

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.

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SUMMARY

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

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

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

[0009] In a further aspect, the present disclosure refers to a method of fermenting bran and/or meal, the method comprising inoculating the bran and/or meal with one or two or all bacterial strains selected from the group consisting of a bacterial strain deposited under deposition number DSM33837, a bacterial strain deposited under deposition number DSM33839, and 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.*

[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.

5 [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.

10 [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 method of improving animal digestion and/or absorption of nutrients, the method comprising administering the animal feed ingredient disclosed herein to an animal.

15 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:

20 [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.

25 [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.

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[0022] **Fig. 4a and 4b** 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 wheat bran. **Fig. 4a** shows the change in wheat bran reducing sugar content after incubation with DSM33837. An increase in the level of reducing sugars in wheat bran can be seen after incubation with DSM33837. **Fig. 4b** shows the change in in wheat bran reducing sugar content after incubation with DSM33838 or DSM33839. An increase in the level of reducing sugars in wheat bran can be seen after incubation. These results demonstrated that incubation of wheat bran in water (1:1 ratio, w/w) with the isolated strains lead to an increase in hydrolysis of carbohydrate polymers in less than 24 hours and as early as 6 hours. The control is wheat bran incubated with water only in 1:1 ratio (w/w).

[0023] **Fig. 5a and 5b** show the results of an exemplary application of the isolated *Bacillus spp.* strains. **Fig. 5a** and **Fig. 5b** show results of sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE) analyses of protein hydrolysis in defatted rice bran meal using crude enzymes prepared from liquid cultures of DSM33839. Secreted enzymes from DSM33839 were shown to increase protein hydrolysis in defatted rice bran meal. Fig. 5a shows the results of rice bran meal proteins after the first extraction, with Fig. 5b showing the results of rice bran meal (RBM) proteins after the second extraction. These results show that DSM33839 secretes enzymes that increase protein hydrolysis in defatted rice bran meal. Asterisks indicate protein bands that are absent or reduced in the treated defatted rice bran meal. The defatted rice bran meal was first incubated in water with or without crude enzymes from DSM33839, the water extract was collected after the incubation for SDS-PAGE analysis as first extraction. The treated defatted rice bran meal was then further incubated with Tris-buffer for 60 min to obtain the second extraction fraction.

[0024] **Fig. 6a and 6b** show the results of an exemplary application of the isolated *Bacillus spp.* strains. **Fig. 6a** and **Fig. 6b** show results of sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE) analyses of protein hydrolysis in defatted rice bran meal using crude enzymes prepared from liquid cultures of DSM33837. Secreted enzymes from DSM33837 increase protein hydrolysis in defatted rice bran meal. Fig. 6a shows the results of rice bran meal (RBM) proteins after the first extraction, with Fig. 6b showing the results of rice bran meal proteins after the second extraction. These results show that DSM33837 secretes enzymes that increase protein hydrolysis in defatted rice bran meal. Asterisks indicate protein bands that are absent or reduced in the treated defatted rice bran meal. The defatted rice bran meal was first incubated in water with or without crude enzymes from DSM33837, the water extract was collected after the incubation for SDS-PAGE analysis as first extraction. The treated defatted rice bran meal was then further incubated with Tris-buffer for 60 minutes to obtain the second extraction fraction.

[0025] **Fig. 7a and 7b** show the results of an exemplary application of the isolated *Bacillus spp.* strains. **Fig. 7a** and **Fig. 7b** show characterization of two dried, modified products of defatted rice bran meal (after drying at 60°C for 48 hours in a 20-gram scale). One product was treated with DSM33837 for 8 hours, the other treated for 24 hours with the same strain. Fig. 7a shows that in both modified defatted

rice bran meal products, live colony-forming units of *Bacillus* were recovered. Fig. 7b shows increased reducing sugar content and detectable xylanases activity in both products as compared to control. These results show that defatted rice bran meal incubated with DSM33837 leads to enhanced functionality of defatted rice bran meal. “No DSM33837” control is defatted rice bran meal treated with water only.

5 [0026] **Fig. 8** shows characterization of a dried, modified product of wheat bran (dried at 60°C for 48 hours in a 20-gram scale before being treated with DSM33837 for 24 hours). In the modified wheat bran, live colony-forming units of *Bacillus* were recovered, accompanied by increased reducing sugar content and detectable xylanases activity as compared to a control. These results show that wheat bran incubated with DSM33837 leads to enhanced functionality of wheat bran. “No DSM33837” control is wheat bran
10 treated with water only.

[0027] **Fig. 9** shows the results of a sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE) analysis of fermented wheat bran (20g-scale). The dried fermented wheat bran was rehydrated and washed with water to remove bacteria, and further incubated with amylase “BAN” (Bacterial Amylase Novo) to remove starch, for better visualization of protein for SDS-PAGE analysis.
15 The DSM33837-fermented wheat bran was shown to have reduced protein bands (highlighted by asterisks in non-fermented wheat bran) indicating that fermentation with DSM33837 has increased the hydrolysis of the wheat bran-bound proteins.

[0028] **Fig.10** shows the results of exemplarily incubating wheat bran with DSM33827 versus wild-type *Bacillus* strain CK7 (Control *Bacillus*). Wheat bran incubated with DSM33837 showed higher
20 reducing sugar content and xylanase activity after 12 hours and 24 hours of incubation.

