

A BACTERIAL ISOLATE AND ITS USE IN THE PREPARATION OF AN ENRICHED PALM-BASED
ANIMAL FEED

- 5 The present invention relates to a composition comprising *Bacillus subtilis* isolate. In particular, the present invention relates to the use of such compositions for preparing an enriched palm-based animal feed.

Oil palm (*Elaeis guineensis* Jacq.) is a tropical crop cultivated mainly for production of palm
10 oil. Palm kernel cake (PKC) is a by-product from oil palm industries and it is produced annually in large quantities (Index Mundi, 2008a, 2008b). There are two major types of PKC available in the market based on different oil extraction methods (Kini et al, 2020). One from mechanical pressing, or expeller, and is generally regarded as palm kernel expeller (PKE). The other from solvent extraction, which will be referred to as palm kernel meal (PKM) in this
15 report. Both PKE and PKM are also distinguishable by their oil content and physical characteristic. PKE normally comes with 8-10% residual oil from pressing, while PKM has a lower oil content of <3% due to more efficient oil extraction by solvent (Alimon, 2004).

Both PKE and PKM (collectively referred to as PKC here) are widely used as feed ingredient
20 primarily due to its nutritive value for ruminants and as a reliable low-cost source of protein and energy in animal feed (Zahari and Alimon, 2004). However, the inclusion of PKC is often limited by its high fibre content and low metabolizable energy, particularly in the diet of monogastric animals like poultry (Sharmila et al., 2014). PKC has been reported to contain high levels of non-starch polysaccharides (NSPs) mainly in the form of poly-mannan
25 (Dusterhoft et al., 1992). These mannan fibres are considered as anti-nutritional factor for poultry and other monogastric animals (Saeed et al., 2019). Presence of high mannan in feed reduces the rate of nutrient hydrolysis and utilization in the diet by increasing intestinal viscosity and nutrient encapsulation (Choct and Annison, 1992).

- 30 To enhance the economic value of PKC in feed, nutrients utilization needs to be optimized by increasing the fibre digestibility to release the nutritive compounds from the fibre matrix. Supplementation of exogenous fibrolytic enzymes, like mannanases to the PKC-based diets

resulted in inconclusive outcomes in nutrient digestibility and animal performance (Sharmila et al., 2014; Iyayi and Davies, 2005). The inconsistency of the enzyme effects could be due to differences in thermal and pH stability, dosage, or substrate specificity of different mannanases. Most of the commercial mannanases were not developed specifically for feed
5 ingredient like PKC.

An alternative way to improve nutrient digestibility in animal is using direct-fed microbials (DFMs). DFMs are a category of probiotics that are used in the animal industry (Callaway and Ricke, 2011). As microbial additives to feed, probiotics could aid in proper digestion of feed
10 and absorption of nutrients by promoting healthy microbial populations in the gastrointestinal tract, thus reducing pathogen infection and enhancing animal performance (Bajagai et al., 2016). A variety of microorganisms have been deployed as DFM in animal feed, either in the form of single or multispecies. *Lactobacillus* and *Bifidobacterium* are the two most common genera used (Bajagai et al., 2016). Recently, Grant et al. (2018) reviewed that
15 the spore-forming *Bacillus* spp. has a distinct advantage over these two popular beneficial species as DFM. One of the highlighted characteristics of *Bacillus* spp. is their spore tolerance to high temperature of up to 113°C, a feature that is crucial for the bacteria to survive feed processing steps. *Bacillus* spores could also endure harsh gastric environment. Upon entering the animal digestive system, *Bacillus* could promote gut health by competitive exclusion or
20 by production of beneficial metabolites that can protect the host from various pathogens (Grant et al., 2018; Elshaghabee et al., 2017). Furthermore, among bacteria, β -mannanases were mostly produced by Gram positive bacteria, primarily various *Bacillus* species (Chauhan, 2012).

25 It will be of feed industry interest that beneficial features of both enzyme and probiotic can be brought together by developing natural *B. subtilis* isolates that can produce essential mannolytic enzymes to target the PKC mannan fibre.

The listing or discussion of an apparently prior-published document in this specification
30 should not necessarily be taken as an acknowledgement that the document is part of the state of the art or is common general knowledge.

