(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
27 June 2013 (27.06.2013)

WIPO | PCT

(10) International Publication Number
WO 2013/092645 Al

(51) **International Patent Classification:**
A23K 1/00 (2006.01) ***A23K 1/12*** (2006.01)
A23K 1/10 (2006.01) ***A23K 1/16*** (2006.01)

(21) **International Application Number:**
PCT/EP2012/076046

(22) **International Filing Date:**
18 December 2012 (18. 12.2012)

(25) **Filing Language:** English

(26) **Publication Language:** English

(30) **Priority Data:**
11306700.3 19 December 201 1 (19. 12.201 1) EP
61/577,222 19 December 201 1 (19. 12.201 1) US

(71) **Applicant: DEINOVE** [FR/FR]; 22 rue Leon Jouhaux, F-75010 Paris (FR).

(72) **Inventors: GAUFRES, Laure;** c/o Deinove, 22 rue Leon Jouhaux, F-75010 Paris (FR). **HIVIN, Patrick;** c/o Deinove, 22 rue Leon Jouhaux, F-75010 Paris (FR). **KREL, Michael;** c/o Deinove, 22 rue Leon Jouhaux, F-75010 Paris (FR). **LEONETTI, Jean-Paul;** 9 rue Monet, F-34170 Castelnau-le-Lez (FR).

(74) **Agents: BECKER, Philippe** et al; Becker & Associes, 25 rue Louis Le Grand, F-75002 Paris (FR).

(81) **Designated States** *(unless otherwise indicated, for every kind of national protection available):* AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available):* ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

— *with sequence listing part of description (Rule 5.2(a))*

WO 2013/092645 A1

(54) **Title:** INGREDIENTS FOR ANIMAL FEED COMPOSITIONS

(57) **Abstract:** The present invention relates to ingredients for animal feed compositions for enhancing animal growth and/or animal health. The invention also relates to methods for producing such ingredients and feed compositions. The methods of the invention further allow improving the palatableness and/or digestibility of feed compositions. More specifically, the invention describes the use of a mix of *Deinococcus* or related bacteria and biomass as a supply of organic constituents in feed compositions.

INGREDIENTS FOR ANIMAL FEED COMPOSITIONS

FIELD OF THE INVENTION

The present invention relates to ingredients for animal feed compositions for enhancing animal growth and/or animal health. The invention also relates to methods for producing such ingredients and to feed compositions. The methods of the invention further allow improving the palatableness and/or digestibility of feed compositions. More specifically, the invention describes the use of a mix of *Deinococcus* or related bacteria and biomass as a supply of organic constituents in feed compositions.

BACKGROUND OF THE INVENTION

The feeding of livestock, such as pets, cattle, ovine, porcine, poultry, fish, -including commercial and ornamental fish-, schrimp and other crustaceans, is a constant problem in the field of animal breeding and agro-food industry. Because of the limited production facilities and lack of improvement in production technology, animal breeding is both costly and time-consuming. To improve the production and the profitability of animal breeding, the most common solution is to provide industrial feed compositions, at least to complete the diet of the animals.

While feedstuffs are not of constant composition, several constituents are required to fulfil the nutritional goal. Indeed, even if the biological response to the feed depends on the ability of the animal to derive useful nutrients from said feed, the feedstuffs used in the feeding generally comprise digestible proteins, lipids, carbohydrates and fiber.

Animal feed compositions are usually formulated with low cost formulations. Of particular importance is the limited supply of digestible energy and the associated cost. The most costly nutrients are proteins and amino acids, including the essential amino acids.

However, the production of animals that contain a significant rate of proteins would be necessary to proportionate the increase in world population.

The use of fish meal or meat and bone meal as protein raw material has been developed because of its low cost. Indeed, meat and bone meal is a product of the rendering industry that comprises about 50% protein, and 8-12% fat. It is primarily used in the formulation of animal feed to improve the amino acid profile of the feed. However, the feeding of bone meal as protein to cattle is thought to have been responsible for the spread of mad cow disease. Consequently, in most parts of the world, the use of bone meal as protein supply is no longer allowed in feed for ruminant animals.

Another raw material used for preparing feed compositions for ruminant animals is cellulosic material, which is a cheap and non direct valuable material. However, even if enzymes in ruminants allow the degradation of cellulose, several pre-treatments are performed to partially hydrolyze the cellulose and hemicellulose in order to help the further degradation by ruminant animals and enhance the digestibility of this raw material. For example, the addition of various exogenous enzymes, such as cellulases and hemicellulases, to the feedstuff comprising cellulosic material is useful to decrease polymer crystallinity and to increase access to the polymer backbone. In the same way, the thermo-chemical pre-treatment of the cellulosic material is also possible to partially hydrolyse the hemicellulose and cellulose portions.

