BACTERIAL STRAINS AND THEIR USES

FIELD OF THE INVENTION

[0001] The present invention relates generally to the field of microbiology. In particular, the present invention relates to the use of bacterial strains and their uses in fermentation.

BACKGROUND

[0002] High fibre content in feed ingredients is often regarded as anti-nutritional due to their entanglement effect on nutritional components like protein, especially for mono-gastric animals.

[0003] Non-starch polysaccharides make up of 20-30% dry weight of defatted rice bran and wheat bran. Protein associated with the fibre matrix can get entangled and hence limit its availability for mono-gastric animals.

[0004] Protein in the feed need to be digested to peptides and amino acids to facilitate absorption in animal gut. In the presence of fibre matrix, some of these proteins are entrapped and are not accessible to proteases activity hence decrease their availability to animals.

[0005] Thus, there is an unmet need for providing methods of fermenting grains and parts thereof, as well as improving digestibility of said grains, as well as methods for loading probiotics into the animal digestive system.

SUMMARY

[0006] In one aspect, the present disclosure refers to a bacterial strain deposited under deposition number DSM33837.

[0007] In one example, the present disclosure refers to a bacterial strain deposited under deposition number DSM33838.

[0008] In another example, the present disclosure refers to a bacterial strain deposited under deposition number DSM33839.

[0009] In another aspect, the present disclosure refers to a method of fermenting bran and/or meal, the method comprising inoculating the bran and/or meal with a bacterial strain deposited under deposition number DSM33837 alone or in combination with a bacterial strain deposited under deposition number DSM33839 and/or a bacterial strain deposited under deposition number DSM33838.

[0010] In yet another aspect, the present disclosure refers to a method of solid-state fermentation of bran and/or meal, the method comprising inoculating the bran and/or meal with a bacterial strain of *Bacillus spp*, wherein the bacterial strain is a bacterial strain deposited under deposition number DSM33837.

[0011] In another aspect, the present disclosure refers to a method of enriching grain, the method comprising inoculating grain with the bacterial strain as defined herein.

[0012] In a further aspect, the present disclosure refers to a method of enriching grain-based feed ingredients, the method comprising inoculating grain with the bacterial strain as defined herein.

[0013] In one aspect, the present disclosure refers to a dry fermented product of grain, wherein the product is coated with the bacterial strain as disclosed herein.

[0014] In another aspect, the present disclosure refers to a composition comprising the dry fermented product of grain as disclosed herein.

[0015] In yet another aspect, the present disclosure refers to an animal feed ingredient comprising the dry fermented product of grain as disclosed herein.

[0016] In a further aspect, the present disclosure refers to the bacterial strain as disclosed herein for use in the preparation of grain for animal-feed ingredient purpose.

[0017] In another aspect, the present disclosure refers to a use of the animal feed ingredient disclosed herein in the manufacture of a medicament for improving animal digestion and/or absorption of nutrients.

BRIEF DESCRIPTION OF THE DRAWINGS

[0018] The invention will be better understood with reference to the detailed description when considered in conjunction with the non-limiting examples and the accompanying drawings, in which:

[0019] **Fig. 1** is a table showing the name of the individual isolated *Bacillus spp.* strains, DSM33837 (also referred to herein as GM1), DSM33839 (also referred to herein as RM4) and DSM33838 (also referred to herein as WB3), along with the results of the 16S rRNA analysis.

[0020] **Fig. 2** is a schematic showing the isolated *Bacillus spp.* strains DSM33837 (GM1), DSM33838 (WB3) and DSM33839 (RM4), and exemplary applications. The strains disclosed herein can be used for single-strain or multi-strain fermentation to produce modified wheat bran or defatted rice bran meal enriched with xylanase activity, increased reducing sugar, loaded with *Bacillus* bacteria counts, and increased protein hydrolysis.

[0021] **Fig. 3a to 3c** show the results of an exemplary application of the isolated *Bacillus spp.* strains. The example shown here is the example of a solid-state fermentation of defatted rice bran meal. **Fig. 3a** shows an increase in the level of reducing sugar in defatted rice bran meal after incubation with the isolated *Bacillus spp.* strains DSM33837 or DSM33839. **Fig.3b** and **Fig.3c** show the enrichment of xylanase activity in the defatted rice bran meal after incubation with the isolated *Bacillus spp.* strains DSM33837 and DSM33839, respectively. These results show that upon incubation of the defatted rice bran meal with the isolated strains in water (1:1 ratio, w/w), hydrolysis of carbohydrate polymers and an increase in hemicellulase activity were detected in less than 24 hours and as early as 8 hours in defatted rice bran meal. The control is defatted rice bran meal incubated with water only in 1:1 ratio (w/w). In the xylanase activity test, the “no substrate control” refers to assays performed with only the enzymes extracted from the defatted rice bran meal without the substrate.

CLAIMS

1. A bacterial strain deposited under deposition number DSM33837.

2. A combination comprising the bacterial strain according to claim 1 and a bacterial strain deposited under deposition number DSM33839 and/or a bacterial strain deposited under deposition number DSM33838.