Any document referred to herein is hereby incorporated by reference in its entirety.

Provided here is the isolation and characterization of a PKC-responsive *B. subtilis* isolate that can be applied in PKC-based feed. The isolate is capable of propagating and secreting
5 mannolytic enzymes upon contact with PKC, especially for solvent-extracted palm kernel meal.

The use of PKC as animal feed has been limited by its high content of mannan, especially in monogastric animals. Direct supplementation of mannan-degrading enzymes together with
10 PKC-containing feed faces some challenges like enzyme substrate specificity, its thermal and pH stabilities during feed pelleting and during passage of the digesta in the animals, respectively. This invention identified beneficial bacteria strain/isolate that is capable of secreting mannolytic enzymes upon in contact with high-mannan feed ingredients like PKC. The isolate could be used either for PKC fermentation/blending or as direct fed microbials to
15 be fermented/blended with PKC. It is reported here the isolation and identification of a native *Bacillus subtilis* isolate that is responsive to PKC and which serves as potential probiotics for animal feed.

In an aspect of the present invention, there is *Bacillus subtilis* strain isolate DSM33646 capable
20 of secreting mannolytic enzymes upon contact with non-starch polysaccharides feed ingredients. DSM33646 is deposited at Leibniz Institute, DSMZ-German Collection of Microorganisms and Cell Cultures GmbH on 24 September 2020 by Wilmar International Limited, Singapore. Non-starch polysaccharides constitute 70 to 90% of plant cell walls. By “non-starch polysaccharides (NSPs)”, it is meant to include any polymeric carbohydrates
25 which differ in composition and structure from starch and possess chemical cross linking among them therefore, are not well digested by animals. They include cellulose, mannans, pectins, gums, glucans, inulin, chitin.

In another aspect of the invention, there is provided a kit comprising the composition
30 comprising *Bacillus subtilis* strain isolate DSM33646, and instructions for using the kit. These set of instructions may include the method for producing an enriched palm-based animal feed according to an aspect of the invention as will be described below.

In yet another aspect of the invention, there is provided a method for producing an enriched palm-based animal feed loaded with live probiotic bacteria and its secreted enzymes capable of hydrolyzing at least three forms of mannan polymers, namely glucomannan, galactomannan, and linear mannan, the method comprising: (a) providing a palm kernel meal; (b) contacting the palm kernel meal with a *Bacillus subtilis* strain to form a blended or fermented product; and (c) drying the blended or fermented product, wherein the *Bacillus subtilis* strain is DSM33646. Preferably, the isolated *Bacillus subtilis* strain of the invention was selected for its ability to grow on solvent-extracted palm kernel meal. While the terms PKE and PKM are used interchangeably in the industry, PKE refers to palm kernel cake that is mechanically extracted.

The palm kernel meal (PKM) may be prepared through any suitable solvent extraction method known to the skilled person. Advantageously, the invention provides for a unique *B. subtilis* strain isolate that is capable of effectively and efficiently secreted the suitable enzymes for hydrolyzing the mannan polymers present in the PKM.

By “blending”, it is meant to include any action to mix and/or combine the various components / reactants used in the manufacturing steps carried out by the invention. Such mixing or blending can include any “fermentation” action which includes any breakdown (for example, a chemical breakdown) of the polymers in the PKM under any suitable conditions. Such suitable conditions are described below.

By “solvent-extracted palm kernel meal”, it is meant to include PKM that is solvent extracted and has less than or equal to 3% oil content.

In various embodiments, the palm kernel meal has an oil content of less than or equal to 3 wt%.

In various embodiments, the method further comprises adding water to step (b), the ratio of palm biomass to water is about 1:1.5.

In various embodiments, the concentration of the *Bacillus subtilis* strain is about between 6×10^6 to 6×10^{10} cfu per gram of the palm kernel meal.

5 In various embodiments, the step of contacting the palm kernel meal with a *Bacillus subtilis* strain comprises fermenting/blending the strain with the meal at a temperature of about between 25°C to 40°C for about between 4 to 72 hours. In various embodiments, the fermentation and blending may be carried out between 4 to 48 hours, or 4 to 24 hours. In various embodiments, the fermentation and blending may be carried out between 6 to 24 hours.