Although these treatments improve carbohydrate digestibility and more generally the bio-availability of nutriments of the cellulosic material, they can be expensive and economically prohibitive. In addition, even if pretreated, the cellulosic material is still indigestible and is therefore incapable of providing nourishment for the non ruminant animals, such as pigs and poultry. Furthermore, these treatments do not provide proteins, which are the most expensive constituent in animal feed compositions.

Consequently, there is a need of less expensive, safer and more nutritious food to commercial farm and battery animals, including both ruminant and non ruminant animals, commercial fish and schrimp aquaculture, and pets.

SUMMARY OF THE INVENTION

The present invention relates to mixes of *Deinococcus* or related bacteria and biomass, such as animal and/or vegetal and/or algal biomass, and the use thereof in animal feeding, e.g., as part of animal feed compositions. The invention also relates to a method of preparing feedstuff using biomass and *Deinococcus* or related bacteria as feedstock. The invention also relates to methods for increasing the nutritive value, palatableness, or digestibility of biomass. The compositions and methods of the invention are suitable for nourishment of any animal, including preferably livestock (including ruminants and non-ruminants), breeding animals, avian animals, fishes or companion animals.

The inventors have discovered that *Deinococcus* or related bacteria in combination with non usable raw materials, such as lignocellulosic biomass, can be used as a safe and low cost nutriment for animals. Both ingredients cooperate to produce high value feedstuff. Both the biomass and the Deinococcus bacteria are transformed, by the invention, from low nutritive value products into useful feedstuff ingredients. The invention shows that Deinococcus bacteria may be used in feedstuff, are safe and contain valuable nutritive agents. The invention also shows that biomass serves as a growing medium for Deinococcus bacteria, thereby allowing their expansion and enrichment, while the Deinococcus bacteria are able to digest the biomass, thereby converting the biomass into high nutritive value products with better digestibility. The feedstuff ingredients according to the invention therefore includes organic (e.g., proteins, amino acids, sugars) and inorganic compounds (e.g., minerals) which are utilized in the normal metabolism of animals and fish and provide essential nutrients that are not synthesized in sufficient amount or not synthesized such as essential amino acids.

Therefore, an object of the invention relates to an ingredient for feedstuff comprising a mix of biomass and a *Deinococcus* or related bacterium, said biomass being at least partially digested by said *Deinococcus* or related bacterium.

The invention also relates to the use of a mix of biomass and a *Deinococcus* or related bacterium to prepare a feedstuff.

The invention also relates to a feedstuff composition comprising an ingredient as defined above. Typically, the feedstuff composition comprises at least 10% by weight of said ingredient. The feedstuff composition may further comprise additional ingredients such as, without limitation, alfalfa, barley, blood meal/meat meal, fat animal poultry, potato wastes, or tomato wastes .

A further object of this invention relates to the use of a composition comprising a biomass and *Deinococcus* or related bacteria, for animal feeding.

A further object of this invention relates to the use of a mix of biomass and *Deinococcus* or related bacteria, said biomass being at least partially digested by said *Deinococcus* or related bacteria, as an ingredient for feedstuff.

A further object of this invention relates to the use of a mix of biomass and *Deinococcus* or related bacteria, said biomass being at least partially digested by said *Deinococcus* or related bacteria, as a protein supply-for feedstuff. As further illustrated in the examples, *Deinococcus* bacteria are themself high source of proteins and, in addition, they hydrolyse proteins of biomass providing therefore available amino acids.

The invention also relates to the use of Deinococcus bacteria as a source of amino acids for animal feeding.

The invention also relates to a method for preparing a feedstuff comprising:

- submitting a biomass to at least partial digestion by *Deinococcus* or related bacteria, and
- formulating a mix of at least partially digested biomass and bacteria, as ingredient for feedstuff.

In a particular embodiment, the method comprises a further step of adding to the mix other ingredients commonly used for feedstuff.

In an alternative embodiment, the method comprises:

(a) combining a biomass and *Deinococcus* or related bacteria to obtain a mix,

(b) maintaining the mix under conditions allowing digestion of the biomass by said Deinococcus or related bacteria,

(c) collecting the mix of (a) or (b), and

(d) optionally mixing the mix of (c) to one or several further ingredients.

The feedstuff may be packaged in any suitable form or container.