3. The bacterial strain of claim 1, or the combination of claim 2, as a freeze-dried product.

4. The bacterial strain of claim 1, or the combination of claim 2, as a liquid culture product.

5. A method of fermenting bran and/or meal, the method comprising inoculating the bran and/or meal with the bacterial strain deposited under deposition number DSM33837 alone, or in combination with a bacterial strain deposited under deposition number DSM33839 and/or a bacterial strain deposited under deposition number DSM33838.

6. The method of claim 5, wherein the fermentation is solid-state fermentation.

7. A method of solid-state fermentation of bran and/or meal, the method comprising inoculating the bran and/or meal with a bacterial deposited under deposition number DSM33837.

8. The method of claim 7, wherein the method further comprises inoculating the bran and/or meal with bacteria of deposition number DSM33839 and/or bacteria of deposition number DSM33838.

9. The method of claim 7 or 8, wherein the inoculation is performed using the bacterial strain in a concentration of between $1.6x10^6$ to $1.6x10^{10}$ CFU.

10. The method of claims 5 to 9, wherein the fermentation or solid-state fermentation is batch fermentation.

11. The method of claims 5 to 10, wherein the fermentation or solid-state fermentation is continuous fermentation.

12. The method of claims 7 to 11, wherein the fermentation or solid-state fermentation is carried out at a temperature between 25°C to 37°C.

13. The method of claims 7 to 12, wherein the fermentation or solid-state fermentation is carried out at a ratio of at least 1:1 of bran or meal to water.

14. The method of claims 7 to 13, wherein the fermentation or solid-state fermentation comprises a blending step.

15. A method of enriching grain, the method comprising inoculating grain with the bacterial strain as defined in claim 1, or the combination of claim 2.

16. A method of enriching grain-based feed ingredients, the method comprising inoculating grain with the bacterial strain as defined in claim 1, or the combination of claim 2.

17. The method of any one of claims 5 to 16, wherein the method results in an increase in enzyme activity.

18. The method of claim 17, wherein the enzyme is protease and/or xylanase.

19. The method of claim 17 or 18, wherein the activity is hydrolysis.

20. The method of any one of claims 5 to 19, wherein the inoculation takes place prior to fermentation, or prior to administration to an animal.

21. The method of any one of claims 5 to 20, wherein the bacterial strain increases any one or more of the factors selected from the group consisting of hydrolysed protein content, concentration of reducing sugars, soluble protein content, and xylanase activity.

22. The method of any one of claims 5 to 14, or the method of any one of claims 15 to 21 wherein the bran and/or meal, or the grain, is selected from the group consisting of oilseed, nut, and legume.

23. The method of any one of claims 5 to 14 or 22, or the method of any one of claims 15 to 22, wherein the bran and/or meal, or the grain, is selected from the group consisting of wheat, wheat germ, white rice, brown rice, germinated brown rice, barley, oats, red rice, sticky black rice, sticky rice, rice bran, rye, triticale, spelt, sorghum, soybean, black soybean, black bean, kidney bean, white bean, pinto bean, navy bean, lima bean, mung bean, adzuki bean, cocoa bean, broad bean, flageolet bean, mogette bean, coffee bean, pea, chickpea, black-eyed pea, garden pea, snow pea, snap pea, pigeon pea, quinoa, lentils, adlay, corn, millet, fonio, teff, sesame seed, rapeseed, sunflower seed, nutmeg, poppy seed, grape seed, chia seed, flax seed, pumpkin seed, hemp seed, mustard seed, cottonseed, peanut, cashew nut, almond, pistachio, walnut, macadamia nut, pecan, hazelnut, brazil nut, oak nut, hickory nut, kola nut, araucaria nut, bunya bunya nut, candlenut (buah keras), oil palm kernel, palm press fiber, copra meal, karuka nut, mongongo nut, paradise nut, red bopple nut, pine nut, chest nut, parts thereof, and combinations thereof.

24. A dry fermented product of grain, wherein the product is coated with the bacterial strain of claim 1, or the combination of claim 2.

25. A composition comprising the dry fermented product of grain of claim 24.

26. An animal feed ingredient comprising the dry fermented product of grain of claim 24.

27. The bacterial strain of claim 1, or the combination of claim 2, for use in the preparation of grain for animal-feed ingredient purpose.

28. The product of claim 24, the composition of claim 25, the animal feed ingredient of claim 26, or the grain of claim 27, wherein the grain is wheat bran, rice bran, meal thereof, or a combination of wheat bran and rice bran, or meal thereof.

29. The product, the composition, the animal feed ingredient, or the grain of claim 28, wherein the rice bran is defatted rice bran.

30. Use of the animal feed ingredient according to any one of claims 26 or 28 to 29 in the manufacture of a medicament for improving animal digestion and/or absorption of nutrients.

31. The use of any one of claims 20 or 30, wherein the animal is selected from the group consisting of a domesticated or wild (undomesticated) animal, livestock, and aquatic animal.