DETAILED DESCRIPTION

[0029] Fermentation of grains, such as, for example, defatted meals, brans, wheat and rice, prior to feeding allows the nutrients contained in the produce, such as, but not limited to, grains, meal, bran, or
25 combinations thereof to be accessed more easily, and therefore improves digestion and absorption of these nutrients. Also, pre-processing of feed ingredients can also render previously innutritious ingredients into more nutritious ingredients, and thereby further improve digestion. Such an improvement of digestion can ameliorate certain symptoms in animals suffering from, for example, colic, gastrointestinal pain, malnourishment and indigestion. Thus, disclosed herein are bacterial strains which
30 have been identified and shown to enhance digestibility of and provide probiotic load to the products, grains, and brans or meal obtained from grains as disclosed herein, which are, but are not limited to, defatted rice bran meal and wheat bran.

[0030] Without being bound by theory, it is thought that providing or introducing a probiotic load to an animal digestive system enhances feed digestibility and improves gut health in the animal by assailing
35 any digestibility issues that may arise from the consumption of grains. Thus, pre-treatment of grains prior to feeding or administration to the animal, for example, by fermenting the grains disclosed herein or by

coating the grains prior to feeding, has been shown in the data disclosed herein to improve digestibility, and thereby increase absorption of nutrients in the animal digestive tract.

[0031] It was found that the isolated bacterial strains disclosed herein displayed increased enzymatic activity compared to, for example, other strains known in the art. Specifically, the isolated *Bacillus spp.* strains disclosed herein are shown to have increased xylanase activity, increased reducing sugar content, and increased protein hydrolysis. It is shown in the present application that the isolated *Bacillus spp.* strains affect detectable xylanases activity upon in contact with defatted rice bran (Fig. 3b and 3c). Fermentation using the selected strains also resulted in increase of reducing sugar in wheat bran (Fig. 4a and 4b).

[0032] A fermentative (bacterial) strain needs to be equipped with several important features, for example, being able to produce essential fibre hydrolysing activity, thereby increasing the reducing sugar content of the grains, meals, and brans. Another feature is having detectable enzyme activity for testing using standard substrate and enzyme assay. Also, a fermentative strain must be capable of providing live colony forming units (CFU) upon completion of the fermentation and product drying. This is to ensure that the bacteria can have a probiotic function, thus being able to continue functioning in the gastrointestinal tract and provide benefit to an animal in terms of gut health.

[0033] For example, regarding proteins associated with defatted rice bran meal fibre matrix, these proteins were shown to be more accessible for digestion, absorption and utilization upon treatment with enzymes secreted by the isolated *Bacillus spp.* strains described herein. (Fig. 5a, 5b, 6a and 6b)

[0034] These features are thought to increase the digestibility of the grain, or meals and brans of grains, in question, as an increase, for example, in the level of hydrolysed proteins and reducing sugars indicates the breakdown of complex nutrients into simple, less complex nutrients, thereby reducing the effort on the gastrointestinal tract to break down these nutrients, as well as being more easily taken up in the intestinal tract. These features, whether taken alone or in combination, were not shown to be common to all *Bacillus* strains of bacteria, as shown for example in the comparison of the isolated *Bacillus spp.* strains disclosed herein with a *Bacillus* strain CK7 (see Fig. 10).

[0035] Thus, in one example, the bacterial strain as disclosed herein is an isolated bacterial strain. In another example, the bacterial strain is a strain deposited under any one of the following deposition numbers DSM33837, DSM33838, and DSM33839. In another example, the bacterial strain is a strain with the deposition number DSM33837 (also referred to herein as GM1). In another example, the bacterial strain is a strain with the deposition number DSM33839 (also referred to herein as RM4). In another example, the bacterial strain is a strain with the deposition number DSM33838 (also referred to herein as WB3).

[0036] Using various screening methods, the isolated *Bacillus spp.* strains disclosed herein are capable of secreting complex enzymes targeting protein and fibre components of the wheat bran and defatted rice bran.

[0037] In one example, the present disclosure describes isolated strains of *Bacillus spp.* and their uses, for example, in the preparation of enriched grains, meals, brans, and combinations thereof. Such grains, meals, brans, and combinations thereof are, for example, but are not limited to, wheat bran and defatted rice bran meal. Such enriched grains, meals, brans, and combinations thereof can be used in, for example, animal feed.

[0038] Bacterial strains can be transported and provided in many different forms. A person skilled in the art will appreciate that the form of the bacterial strain can be tailored to the required application, so long as the bacterial strain is viable upon receipt and application. For example, such a form can be an inactive, but viable form of the bacterial strain. In one example, the bacterial strains disclosed herein are provided as a freeze-dried product. In another example, the bacterial strains disclosed herein are provided as a liquid culture product.

[0039] In one example, there is disclosed a method of fermentation comprising application of the bacterial strains disclosed herein. In another example, there is disclosed a method of enriching grain, meal, bran or combinations thereof by inoculating grain, meal, bran, or combinations thereof with the bacterial strain as described herein. In a further example, there is disclosed a method of enriching grain-based feed ingredients, the method comprising inoculating grain, meal, or bran or combinations thereof with the bacterial strain as defined herein. Also disclosed herein are methods of improving animal digestion and/or absorption of nutrients, the method comprising administering to an animal an animal feed ingredient as described herein.

[0040] In the context of the present application, the term “enriching” refers to edible products which have been modified to be increased in nutrients, or in which nutrients have been made more accessible to the digestive system, thereby increasing the amount of nutrients that can be taken up by the gastrointestinal tract.