The biomass for use in the invention may comprise vegetal biomass, animal biomass and/or algal biomass. In a particular embodiment, the biomass is a lignocellulosic biomass. In another embodiment, the biomass is a protein-containing biomass. The digestion of the biomass may be performed in aerobic or anaerobic conditions, preferably in a reactor, for managing easily the reaction parameters (e.g., time of reaction, temperature, or feedstock quantities).

The invention also relates to a method for improving the nutritive value of a biomass, comprising subjecting said biomass to at least partial digestion by *Deinococcus* or related bacteria. Said method may further comprise a step wherein the nutritive value of said at least partially digested/hydrolyzed biomass is analysed or controlled.

A further object of this invention relates to a method for improving the palatableness and/or digestibility of a biomass, comprising subjecting said biomass to at least partial digestion by *Deinococcus* or related bacteria, in order to at least partially degrade complex sugars of said biomass. Said method may further comprise a step wherein the degradation of the complex sugars is controlled.

The compositions and methods of the invention may be used as nutrient or nutrient additive for any animal, especially non-human mammals such as livestock (including ruminants and non-ruminants), breeding animals, or companion animals; avian animals, or fishes.

LEGEND TO THE FIGURES

Figure 1: Growth of strain DG01 (D. Geothermalis 01) on 1% pre-treated rapeseed straw with or without addition of cellulase and β-glucosidase.

Figure 2: TLC analysis showing consumption of xylose and glucose removed from 1% pre-treated rapeseed straw inoculated with DG01. Control consists on the pre-treated rapeseed straw without bacteria. Enzymes are added at TO in the culture (0.1 lg of cellulase/g of cellulose and 0.05g of β-glucosidase/g of cellulose). The total enzymatic hydrolysis is performed using l.lg of cellulase/g of cellulose, 0.5g of β-glucosidase/g of cellulose and 5.5g of xylanase/g hemicelluloses.

Figure 3: Growth of strain DG01 on whole wheat (1%, with or without Termamyl, and 6%) or fermentation residues (6%) showing consumption of gluten (%) contained in the medium. Strain DG01 is able to hydrolyze gluten from whole wheat or fermentation residues.

DETAILED DESCRIPTION OF THE INVENTION

The invention relates, generally, to the use of *Deinococcus* or related bacterial strains to produce animal feed or additives with biomass. More particularly, the invention provides a low cost and high level supply for proteins and amino acids, based on the growth of Deinococcus bacteria and degradation/transformation of the biomass. The mix of *Deinococcus* or related bacteria and a biomass according to the invention may be used to provide animals low cost and/or high level source of valuable mono and di-acids or any metabolites derived from these mono or di-acids, derived from sugars such as xylan or hemicellulose hydrolysate contained in the biomass. According to the invention, unprocessed material of biological origin, including vegetal, algal or animal biomass, or secondary biomass, which essentially contains hydrolyzed pre-treated biomass products,

may be used in combination with Deinococcus bacteria as nourishing/nutritive feedstock, animal feed or additive.

It is therefore an object of the invention to provide an ingredient for feedstuff comprising a mix of biomass and *Deinococcus* or related bacteria, said biomass being at least partially digested by *Deinococcus.*

A further object of this invention relates to the use of a composition comprising a biomass and *Deinococcus* or related bacteria, for animal feeding.

Definitions

The term *"biomass"* according to the invention typically designates any biological material. In particular, the term biomass includes organic material of biological origin, including vegetal, algal or animal origin, which may be unprocessed or pretreated. Examples of biomass include, without limitation, forestry products, including mature trees unsuitable for lumber or paper production, pulp, recycled paper, organic waste, agricultural products, such as grasses, straw, crops and animal manure, and aquatic products, such as algae and seaweed. Examples of biomass include wood or vegetal material derived from numerous types of plants, including miscanthus, hemp, switchgrass, sugarbeet, wheat, barley, corn, rice, soy, rapeseed (including canola), sorghum, sugarcane, peanut, cotton, lupine, and a variety of tree species, ranging from eucalyptus to oil palm, poplar, willow. Specific sources of biomass include, without limitation, plant residues, hardwood or softwood stems, cobs, straw, grass, leaves, seeds, paper, etc. (see for instance Sun et al, Bioresource Technology 83 (2002) 1-11). The term biomass also encompasses transformed biomass or secondary biomass, which essentially contains hydrolysed pre-treated biomass products. In a preferred embodiment, biomass according to the invention comprises any lignocellulosic material, for example, cellulose, hemicelluloses and/or xylan.