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In various embodiments, the fermentation/blending is carried out with water and at its resultant pH of between 4.0 to 8.0 upon in contact with the meal.

15 In various embodiments, drying the fermented product is carried out at a temperature of about between 60°C to 80°C until the moisture content of the fermented product is 10% or less.

Advantageously, the fermented/blended product produced by the invention has hydrolyzing activity on non-starch polysaccharides.

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In various embodiments, the palm kernel meal comprises a high content of non-starch polysaccharides.

25 In various embodiments, the non-starch polysaccharides are glucomannan, galactomannan or linear mannan.

The method of the invention may be carried out in a continuous or batch-wise process.

30 In various embodiments, the blended/fermented product is further fermented/blended with a fresh amount of palm kernel meal at a ratio range between 1:1 to 1:10 prior to the drying step (c). In various embodiments, the blended/fermented product may be further fermented/blended with water in the ratio 1:1.5. By "fresh" PKM, is meant to include the

solvent-extracted PKM that has yet to be exposed to the *B. subtilis* isolate or to be subject to any enzyme treatment, i.e. step (a) in the method steps outlined above.

5 In another aspect of the invention, there is provided an enriched palm-based animal feed loaded with live probiotic bacteria and its secreted enzymes that can hydrolyze at least three forms of mannan polymers, wherein the feed is obtained or obtainable by a method of the invention.

10 In an aspect of the invention, there is provided a method for preparing enriched palm-based feed comprising contacting or treating palm biomass with an isolate of a *Bacillus subtilis* strain deposited at Leibniz Institute, DSMZ-German Collection of Microorganisms and Cell Cultures GmbH having deposit number DSM 33646.

15 In various embodiments, the strain is selected based on its ability to grow solely on solvent-extracted palm biomass. The strain is included in animal feed as a probiotic. The strain is specific to palm kernel meal. The strain has mannan-degrading ability. Fermentation is accelerated by the strain of at least 4-6 hours from start of fermenting/blending.

20 The strain is introduced by fermenting/blending with a mixture of PKM and water wherein the PKM to water ratio is 1:1.5 or at higher ratios. The strain is used at 6×10^8 cfu/ g of PKM but dosage could vary between 6×10^6 to 6×10^{10} cfu. Different ratios may be used. Here, the water content was kept as low as possible because a high amount of water will increase the cost of drying.

25 In various embodiments, the strain and mixture are fermented/blended for between 6 to 24 hours. To increase production volume, it would be preferable to have the shortest time possible, in this case 6h. This is different from traditional fermentation process that normally takes about 24 hours and above.

30 In various embodiments, the strain and mixture is fermented/blended at a temperature range 25 to 40 degrees Celsius. In various embodiments, the temperature is 37 degrees Celsius, including the heat buildup of the apparatus.

In various embodiments, the palm biomass is palm kernel meal having less than 3% oil content.

- 5 In various embodiments, the enriched palm-based feed is manufactured in a continuous accelerated fermentation/blending manner in a manufacturing plant.

In various embodiments, the enriched palm-based feed is manufactured in a batch accelerated fermentation/blending manner in a manufacturing plant.

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In another aspect of the invention, there is provided a fermented/blended enriched palm-based feed of an aspect of the invention.

- 15 In various embodiments, the feed is enriched with hydrolyzing activity capable of hydrolyzing at least 3 different forms of mannan polymers.

In various embodiments, the 3 different forms of mannan polymers are glucomannan, galactomannan and linear mannan.

- 20 In various embodiments, the fermented/blended enriched palm-based feed is in direct feed form.

In various embodiments, the fermented/blended enriched palm-based feed is in dried form.

- 25 In another aspect of the invention, there is provided a further fermented/blended enriched palm-based feed of an aspect of the invention.

- In various embodiments, wherein the product is further fermented/blended with fresh PKM at a ratio range between 1:1 to 1:10. Each round of fermenting/blending enhances DSM33646 growth, the 1:10 ratio limit is to ensure sufficient growth of DSM33646 to reach optimal enrichment of the mannan hydrolyzing activity.
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For the water to PKM ratio, it is either 1:1.5 (optimized) or other ratios.