[0041] As described herein, the fermentation can be performed using one, or two, or all bacterial strains disclosed herein. In one example, the method of fermenting as disclosed herein is a method of fermenting bran and/or meal, or grain. In another example, the method comprises inoculating the bran and/or meal with one or two or all bacterial strains selected from the group consisting of a bacterial strain deposited under deposition number DSM33837, a bacterial strain deposited under deposition number DSM33839, and a bacterial strain deposited under deposition number DSM33838.

[0042] Thus, in one example, there is disclosed the use of the isolated *Bacillus spp.* strains described herein in the methods described herein. One such method is, for example, grain fermentation. Another such example is the fermentation of bran and/or meal. In another example, the bacterial strain of *Bacillus spp.* used in the method disclosed herein is one, two or all of the bacterial strains of bacteria of the deposition number DSM33837, bacteria of deposition number DSM33839, and bacteria of deposition number DSM33838.

[0043] The term “fermentation”, as used herein, refers to a metabolic process that produces chemical changes in organic substrates through the action of enzymes. In microorganisms, fermentation is the primary means of producing adenosine triphosphate (ATP) by the degradation of organic nutrients anaerobically. In food production, can refer to any process in which the activity of microorganisms (for example, but not limited to yeast and bacteria,) brings about a desirable change to a foodstuff or beverage. Such a desirable change can be, for example, the metabolism of sugars to alcohol, or the breaking down of indigestible components, such as fibres, into smaller and possible digestible metabolites. For example, fermentation is used for preservation in a process that produces lactic acid found in such sour foods as pickled cucumbers, kombucha, kimchi, and yogurt, as well as for producing alcoholic beverages such as wine and beer. Fermentation also occurs within the gastrointestinal tracts of all animals, including humans. Depending on the bacterium used for fermentation, the fermentation can take place aerobically or anaerobically.

[0044] Most industrial fermentation uses batch or fed-batch procedures, although continuous fermentation can be more economical if various challenges, particularly the difficulty of maintaining sterility, can be met.

[0045] In a batch process, all the ingredients are combined, and the reactions proceed without any further input. Batch fermentation goes through a series of phases. There is a lag phase, in which microorganisms adjust to their environment; then a phase in which exponential growth occurs. Once many of the nutrients have been consumed, the growth slows and becomes non-exponential, but production of secondary metabolites (including, but not limited to, antibiotics and enzymes) accelerates. This continues through a stationary phase after most of the nutrients have been consumed, after which all the microorganisms present die.

[0046] In continuous fermentation, substrates are added and final products are removed continuously. There are three varieties of continuous fermentations: chemostats (which hold nutrient levels constant), turbidostats (which keep microorganisms mass constant) and plug flow reactors (in which the culture medium flows steadily through a tube while the microorganisms are recycled from the outlet to the inlet). If the process works well, there is a steady flow of feed and effluent, and the costs of repeatedly setting up a batch are avoided. Also, continuous fermentation can prolong the exponential growth phase of the bacteria, thereby avoiding by-products that inhibit the reactions by continuously removing them. However, as appreciated in the art, it is difficult to maintain a steady state and avoid contamination, and the continuous fermentation designs tend to be complex.

[0047] The term “solid state fermentation” refers to a fermentation process in which the microorganisms required for fermentation are grown on a solid support. This technology for the culture of microorganisms is an alternative to liquid or submerged fermentation, used predominantly for industrial purposes.

[0048] In one example, the fermentation is solid-state fermentation. In another example, the fermentation is batch or continuous fermentation. In yet another example, the fermentation is a solid-state batch fermentation, or a solid-state continuous fermentation.

[0049] In one example, the fermentation is carried out at a temperature between 20°C to 40°C, or, for example, between 25°C to 37°C. In another example, the fermentation is carried out at a temperature of about 24°C, about 25°C, about 26°C, about 27°C, about 28°C, about 29°C, about 30°C, about 31°C, about 32°C, about 33°C, about 34°C, about 35°C, about 36°C, about 37°C, about 38°C, about 39°C, or about 40°C.

[0050] In one example, the fermentation is carried out in a reaction volume of at least 1 gram. In another example, the reaction volume is between 1 gram and 20 grams. In another example, the reaction volume is selected from, but not limited to, about 1 gram, about 2 grams, about 3 grams, about 4 grams, about 5 grams, about 6 grams, about 7 grams, about 8 grams, about 9 grams, about 10 grams, about 11 grams, about 12 grams, about 13 grams, about 14 grams, about 15 grams, about 16 grams, about 17 grams, about 18 grams, about 19 grams, or about 20 grams. A person skilled in the art will appreciate that the methods and products disclosed herein are up scalable. That is to say that the methods disclosed herein can be performed on an experimental scale (for example, but not limited to laboratory application) up to an industrial scale, with reaction volumes ranging from grams to tonnes, as required.

[0051] The fermentation disclosed herein is performed in the presence of water, or in a humid environment. In one example, the fermentation is carried out on bran and/or meal, whereby the bran and/or meal is presented in a ratio of at least 1:2 of bran and/or meal to water. In another example, the ratio of bran and/or meal to water is at least 1:1.

[0052] As the fermentation described herein is performed in the presence of water, adjustment of the pH of the fermentation reaction is not required. In other words, the fermentation takes place at the pH of water.

[0053] In another example, the fermentation disclosed herein comprises a blending step. Such a blending step can be, for example, a step of adding of bran and/or meal, or grain (for example, but not limited to, wheat bran or defatted rice bran meal) with the bacterial strains disclosed and water. This blending (also referred to as a mixing step) can also form part of the process of initiating fermentation. In other words, the blending step, as referred to herein, pertains to the bacterial strains, as disclosed herein, coming into contact with bran and/or meal, or grain (for example, but not limited to, wheat bran or defatted rice bran meal) in the presence of water. It is also of note that the fermentation to be initiated here is, in this example, primarily an aerobic fermentation. It is also contemplated within the scope of the present application that such “blended” or “mixed” products can be fed directly to animals without having gone through the fermentation process disclosed herein.