The biomass according to the invention may comprise raw biomass and/or secondary biomass. The "raw *biomass"* is unprocessed material from biological matter. Examples include, without limitation, forestry products, such as mature trees unsuitable for lumber or paper production, agricultural products, such as grasses, crops and animal manure, and aquatic products, such as algae and seaweed. The *"secondary biomass"* is any material initially derived from raw biomass, which has undergone significant chemical and physical changes. Examples include, without limitation, paper, leather, cotton, hemp, natural rubber products, food processing by-products, fish and animal meals, and used cooking oils.

The term *"lignocellulosic biomass"* according to the invention designates an organic biological materialcontaining lignin, cellulose, hemicellulose and/or xylan. The term lignocellulosic biomass generally designates unprocessed material of biological origin, e.g., raw biomass. Examples of lignocellulosic biomass include, without limitation, wood or vegetal material derived from numerous types of plants, including miscanthus, rapeseed, switch grass, hemp, sugarbeet, wheat, wheat straw, corn, poplar, willow, sorghum, sugarcane, and a variety of tree species, ranging from eucalyptus to oil palm.

As used herein, the term *"biomass derivatives"* designates all molecules derived from raw biomass and/or from secondary biomass, as defined above.

In the context of the present application, the term *"Deinococcus* bacterium" includes wild type bacterium, or natural variant strains of *Deinococcus*, e.g., strains obtained through accelerated evolution, by DNA-shuffling technologies, as well as recombinant strains obtained by insertion of eukaryotic, prokaryotic and/or synthetic nucleic acid(s).

A bacterium *"related'* to *Deinococcus* designates a bacterium which (i) contains a 16SrDNA which, upon amplification using primers GTTACCCGGAATCACTGGGCGTA (SEQ ID NO: 1) and GGTATCTACGCATTCCACCGCTA (SEQ ID NO: 2), generates a fragment of about 158 base pairs and/or (ii) resists a UV treatment of 4 mJ/cm^2. In a particular embodiment, *Deinococcus-re*lated bacteria are bacteria having a 16S rDNA molecule which is at least 70%, preferably at least 80% identical in sequence to a Deinococcus 16S rDNA sequence.

In the context of the invention, *"proteins"* mean all biochemical compounds comprising one or more polymer chain(s) of amino acids residues bonded together. The protein supply of the invention includes proteins, peptides, polypeptides, amino acids and related derivatives.

In the context of the invention, an "exogenous component", such as an "exogenous protein" refers to a component originating from the biomass or a culture medium and, conversely, an "endogenous component" refers to a component originating from the bacterium.

In the context of the invention, *"starch"* refers to a carbohydrate consisting of a large number of glucose units joined together by 1-4 and 1-6 glycosidic bonds. Starch is an energy storage molecule accumulated by many plants and bacteria, and starch molecules arrange themselves in the plant in semi-crystalline granules.

Digestion of the biomass by the bacteria to produce high value feedstuff

The present invention is based, inter alia, on the combination of biomass and Deinococcus or related bacteria which cooperate to generate high nutritive value, and high digestibility products suitable for feedstuff. The invention shows biomass supports the growth and expansion of the bacteria, increasing the nutritive value of the mix, while the bacteria are able to digest the biomass, further increasing the nutritive value, digestibility and palatebleness of the mix.

According to the invention, the biomass may be at least partially digested by the *Deinococcus* and/or related bacteria.

The term digested or digestion includes all biological modification or transformation of the biomass, such as degradation or hydrolysis of components of the biomass such as raw material, cell walls, polymers (e.g., sugars, proteins), etc. Digestion may be partial, meaning that only a portion of some of the components of the biomass is digested,

typically 5%, 10%, or more. Partial digestion indicates that at least part of the biomass has been modified or transformed, which typically results in an increased nutritive value, digestibility or palatableness.

Deinococcus or related bacteria may advantageously catalyze (or contribute to the catalysis of) the degradation of various components of a biomass, such as sugar polymers like starch, xylan or cellulose into oligosaccharides of smaller degree of polymerization and monosaccacharides. Indeed, Deinococcus bacteria which express particular enzymes and/or have the ability to transform raw biomass have been disclosed in the art by Applicant. In this regard, preferred Deinococcus bacteria for use in the invention synthezise xylanases and/or cellulases and/or amylases. Xylanases are enzymes that catalyze the hydrolysis of xylan, a major component of hardwood and softwood hemicelluloses. Amylases are involved in the hydrolysis of starch polymers. Cellulases are enzymes that catalyze the hydrolysis of cellulose or hemicellulose, a major component of hardwood and softwood.

In a particular embodiment, the digestion of the biomass designates a reduction in the level of cellulose or hemicellulose of the biomass, preferably a reduction by at least 5%. Such a decrease facilitates digestibility of the biomass by all kinds of animals, including non ruminant animals.