In various embodiments, the product is characterized by the hydrolyzing of at least 3 different forms of mannan polymers.

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In various embodiments, the 3 different forms of mannan polymers are glucomannan, galactomannan and linear mannan.

10 In various embodiments, the further fermented/blended enriched palm-based feed is in direct feed form.

In various embodiments, the further fermented/blended enriched palm-based feed is in dried form.

15 In yet another aspect of the invention, there is provided a composition comprising the fermented/blended enriched palm-based feed of an aspect of the invention and the further fermented/blended enriched palm-based feed of an aspect of the invention where the fermenting/blending time is from 2.5 to 24 hours.

20 In various embodiments, the fermented/blended enriched palm-based feed and the further fermented/blended enriched palm-based feed can be further fermented/blended.

In another aspect of the invention, there is provided an enriched palm-based animal feed comprising a *Bacillus subtilis* strain isolate DSM33646. The animal feed further comprising a
25 solvent-extracted palm kernel meal.

In various embodiments, the feed has enzymatic hydrolyzing activity of at least 3 different forms of mannan polymers, the 3 different forms of mannan polymers are glucomannan, galactomannan and/or linear mannan.

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The animal feed may be in direct feed form, or in dried form.

In various embodiments, the animal feed is further blended/fermented with the addition of water and palm kernel meal.

Advantageously, the present invention relates to a highly responsive bacillus strain towards PKM that can secrete enzymes to breakdown mannan components of PKM in very short time. Current bacillus isolates are not used specifically for solvent extracted PKM (they were mostly for PKE and were not demonstrated to be “responsive” to PKE either), that mean they are very likely not responsive to PKM. None of the prior art isolates have shown they can secrete mannanases if incubated directly with palm kernel cake, especially solvent extracted PKM to the extent of our reported levels. It is about the responsiveness of the strain towards the biomass. It is the “responsiveness” of the isolated DSM33646 strain that make the illustrated production of “fermented/blended” PKM possible, and thus make the whole process unique from other (shortened fermentation duration is made possible through this invention instead of lengthy fermentation).

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In order that the present invention may be fully understood and readily put into practical effect, there shall now be described by way of non-limitative examples only preferred embodiments of the present invention, the description being with reference to the accompanying illustrative figures.

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In the Figures:

Figure 1 is an illustration summarizing a method according to an embodiment of the present invention;

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Figures 2, 3 and 4 show results obtained from Example 3;

Figure 5 shows results obtained from Example 4;

30 Figure 6 shows results obtained from Example 5; and

Figure 7 shows results obtained from Example 6

In Figure 1, a general schematic of carrying out the method according to an embodiment of the present invention is shown. Here, it can be seen that the fermented/blended PKM product is obtained from solvent-extracted PKM by incubating it with the *B. subtilis* isolate of the invention which is capable of producing the necessary enzymes in a short time to breakdown different types of mannans.

EXAMPLE 1

10 *Materials and Methods*

1. Samples, microorganisms, and chemicals

PKM was obtained from palm kernel solvent extraction plant located in East Java, Indonesia. The bacteria populations used to inoculate PKM were obtained from the exocarps of palm fruits from different oil palm plantations of Wilmar International. The *Bacillus subtilis* CK7 strain was obtained from PT. Wilmar Bernih Indonesia. Glucomannan (konjac; high viscosity) and mannan (1,4-β-D-mannan) (Megazyme, Wicklow, Ireland), locust bean gum from Ceratonia siliqua seeds (LBG), and DNS reagent component (3,5-dinitrosalicylic acid, potassium sodium tartrate tetrahydrate and sodium hydroxide) (Sigma Chemical Co., St. Louis, MO, USA), were purchased and used.

2. Isolation and identification of mannan-responsive bacteria

1g of PKM in 5 mL of water was inoculated with microbes from exocarps of palm fruits and incubated in a 37°C incubator. The grown bacteria from >2 weeks old PKM-only culture tube was streaked on a Luria-Bertani (LB) agar plate to select for single colonies. Each bacteria colony was grown and re-inoculated into 100 ml 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) LBG as sole carbon source (LBG-medium). The tubes were incubated at 37°C for 48 hours and cell-free supernatant was collected as crude enzymes and their mannolytic activity was assessed by the assay as described below. The genomic DNA of isolates with high mannolytic activity was

extracted (Hoffman, 2003) and was used as template in PCR for 16S rRNA partial gene amplification (~500bp) using the following universal primers: 16s Forward (5'-CCTACGGGAGGCAGCAG-3') and 16s Reverse (5'-GGACTACHVGGG TWTCTAAT-3') (Takahashi et al., 2014). 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) (<https://www.ncbi.nlm.nih.gov/>).