[0054] As disclosed herein, the results of fermentation with the isolated *Bacillus spp.* strains described herein are, but are not limited to, an increase in the concentration of reducing sugar released, an

increase in protein hydrolysis, an increase in xylanase activity, and combinations thereof. It is of note that the soluble or hydrolysed protein content and protein hydrolysis (as the result of, for example, enzymatic or bacterial activity) are interrelated. An increase in protein hydrolysis will result in an increase in soluble or hydrolysed protein content/concentration. Thus, due to this direct correlation between these two concepts, the terms “soluble protein”, “hydrolysed protein”, and “protein hydrolysis” can be used interchangeably in the present disclosure.

[0055] As used herein, the term “inoculation” or “inoculating” refers to the contact of a microorganism with a culture medium or a substrate. An inoculum is that with which the inoculation is performed.

[0056] In the method disclosed herein, the bacterial strains disclosed herein are applied or used to inoculate grains, meals, brans, parts, or combinations thereof, as described herein, in an amount that is sufficient to initiate fermentation of the same in the appropriate conditions.

[0057] As described herein, the concentration of bacteria is at least 1.6×10^6 colony forming units (CFUs). In another example, the concentration of bacteria is between 1.6×10^6 to 1.6×10^{12} colony forming units. In yet another example, the concentration of bacteria is between 1.6×10^6 to 1.6×10^{10} colony forming units. In a further example, the concentration of bacteria is about 1.5×10^6 , 1.5×10^7 , 1.5×10^8 , 1.5×10^9 , 1.5×10^{10} , 1.5×10^{11} , or 1.5×10^{12} colony forming units. In another example, the concentration of bacteria is about 1×10^6 , 1×10^7 , 1×10^8 , 1×10^9 , 1×10^{10} , 1×10^{11} , 1×10^{12} colony forming units. A person skilled in the art will be capable of selecting the concentration of bacteria required for fermentation in order to obtain the result as disclosed herein.

[0058] In one example, the inoculation takes place prior to fermentation.

[0059] The method disclosed herein require that an appropriate strain selected for the fermentation of grain, meal, bran, or combinations thereof, for example, wheat bran and defatted rice bran is selected. Not all other *Bacillus spp.* strains will provide the described enrichment of the grain, meal, bran, or combinations thereof as disclosed herein.

[0060] In one example, in the dried products, the presence of recoverable *Bacillus* colony forming units upon rehydration was shown in products containing the isolated *Bacillus spp.* strains (Figs. 7a and 8), indicating that live probiotic bacteria are to be administered together with the meal for uptake into the gastrointestinal tract. This has been shown to contribute to improved gut health in, for example, the animals.

[0061] Thus, in one example, dried fermented products of wheat bran and rice bran meal are loaded with recoverable live bacteria. Such loading also accompanied by increased xylanase activity, an increase in the level of reducing sugars, as well as an increase in xylanase/protease activity.

[0062] As used herein, the term “grain” refers to a small, hard, dry seed, with or without an attached hull or fruit layer, harvested for human or animal consumption. The terms “seed” and “grain” can be used interchangeably in the context of the present application. A grain crop is, for example, a grain-producing

plant. The two main types of commercial grain crops are, for example, cereals, and legumes. Grains and cereal are synonymous with caryopses, the fruits of the Poaceae (grass) family. Specifically in the art of agronomy and commerce, seeds or fruits from other plant families are called grains if they resemble caryopses. For example, amaranth is sold as "grain amaranth", and amaranth products may be described as "whole grains".

[0063] Cereal grains, for example, come from crops which are members of the Poaceae (grass) family. Cereal grains contain a substantial amount of starch, a carbohydrate that provides dietary energy. Examples of grains are, but are not limited to finger millet, fonio, foxtail millet, Japanese millet, Job's tears (also referred to as adlay or adlay millet), kodo millet, maize (also referred to as corn), millet, pearl millet, proso millet, sorghum, quinoa, barley, oats, red rice, sticky black rice, sticky rice, rice bran, rye, spelt, teff, Triticale, wheat, wheat germ, white rice, brown rice, germinated brown rice, and wild rice.

[0064] Pulses (also known as grain legumes or legumes) are the grains or seeds of the members of the Fabaceae or Leguminosae family. Pulses have a higher protein content than most other plant foods, usually at around 20%, while soybeans have as much as 35%. As is the case with all other whole plant foods, pulses also contain carbohydrate and fat. Examples of pulses, or grain legumes, are, but are not limited to, chickpea, common bean, common pea (garden pea), fava bean, lentils, lima bean, lupin, mung bean, adzuki bean, cocoa bean, peanut, pigeon pea, runner bean, soybean, black soybean, black bean, kidney bean, white bean, pinto bean, navy bean, broad bean, flageolet bean, mogette bean, coffee bean, pea, black-eyed pea, snow pea, and snap pea.

[0065] As used herein, the term "oilseed" refers to grains which are grown primarily for the extraction of their edible oil. Vegetable oils provide dietary energy and some essential fatty acids. Plant families that fall within the category of oilseeds are, but are not limited to, plants of the Brassicaceae (mustard), Asteraceae (aster), Linaceae (flax), Cannabaceae (hemp) and Papaveraceae (poppy) families. As such, examples of oilseeds are, but are not limited to, black mustard seed, India mustard seed, white mustard seed, rapeseed (including canola), safflower, sunflower seed, flax seed, hemp seed, sesame seed, sunflower seed, grape seed, chia seed, pumpkin seed, cottonseed and poppy seed.

