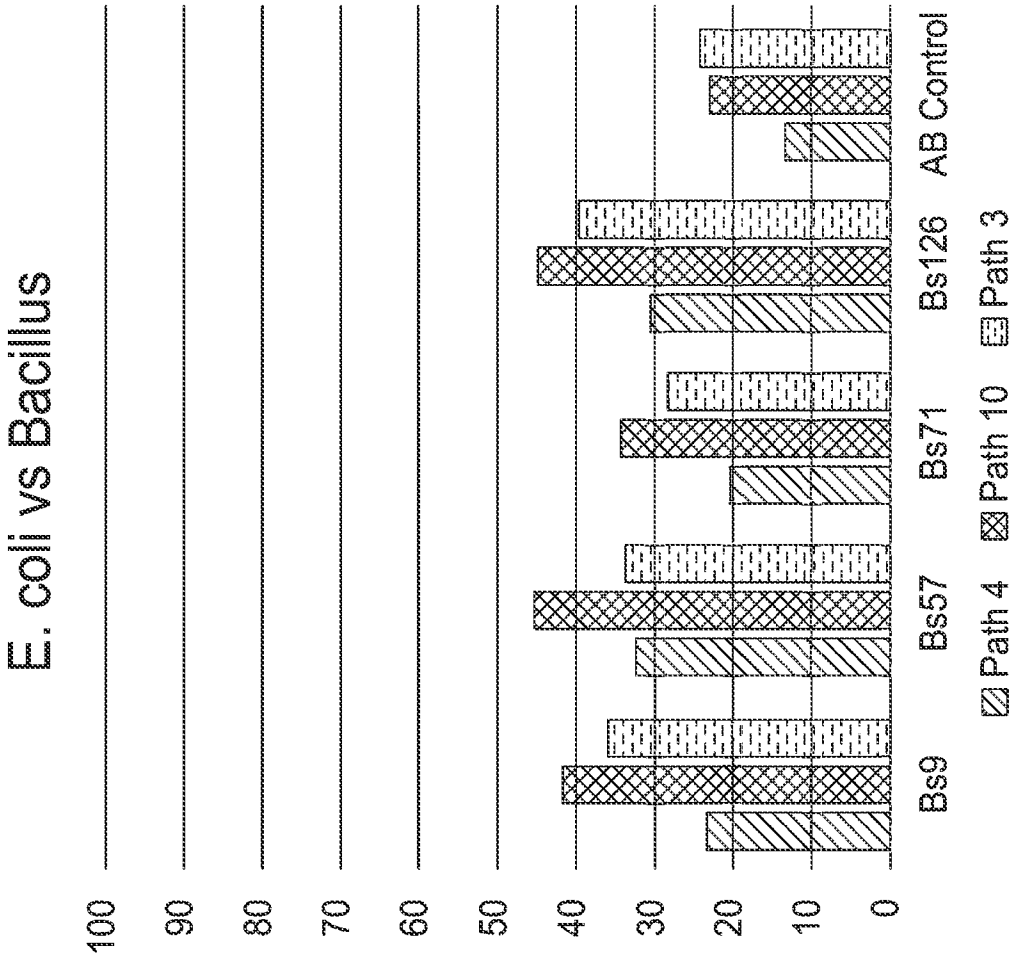


Fig. 1A

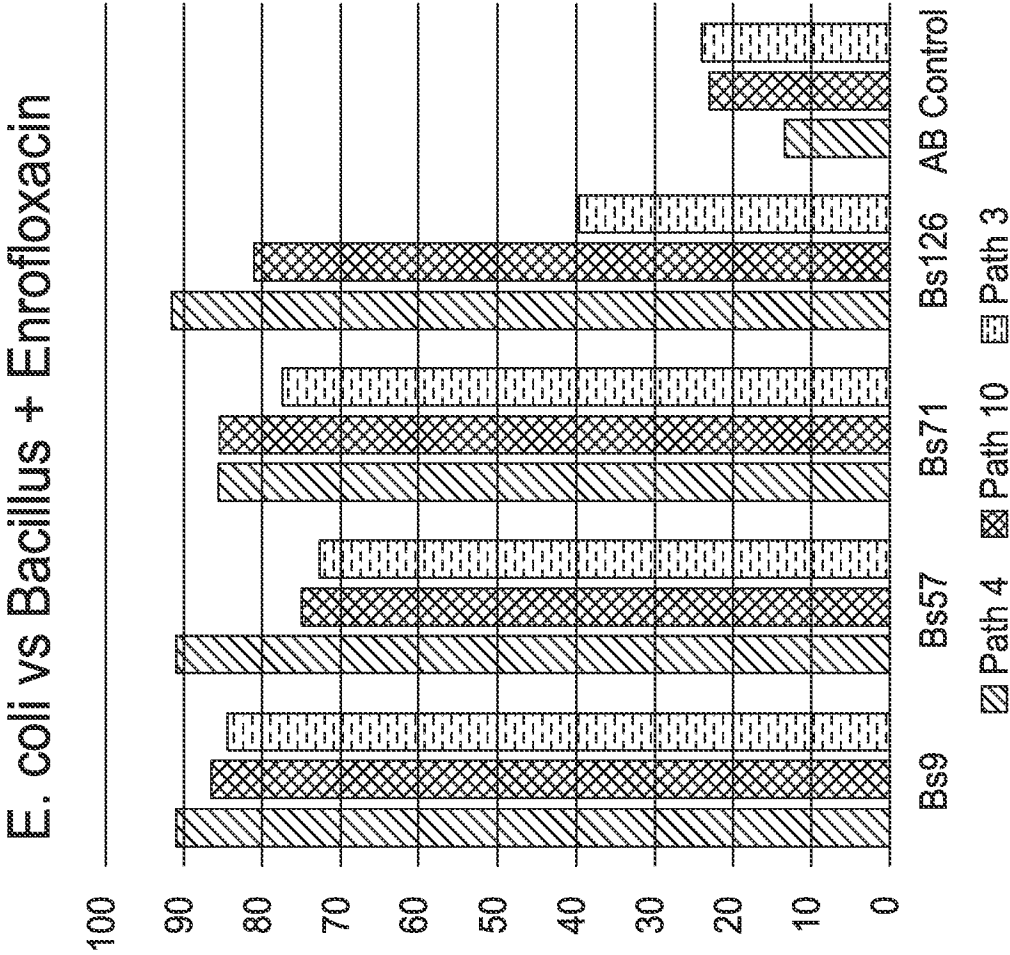


Fig. 1B

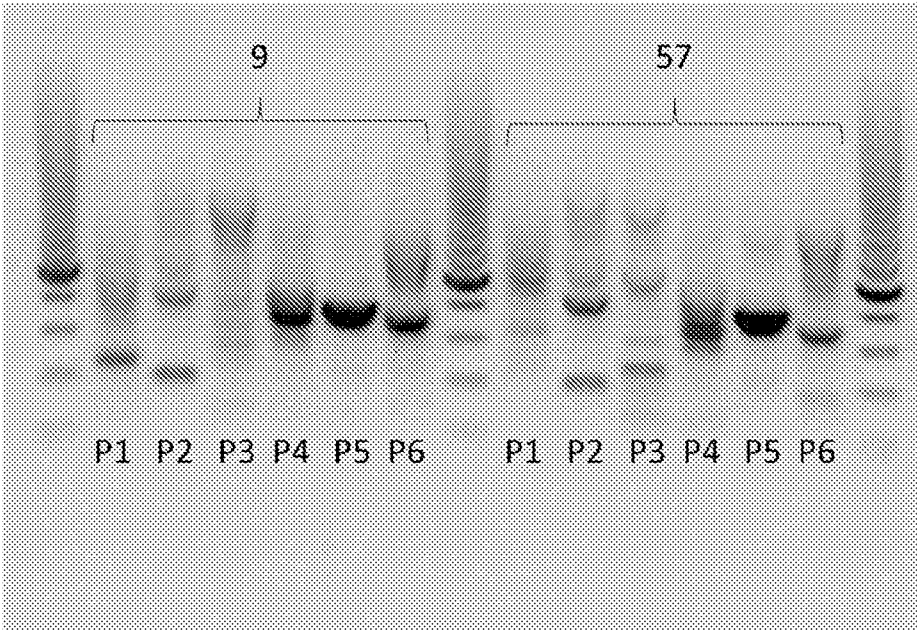


Fig. 2

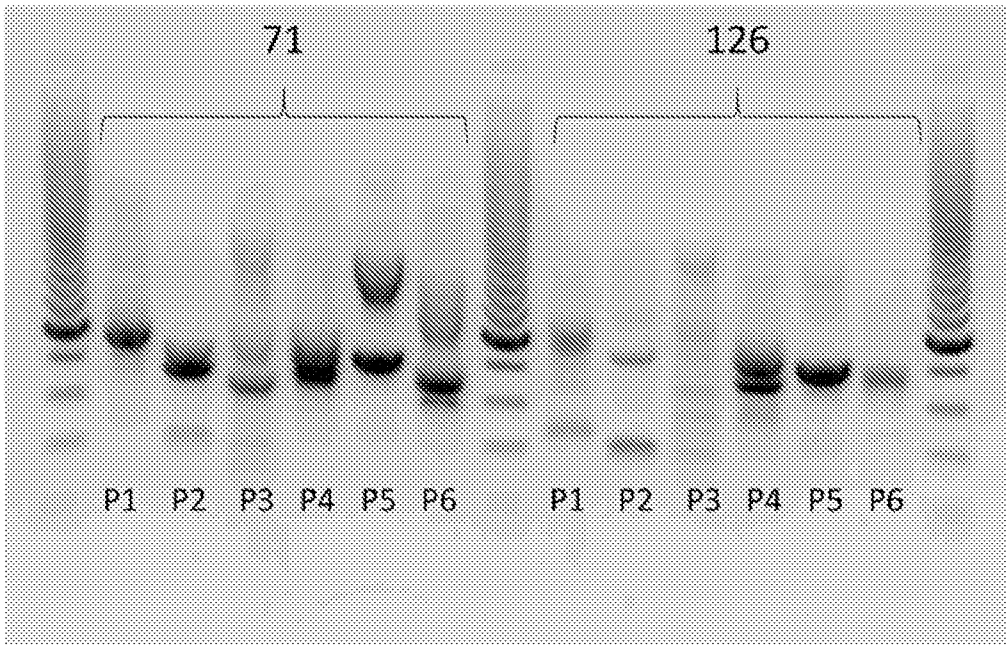


Fig. 3

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2021/046452

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A23K 10/18; A23K 20/195; A23K 50/00; A61K 35/742; C12R 1/07 (2021.01)

CPC - A01N 63/22; A23K 10/18; A23K 20/195; A23K 50/00; A61K 35/742; C12N 1/205; C12R 2001/07 (2021.08)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

see Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

see Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

see Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2019/141815 A1 (CHR. HANSEN A/S) 25 July 2019 (25.07.2019) entire document	1-18
A	US 2020/0015497 A1 (MICROBIAL DISCOVERY GROUP LLC) 16 January 2020 (16.01.2020) entire document	1-18
A	WO 2019/152791 A1 (NOVOZYMES A/S et al) 08 August 2019 (08.08.2019) entire document	1-18
A	US 2020/0093158 A1 (OMNIGEN RESEARCH LLC) 26 March 2020 (26.03.2020) entire document	1-18
P, A	WO 2021/035137 A1 (MICROBIAL DISCOVERY GROUP LLC) 25 February 2021 (25.02.2021) entire document	1-18

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

28 October 2021

Date of mailing of the international search report

DEC 06 2021

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