3. Determination of mannolytic activity

The activity of crude enzymes was determined by measuring the release of reducing sugar by DNS method described by Miller (1959) with minor modifications using mannose as the standard. The reaction mixture comprised 950 µl of 100 mM sodium acetate buffer (pH 5.0) containing 0.5% substrate (w/v), and 50 µl of crude enzymes. The absorbance at 540 nm was measured with a Libra S22 UV/Vis Spectrophotometer (Biochrom, UK).

5. PKC hydrolysis assay

PKC was sterilized at 121°C for 15 min. In each well of a 6-well plate, 1g of PKC was inoculated with 6×10^8 CFU of the selected *Bacillus* isolate or *B. subtilis* CK7 and 1.5 mL of water. PKC with water only was used as the negative control. Subsequently, PKC was incubated at 37°C for different time points, for example between 4 to 72 hours, or between 6 to 23 hours.. After incubation, the PKC samples were diluted with water (1:4) and centrifuged at 12,000 rpm for 5 min to obtain the cell-free supernatant as PKC crude extract for analysis of mannolytic activity on LBG, konjac glucomannan and 1,4-β-D-mannan. All the experiments were conducted in triplicates.

6. Drying of the fermented product

The fermented product could be dried at a temperature range of 60°C to 80°C, until the moisture content reaches 10% or less.

The drying process could be accompanied by vacuum treatment.

The fermented/blended products could be sun-dried if the fermenting/blending is carried out
5 at farm and for short-term storage at the farm.

Results and Discussion

We sought to identify natural beneficial bacteria isolates that could be used to enhance the nutritive profile of PKC and be applied together with PKC as direct fed microbial. Most of the studies have largely focused on the utilization of fermentative strains obtained from cell collection centers or from strains isolated directly from the expeller cake. In 2018, Virginia et. al. identified a mannanase-producing *Bacillus subtilis* strain, CK7, isolated from palm oil mill area. The crude enzymes secreted by the CK7 was shown to be able to release reducing sugar from PKC (Virginia et. al., 2018). However, the responsiveness of this strain upon direct contact with PKC has not been assessed. In PKE (8-10% oil), higher oil content could have facilitated the selection of strains that are capable of using oil as energy source; while in solvent-extracted PKM (<3% oil), with a greater reduction in oil content, limited energy source could have facilitated selection of strains that can secrete higher level of cellulolytic enzymes for utilization of the mannan-rich fiber. In this invention, low-oil PKM was used as a substrate to screen for mannan-degrading bacteria. Bacterial communities from the exocarps of palm fruits from different plantations were used to inoculate PKM. Bacterial isolates that were enriched by PKM incubation were further examined for their mannolytic activity. Among the PKC-responsive bacteria isolates, DSM33646 was identified as *Bacillus subtilis* (98% pairwise identity upon comparison of the 16S sequences with database in the GenBank).

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To further evaluate the responsiveness of the DSM33646 isolate to PKC, the ability of DSM33646 isolate to propagate and secrete mannolytic enzymes upon incubation with PKC was examined and compared to that of the CK7 strain. DSM33646 and CK7 were reintroduced into PKM (6×10^8 cfu/g). The water extracts of the PKM inoculated with DSM33646 isolate at different fermenting/blending times (starting from 6h after fermenting/blending) showed significant increase in mannolytic activities (against at least 3 forms of mannan substrates LBG, konjac, and linear mannan), while inoculation of CK7 strain showed no increase in the activity. The mannolytic activities of the DSM33646 strain persisted and increased at longer fermenting/blending times.

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In conclusion, this invention has identified a *B. subtilis* isolate that is highly responsive to mannan-rich PKC. This isolate could be further developed for use in the feed industry, either in fermentation/blending of PKC or direct inclusion in PKC-based diet as DFM.