[0066] As used herein, the term "nuts" refers to a fruit composed of an inedible hard shell and a seed, which is generally edible. It is noted that the usage of the term "nuts" is less restrictive, and many nuts (in the culinary sense), such as acorns, almonds, cashews, pecans, pistachios, walnuts, and Brazil nuts, are not nuts in a botanical sense, but rather drupe fruits, (as is the case with almonds, cashews, pecans, pistachios, walnuts) or capsule seeds (Brazil nut). Thus, the term "nuts", as used herein, encompasses both nuts in the biological sense, as well as nuts in the culinary or food-industrial sense. Non-limiting examples of nuts are 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), nutmeg, oil palm kernel, palm press fiber, copra meal, karuka nut, mongongo nut, paradise nut, red bopple nut, pine nut, and chestnut.

[0067] As used herein, the term “bran”, also known as miller's bran, refers to the hard outer layers of cereal grain. It consists of the combined aleurone and pericarp. Along with germ, it is an integral part of whole grains, and is often produced as a by-product of milling in the production of refined grains. Bran is present in cereal grain, including rice, corn (maize), wheat, oats, barley, rye and millet. Bran is not the same as chaff, which is a coarser scaly material surrounding the grain but not forming part of the grain itself, and which is indigestible to humans.

[0068] The term “meal”, as used herein, refers to the coarse-ground, edible part of various grains. Meal is often used in animal feed and can also be characterised as being a coarser blend than flour, whereby flour refers to a powder made by grinding raw grains, roots, beans, nuts, or seeds. It is noted that this does not refer only to the wheat flour commonly used, but refers to the ground product of raw grains, roots, beans, nuts, or seeds. As used herein, the term “meal” also refers to products that remain after oil extraction of grains, brans or oilseeds has been completed. A meal may or may not contain further additives and agents for, for example, enriching the meal, or preventing caking after grinding.

[0069] In one example, the meal referred to herein is a defatted meal. As used herein, the term “defatted” refers to the removal of fat from the product in question. When used in conjunction with meal, for example, defatted meal refers to meal which has a lower fat content than otherwise untreated (non-defatted) meal.

[0070] As used herein, the term “modified”, in the context of the present disclosure and when used in conjunction with, for example, defatted rice bran or wheat bran, is used synonymously with the term “fermented” and refers to the resulting product from treatment of the bran/meal or grain as disclosed herein with one or more bacterial strains disclosed herein. For example, the term “modified defatted rice bran meal” or “modified wheat bran” refers to either defatted rice bran or wheat bran which has been treated with a bacterial strain as disclosed herein. This term can refer to such a mixture of, for example, modified wheat bran and a bacterial strain as disclosed herein after mixing but before fermentation, or after fermentation of the same. The term “modified” is also used to refer to the dried products of the treatment and methods disclosed herein.

[0071] In one example, the grain, bran, meal, or parts of the grain, disclosed herein are, but are not limited to, oilseed, nut, and legume.

[0072] In another example, the grain, bran, meal, or parts of the grain is, but is not limited to, 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, 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, copra meal, karuka nut, mongongo nut, paradise nut, red bopple nut, pine nut, chest nut, parts thereof, and combinations thereof. In another example, the grain is wheat and rice. In another example, the grains are wheat bran and defatted rice bran. In another example, the grains are defatted rice bran meal.

5 [0073] In one example, the subject of the methods of fermentation as disclosed herein are part of the grain. Such a part of a grain can be, but are not limited to, bran, germ, endosperm and combinations thereof. In another example, the bran is obtained from any of the grains disclosed herein, for example, but not limited to 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, 10 kidney bean, white bean, pinto bean, navy bean, lima 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 15 nut, bunya bunya nut, candlenut (buah keras), oil palm kernel, copra meal, karuka nut, mongongo nut, paradise nut, red bopple nut, pine nut, chest nut, parts thereof, and combinations thereof. In yet another example, the bran is wheat bran, or rice bran, or a combination of rice bran and wheat bran. In another example, the bran is defatted rice bran.

[0074] The parts of the grains or the grains disclosed can be pre-processed prior to use in 20 fermentation. Such pre-processing can include, but is not limited to, grinding, mashing, mulching, dried prior to grinding, defatted, enriched, frozen, dried, blended, and combinations thereof. In one example, the bran is defatted.

[0075] In addition to the fibre hydrolysis, the *Bacillus spp.* strains disclosed herein were shown to increase protein hydrolysis. The liquid culture media used for producing the isolated *Bacillus spp.* strains 25 disclosed herein was also shown to be enriched in proteases. Without being bound by theory, such proteases are thought to assist in degrading proteins present in the grain, and hence are thought to facilitate uptake of nutrients from the grains, and also increase protein utility and absorption. As shown in data present herein, crude enzyme preparation from the liquid culture medium of the isolated *Bacillus spp.* strains were able to hydrolyse protein in the defatted rice bran (Fig. 5a, 5b, 6a and 6b). In a separate 30 example, wheat bran fermented with the isolated *Bacillus spp.* strains showed increased protein hydrolysis in the final product (Fig. 9).

[0076] Increase protein hydrolysis in the wheat bran and defatted rice bran meal provides more hydrolysed protein for animal digestion and absorption, thus increase the value of the wheat bran and defatted rice bran meal as animal feed ingredient or additive.