5 EXAMPLE 2: An inventive process to select for PKM-specific strain

Bacteria associated with loose palm fruits were inoculated into PKM and water (1:5, w/w), incubated for >2 weeks at 37°C for selection of PKM-specific strain. Viable bacteria in the culture were then propagated and tested. DSM33646, a *Bacillus subtilis* strain was isolated from the process.

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Other mannan-degrading bacteria were either screened from a bacteria collection or isolated directly from PKE (with 8-10% oil content), food, environment without going through the inventive process. Low-oil PKM serves as a special natural feedstock to isolate microbes that could use mannan fiber efficiently, as the oil content is significantly lower than PKE, avoiding isolating bacteria that could survive based on oil as carbon source. A published *Bacillus subtilis* strain CK7, which was isolated from the environment and found to have mannan-degrading ability was used as a control to show the advancement of the strain screened through the inventive process.

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EXAMPLE 3: Loaded enzyme activities of the DSM33646 fermented/blended PKM

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1 g of PKM was fermented/blended with 6×10^8 cfu of DSM33646 *Bacillus subtilis* or *Bacillus subtilis* CK7.

One unit (U) of the enzyme activity ($\mu\text{mol}/\text{min}$) is defined as the amount of the enzymes required to release 1 μmol of mannose equivalent from 0.5% of respective substrates (Konjac/glucomannan, LBG/galactomannan, and Linear mannan) per minute after incubation at 55°C (normalized to per gram of the fermented/blended product).

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EXAMPLE 4: Loaded enzyme activity in the PKM fermented/blended product obtained from a method of the invention.

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Examples 4 and 5 below describe how we can scale up the fermenting/blending while retaining enzyme activity in the fermented/blended meal. The enzyme activity is measured by the amount of

reducing sugar released from the enzymatic assay per minute using enzymes harvested/recovered from the fermenting/blending in DNS assay. The assay was conducted as follows:

- a. Reaction mixture: 50ul of extracted enzymes harvested/recovered from the PKM (with necessary dilutions) + 950ul linear mannansolution (0.5% linear mannan, 0.1M pH 5.0 buffer)
- 5 b. Reaction condition: 55°C, 1000rpm, 1hr

DNS test: 300ul reaction mixture + 900ul DNS solution; heated at 100dc for 5mins, the absorbance is measure at 540nm.

- 10 0.5 g of 6-h fermented/blended product from Claim 2 was further fermented/blended with PKM in 1:10 ratio for 17h. The loaded enzyme activity in the 5.5 g of final product was determined. One unit (U) of the enzyme activity ($\mu\text{mol}/\text{min}$) is defined as the amount of the enzymes required to release 1 μmol of mannose equivalent from 0.5% of linear mannan per minute after incubation at 55°C (normalized to per gram of the fermented/blended product). Positive control (Pos ctrl) is 0.5 g of 23h-
- 15 fermented/blended product.

EXAMPLE 5: Loaded enzyme activity in the PKM fermented/blended product.

- 1 g of 2.5-h fermented/blended product was further fermented/blended with PKM in 1:4 ratio
- 20 for 2.5h. The 5g of the fermented/blended product was then further fermented/blended with PKM in 1:9 ratio for 19h. The loaded enzyme activity in the 50 g of final product was determined. One unit (U) of the enzyme activity ($\mu\text{mol}/\text{min}$) is defined as the amount of the enzymes required to release 1 μmol of mannose equivalent from 0.5% of linear mannan per minute after incubation at 55°C (normalized to per gram of the fermented/blended product). Positive control
- 25 (Pos ctrl) is 1 g of 24h-fermented/blended product, negative control (neg ctrl) is a 50 g of PKM without strain.

EXAMPLE 6: Loaded enzyme activity in the PKM fermented/blended with different dosages of DSM33646.

- 30 1 g of PKM was fermented/blended with different dosages (6×10^6 to 6×10^{10} CFU) of DSM33646 *Bacillus subtilis*. One unit (U) of the enzyme activity ($\mu\text{mol}/\text{min}$) is defined as the amount of the enzyme capable of releasing 1 μmol of mannose equivalent from 0.5% of

LBG/galactomannan per minute after incubation at 55°C (normalized to per gram of the fermented/blended product).