35 [0077] Thus, in one example, the method disclosed herein results in an increase in any one or more of factors such as, for example, but not limited to, hydrolysed protein content, concentration of reducing sugars, xylanases activity, and combinations thereof. In one example, the method described herein results

in an increase in enzyme activity. Such enzyme activity is, but is not limited to, for example, hydrolysis, xylanase activity, protease activity or combinations thereof. In one example, the enzyme activity is hydrolysis. In another example, the enzyme activity is protease activity. In another example, the enzyme activity is xylanase activity.

5 [0078] The product made using the methods disclosed herein can be used in various applications. One example of such an application is feeding animals. Another exemplary application is the use of the bacterial strains as described herein, or combinations thereof in the preparation of grain, meal, or bran for animal-feed ingredient purpose. The methods disclosed herein can also be used to obtain products such as, but not limited to, a dry fermented product of grain, meal, or bran, as disclosed herein, a composition
10 comprising the dry, fermented product of grain, meal, or bran, and/or an animal feed ingredient comprising the dry, fermented product of grain, meal, or bran. Such products can also be further processed based on their intended application, for example, for use in animal feed.

[0079] The term “livestock”, as disclosed herein, refers to any domesticated animal raised in an agricultural setting to produce labour and commodities such as meat, eggs, milk, fur, leather, and wool.
15 The term is sometimes used to refer solely to those that are bred for consumption, while other times it refers only to farmed ruminants, such as cattle, sheep and goats. The definition of livestock, as used herein, includes, but is not limited to horses, donkey, cows (also referred to as cattle), zebu, bali cattle, yak, water buffalo, pigs, sheep, goats, poultry, fish.

[0080] Thus, in one example, an animal is, but are not limited to, domesticated or wild
20 (undomesticated) animal, livestock, and aquatic animal. As such, the products disclosed herein can be used, for example, in farming, aquaculture, animal rearing, and any situation in which an animal is to be fed.

[0081] The invention illustratively described herein may suitably be practiced in the absence of any element or elements, limitation or limitations, not specifically disclosed herein. Thus, for example, the
25 terms “comprising”, “including”, “containing”, etc. shall be read expansively and without limitation. Additionally, the terms and expressions employed herein have been used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that
30 although the present invention has been specifically disclosed by preferred embodiments and optional features, modification and variation of the inventions embodied therein herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention.

[0082] As used in this application, the singular form “a,” “an,” and “the” include plural references
35 unless the context clearly dictates otherwise. For example, the term “a genetic marker” includes a plurality of genetic markers, including mixtures and combinations thereof.

[0083] As used herein, the term “about”, in the context of concentrations of components of the formulations, typically means +/- 5% of the stated value, more typically +/- 4% of the stated value, more typically +/- 3% of the stated value, more typically, +/- 2% of the stated value, even more typically +/-1% of the stated value, and even more typically +/- 0.5% of the stated value.

5 [0084] Throughout this disclosure, certain embodiments may be disclosed in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the disclosed ranges. Accordingly, the description of a range should be considered to have specifically disclosed all the possible sub-ranges as well as individual numerical values within that range. For example, description of a range such as from
10 1 to 6 should be considered to have specifically disclosed sub-ranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 3, 4, 5, and 6. This applies regardless of the breadth of the range.

[0085] Certain embodiments may also be described broadly and generically herein. Each of the narrower species and sub-generic groupings falling within the generic disclosure also form part of the
15 disclosure. This includes the generic description of the embodiments with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein.

[0086] The invention has been described broadly and generically herein. Each of the narrower species and sub-generic groupings falling within the generic disclosure also form part of the invention. This
20 includes the generic description of the invention with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein.

[0087] Other embodiments are within the following claims and non-limiting examples. In addition, where features or aspects of the invention are described in terms of Markush groups, those skilled in the
25 art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group.

EXPERIMENTAL SECTION

[0088] Provided below are non-limiting examples of the claimed invention.

30 *Isolation and identification of Bacillus strains*

[0089] One gram of agricultural plant biomass and 5 ml of sterile distilled water were added to a 50-ml falcon polypropylene tube and incubated in a 37°C incubator. After several days, a sample of the culture was streaked on a Luria-Bertani (LB) agar plate and incubated at 37°C overnight. Sub-culture with repeated streaking was performed to obtain pure culture. Grown single colonies were picked and
35 inoculated into LB broth overnight at 37°C, 220 rpm. Next day, the culture was collected, washed with water, and inoculated at initial OD600 of 0.2 into M9 minimal medium (6.78 g/l Na₂HPO₄•7H₂O, 3 g/l

KH₂PO₄, 1 g/l NH₄Cl, 0.5 g/l NaCl) containing 0.5% (w/v) beech wood xylan as sole carbon source. The tubes were incubated at 37°C, 220 rpm for several days and their enzyme activity was assessed by a preliminary plate screen with Azurine-cross-linked xylan (AZCL-xylan) and the xylanase assay as described below. The isolates with high xylanase activity were selected and stored in 30 % glycerol at -80°C for further analysis.

Production of crude enzymes

[0090] Enzyme production was carried out by inoculating cells at initial OD₆₀₀ of 0.1 in 100 mL of production medium (1% sucrose, 0.5% yeast extract, 0.1% MgSO₄•7H₂O, 0.12% (NH₄)₂SO₄, 0.02% NH₄Cl, 0.3% Na₂HPO₄, 0.4% K₂HPO₄, 0.004% CaCl₂ and 0.4% KH₂PO₄) under aerobic conditions at 37°C, 220rpm. The optimum culture period for enzyme production was investigated using bacterial cell growth curve and enzyme activity. Next, the cell biomass was removed from the culture by centrifugation at 4000 rpm for 15 minutes under cold condition. The cell-free supernatant was used as a crude enzyme for subsequent enzymatic assays. Protein concentration was determined via Bradford method, with bovine serum albumin (BSA) as a standard.

Identification of microorganisms

[0091] The phylogeny of isolated strains was further identified by 16S rRNA sequencing. Genomic DNA extraction was performed by the methodology as described by Hoffman et al. (1987; Gene; 57; 267-272) and used as template in PCR for 16S rRNA partial gene amplification (~500bp) using the following universal primers: WIC1P2336 (5'- CCT ACG GGA GGC AGC AG -3'; SEQ ID NO: 1) and WIC1P2337 (5'- GGA CTA CHV GGG TWT CTA AT -3'; SEQ ID NO: 2). The amplicons were purified and sequenced. Sequence similarity and homology analysis against the GenBank database were carried out using the basic local alignment search tool (BLASTN) on the National Center for Biotechnology Information (NCBI) (www.ncbi.nlm.nih.gov/).

Determination of xylanase activity

[0092] Xylanase activity assay was performed using the 3, 5-dinitrosalicylic acid (DNS) method as described in the art, with minor modifications. In short, the standard reaction mixture (total volume 1 ml) containing 950 µl 0.5% (w/v) substrate beech wood xylan in Phosphate-buffered saline (PBS) buffer (pH 7) and 50 µl appropriately diluted crude enzyme. As a negative control, crude enzyme was incubated with 950 µl PBS without substrate. The reaction was incubated in a thermomixer at 40°C, 1000 rpm for 60 minutes. After incubation, 300 µl of reaction product was aliquoted and stopped by adding 900 µl of DNS reagent, followed by boiling at 100°C for 5 minutes. The absorbance at 540 nm (A_{540nm}) was measured and the amount of reducing sugar liberated was extrapolated from the xylose standard curve. One unit of xylanase activity was defined as the amount of enzyme required to produce 1 µmol of xylose reducing sugar equivalents per min. The assay was conducted in triplicates.

Measurement of reducing sugar from solid-state fermentation (SSF)

[0093] Using the 3, 5-dinitrosalicylic acid (DNS) method as described in the art, the total reducing sugar liberated during the time course of solid-state fermentation (SSF) was examined. 300 µl of suitably diluted cell-free fermentation crude extract was added to 900 µl of DNS reagent and boiled at 100°C for 5 minutes. Following which, the absorbance at 540 nm (A_{540nm}) was measured and the amount of reducing sugar liberated was calculated outlined as above.

Production of solid-state fermentation (SSF) product

[0094] Solid-state fermentation was upscaled to 20 g of rice bran meal (RBM)/wheat bran (WB) in a stepwise manner. 1 g of sterilized rice bran meal (RBM)/wheat bran (WB) in a 50 ml falcon polypropylene tube was inoculated with 1 ml (1.6×10^8 CFU) of selected strains and allowed to incubate for 3-hours at 37°C. Subsequently, the 1 g of inoculated meal was mixed thoroughly with 19 g of meal in a 500 mL HDPE octagonal bottle, with a meal to moisture ratio of 1:1. Fermentation was carried out for a total of 24 hours at 37°C. Following fermentation, samples were transferred into a 1 L beaker, covered with a double layer of cheesecloth, and dried in a 60°C incubator for 48 hours, until excess moisture was completely evaporated.

Colony count of viable microbes from solid-state fermentation (SSF) product post-drying

[0095] Recovery of viable microbes from solid-state fermentation (SSF) product after drying was examined by re-hydrating 1 g of dried SSF product in 10 mL of distilled water and shaking thoroughly. The suspension was serially diluted to 10^{-4} . Diluted samples were plated onto LB agar plates and incubated at 37°C overnight. The resultant colony-forming units (CFU) were counted and used to calculate CFU/g of meal.

Performance evaluation of selected strains on rice bran meal (RBM) and wheat bran (WB) fermentation

[0096] The ability of selected strains to increase fibre hydrolysis in rice bran meal (RBM) and wheat bran (WB) after fermentation was assessed. Rice bran meal or wheat bran was autoclave sterilized at 121°C for 15 minutes and dried overnight in an oven. The sterilized rice bran meal or wheat bran (1 g) in 50-ml falcon polypropylene tubes were inoculated with 1 ml (1.6×10^8 CFU) of selected strains that were washed and re-suspended in sterile distilled water (1:2 ratio). Rice bran meal or wheat bran with distilled water only was used as the negative control. The rice bran meal or wheat bran was incubated at 37°C for 48 hours, with individual tubes collected at 4-hour intervals up to 24 hours, and subsequently at 12-hour intervals until 48 hours. After incubation, 9 ml of water was added into each experimental tube, mixed thoroughly, and then aspirated into a 15-ml falcon polypropylene tube. Then, the cell-free rice bran meal or wheat bran crude extract was obtained by centrifugation at 12,000 rpm for 2 minutes under cold conditions (4°C), and used for further analysis of protein content, xylanase activity and amount of xylan reducing sugar liberated. All experiments were conducted in triplicates.