- 5 Whilst there has been described in the foregoing description preferred embodiments of the present invention, it will be understood by those skilled in the technology concerned that many variations or modifications in details of design or construction may be made without departing from the present invention.

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CLAIMS

1. A *Bacillus subtilis* strain isolate DSM33646 capable of secreting mannolytic enzymes
5 upon contact with non-starch polysaccharides feed ingredients.
2. A kit comprising isolated DSM33646 and instructions for using the kit.
3. A method for producing an enriched palm-based animal feed, the method comprising:
10 (a) providing a palm kernel meal;
(b) contacting the palm kernel meal with a *Bacillus subtilis* strain to form a blended or fermented product; and
(c) drying the blended or fermented product,
wherein the *Bacillus subtilis* strain is DSM 33646.
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4. The method according to claim 3, wherein the palm kernel meal has an oil content of less than or equal to 3 wt%.
5. The method according to any one of claims 3 or 4, wherein the strain is selected for
20 its ability to grow on solvent-extracted palm kernel meal.
6. The method according to any one of claims 3 to 5, wherein the method further comprises adding water to step (b), the ratio of palm biomass to water is about 1:1.5.
- 25 7. The method according to any one of claims 3 to 6, wherein the concentration of the *Bacillus subtilis* strain is about between 6×10^6 to 6×10^{10} cfu per gram of the palm kernel meal.
8. The method according to any one of claims 3 to 7, wherein contacting the palm kernel meal with a *Bacillus subtilis* strain comprises fermenting or blending the strain with the meal
30 at a temperature of about between 25°C to 40°C for about at least between 4 to 72 hours.

9. The method according to any one of claims 3 to 8, wherein contacting the palm kernel meal with a *Bacillus subtilis* strain comprises fermenting or blending the strain with the meal at a temperature of about between 25°C to 40°C for about at least between 4 to 48 hours.
- 5 10. The method according to any one of claims 3 to 9, wherein contacting the palm kernel meal with a *Bacillus subtilis* strain comprises fermenting or blending the strain with the meal at a temperature of about between 25°C to 40°C for about at least between 4 to 24 hours.
11. The method according to claim 6, wherein the pH of the water is between 4.0 to 8.0.
- 10 12. The method according to any one of claims 3 to 11, wherein drying the fermented or blended product is carried out at a temperature of about between 60°C to 80°C until the moisture content of the fermented or blended product is 10% or less.
- 15 13. The method according to any one of claims 3 to 12, wherein the fermented/blended product has hydrolyzing activity on non-starch polysaccharides.
14. The method according to claim 13, wherein the non-starch polysaccharides are glucomannan, galactomannan and/or linear mannan.
- 20 15. The method according to any one of claims 3 to 14, wherein the method is carried out in a continuous process.
16. The method according to any one of claims 3 to 14, wherein the method is carried out
- 25 in a batch process.
17. The method according to any one of claim 3 to 16, wherein the blended or fermented product is further fermented or blended with a fresh amount of palm kernel meal at a ratio range between 1:1 to 1:10 prior to the drying step (c).
- 30 18. The method according to claim 17, wherein the blended or fermented product is further fermented or blended with water in the ratio 1:1.5.

19. An enriched palm-based animal feed having a reduced mannan polymers content obtained or obtainable by a method according to any one of claims 3 to 18.
- 5 20. An enriched palm-based animal feed comprising a *Bacillus subtilis* strain isolate DSM33646.
21. The animal feed further comprising a solvent-extracted palm kernel meal.
- 10 22. An enriched palm-based animal feed having a reduced mannan polymers content, wherein the feed has enzymatic hydrolyzing activity of at least 3 different forms of mannan polymers, the 3 different forms of mannan polymers are glucomannan, galactomannan and/or linear mannan.
- 15 23. An enriched palm-based animal feed according to any one of claims 19 to 22 in direct feed form.
24. An enriched palm-based animal feed according to any one of claims 19 to 23 in dried form.
- 20 25. An enriched palm-based animal feed according to any one of claims 19 to 24, wherein the animal feed is further blended or fermented with the addition of water and a fresh amount of palm kernel meal.

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