Performance evaluation of crude enzymes on rice bran meal (RBM) protein hydrolysis

[0097] The effect of crude enzymes derived from production medium on proteins present in rice bran meal (RBM) was evaluated using sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-

PAGE). 5 ug of crude enzyme was added to 250 µl reaction mixtures containing 20% (w/v) RBM in distilled water and incubated at 40°C, 1000rpm for the following time intervals 0, 5, 15, 30 and 60 minutes. Subsequently, the reaction was stopped by heating samples at 100°C for 15 minutes to denature crude enzymes. Samples were centrifuged and the supernatant, henceforth referred to as “first fraction” was stored for further analysis via SDS-PAGE. The remaining RBM was then incubated in 30 mM Tris-HCl buffer (pH 7) at 37°C, 2000 rpm for 2 hours to extract the residual bound proteins in RBM, henceforth referred to as “second fraction”. Both fractions were analysed by via SDS-PAGE as outlined below.

Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE) of solid-state fermentation (SSF) product

[0098] 150 mg of solid-state fermentation (SSF) product was washed with 1.5 mL distilled water by vortexing to remove residual microbes. Excess starch in SSF product was hydrolysed by amylase treatment, 5 ug of BAN480L was incubated for 2 hours at 37°C with SSF product resuspended in 1.5 mL of 30 mM Tris-HCl buffer. The Tris-HCl buffer was then replaced with 900 µl of 1x SDS loading dye and heated for 10 minutes at 100°C. The samples were analysed by via SDS-PAGE using gradient 4-20% Mini-PROTEAN TGX Precast Protein Gels. Following SDS-PAGE, gels were stained with Instantblue™ ultrafast Protein Stain and visualised with Bio-rad gel doc.

CLAIMS

1. A bacterial strain deposited under deposition number DSM33837.
- 5 2. A bacterial strain deposited under deposition number DSM33839.
3. A bacterial strain deposited under deposition number DSM33838.
4. A combination comprising the bacterial strains according to claims 1 to 3.
- 10 5. The bacterial strain of any one of claims 1 to 3, or the combination of claim 4, as a freeze-dried product.
6. The bacterial strain of any one of claims 1 to 3, or the combination of claim 4, as a liquid culture
15 product.
7. A method of fermenting bran and/or meal, the method comprising inoculating the bran and/or meal with one or two or all bacterial strains selected from the group consisting of a bacterial strain deposited under deposition number DSM33837, a bacterial strain deposited under
20 deposition number DSM33839, and a bacterial strain deposited under deposition number DSM33838.
8. The method of claim 7, wherein the fermentation is solid-state fermentation.
- 25 9. 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.*
10. The method of claim 9, wherein the bacterial strain of *Bacillus spp.* is one, two or all of the bacterial strains selected from the group consisting of bacteria of deposition number DSM33837,
30 bacteria of deposition number DSM33839, and bacteria of deposition number DSM33838.
11. The method of any one of claims 9 to 10, wherein the inoculation is performed using the bacterial strain in a concentration of between 1.6×10^6 to 1.6×10^{10} CFU.
- 35 12. The method of claims 7 to 11, wherein the fermentation or solid-state fermentation is batch fermentation.
13. The method of claims 7 to 12, wherein the fermentation or solid-state fermentation is continuous fermentation.

14. The method of claims 9 to 13, wherein the fermentation or solid-state fermentation is carried out at a temperature between 25°C to 37°C.
- 5 15. The method of claims 9 to 14, wherein the fermentation or solid-state fermentation is carried out at a ratio of at least 1:1 of bran or meal to water.
16. The method of claims 9 to 15, wherein the fermentation or solid-state fermentation comprises a blending step.
- 10 17. A method of enriching grain, the method comprising inoculating grain with the bacterial strain as defined in any one of claims 1 to 3, or the combination of claim 4.
18. A method of enriching grain-based feed ingredients, the method comprising inoculating grain with the bacterial strain as defined in any one of claims 1 to 3, or the combination of claim 4.
- 15 19. The method of any one of claims 7 to 18, wherein the method results in an increase in enzyme activity.
- 20 20. The method of claim 19, wherein the enzyme is protease and/or xylanase.
21. The method of any one of claims 19 to 20, wherein the activity is hydrolysis.
22. The method of any one of claims 7 to 21, wherein the inoculation takes place prior to fermentation, or prior to administration to an animal.
- 25 23. The method of any one of claims 7 to 22, 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.
- 30 24. The method of any one of claims 7 to 16, or the method of any one of claims 17 to 23 wherein the bran and/or meal, or the grain, is selected from the group consisting of oilseed, nut, and legume.
- 35 25. The method of any one of claims 7 to 16 or 24, or the method of any one of claims 17 to 24, 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
- 40

- 5 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
5 (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.
26. A dry fermented product of grain, wherein the product is coated with the bacterial strain of any one or claims 1 to 3, or the combination of claim 4.
- 10 27. A composition comprising the dry fermented product of grain of claim 26.
28. An animal feed ingredient comprising the dry fermented product of grain of claim 26.
- 15 29. The bacterial strain of any one or claims 1 to 3, or the combination of claim 4, for use in the preparation of grain for animal-feed ingredient purpose.
30. The product of claim 26, the composition of claim 27, the animal feed ingredient of claim 28, or the grain of claim 29, wherein the grain is wheat bran, rice bran, meal thereof, or a combination
20 of wheat bran and rice bran, or meal thereof.
31. The product, the composition, the animal feed ingredient, or the grain of claim 30, wherein the rice bran is defatted rice bran.
- 25 32. A method of improving animal digestion and/or absorption of nutrients, the method comprising administering the animal feed ingredient according to any one of claims 28 or 30 to 31 to an animal.
- 30 33. The method of any one of claims 22 or 32, wherein the animal is selected from the group consisting of a domesticated or wild (undomesticated) animal, livestock, and aquatic animal.