In a preferred embodiment, the digestion of the biomass designates the conversion of hemicellulose from said biomass into smaller oligosaccharides and/or xylose, mannose, arabinose or galactose, and/or the conversion of cellulose and/or starch from said biomass into glucose. Preferably, the digestion encompasses conversion of at least 5% hemicellulose, cellulose, or starch from said biomass.

Such at least partial digestion of the sugars improves the digestibility and/or palatableness of the biomass, since C5 sugars like xylose, which cause the non appetence of the substrate, are digested by *Deinococcus* (Br J nutr. 2010 May; 103(10): 1507-13).

In another embodiment, digestion comprises the cellular sugar oxidation, particularly including DP3-DP7 sugars (DP: degree of polymerization) e.g.; triose, tetrose, pentose, hexose, and heptose, for example selected from xylose, arabinose, glucose, galactose, or fucose residues as monomers.

Simultaneously to the degradation/digestion of the biomass, the growth of the bacteria increases. Consequently, because the level of bacteria increases inside the mix, the level of proteins and lipids increases too. Then, according to the invention, the digestion of the biomass by *Deinococcus* or related bacteria, even indigestible biomass such as wheat bean or canola seed cattle cakes, provides a high level of directly nourishing constituents for animals. The invention allows making the most of several vegetal biomasses that were not used for animal feeding because of their indigestibility and/or few levels of nutriments. It is for example the case for rapeseed cattle cakes which contain a few level of proteins compared to soy bean cattle cakes. Such biomass comprises 1 to 6 g/1 of vegetal proteins and no significant amount of lipids. After a partial digestion of said biomass by *Deinococcus* or related bacterial according to the invention, one may expect about 12 to 25 g/1 of proteins and about 1 to 2.5 g/1 of lipids. In the same way, several animal biomasses without industrial interest, such as poultry feathers, may be advantageously used as biomass to be mixed with *Deinococcus* for providing animal feedstuff ingredient.

In addition, since *Deinococcus* sp. produce naturally carotenoids, the mix biomass /Deinococcus of the invention may contain carotenoids which have a positive impact on both the visual and gustative quality and the health of the animals fed with said mix. For example, based on a vegetal biomass without any carotenoid, one may expect about 15 mg/kg of carotenoids in the mix after the partial digestion of the vegetal biomass and the growth of the bacteria.

The final amount of proteins, amino acids, lipids, vitamins, carotenoids etc. produced or synthesized by *Deinococcus* and recovered in the mix will depend on several industrial parameters, such as the nature and the conditions of the reaction, the time of reaction, the initial amount of bacteria and the kind of biomass used. It will be apparent to one skilled in

the art that various adaptations can be made to adapt the method of the invention to the need.

For example, the digestion level of the biomass may vary depending on the industrial/economic and/or nutritive requirements. If a high level of nutritive constituents is preferred to a low cost production, the time of reaction may be increased for allowing a complete digestion of the biomass. In the final mix, one may expect a lack of vegetal fraction, the bacteria having almost totally digested the biomass. Conversely a low cost ingredient, with a minimum level of proteins, can be desired. The balance between the time of reaction and the level of nutritive constituents may be easily adapted.

The mix of the invention, or final mix, comprises the remaining biomass (the part of the biomass which has not been hydrolyzed/digested by the bacteria) and *Deinococcus* or related bacterial, whose number has increased.

In an embodiment of the invention, the *Deinococcus* or related bacterium used presents a proteoloytic activity, particularly useful to at least partially hydrolyse proteins contained in the biomass.

Proteases, also known as proteinases or proteolytic enzymes, are enzymes that begin protein catabolism by hydrolysis of the peptide bonds that link amino acids together in the polypeptide chain.

The inventors have discovered that *Deinococcus* or related bacteria may present a high proteolytic activity. According to the invention, *Deinococcus* or related bacteria having a proteolytic activity may be advantageously used in combination with several vegetal, algal or animal biomasses containing proteins to provide highly digestible amino acids. The bacteria hydrolyze the proteins, providing amino acids and peptides that are easily absorbed by animals. The resulting mix may be used as an ingredient for feed composition that provides both exogenous amino acids (and/or peptides) and endogenous nutriments (from the bacteria themselves) including proteins and amino acids.

For example, *Deinococcus* bacteria may be contacted with algae such as *Micratinium pusilum* and/or *Chlorella sp* so that the resulting mix may be used instead of fish meal as ingredient for feedstuff containing useful amino acids.

Other examples of biomasses containing proteins include beet pulp, soybeans, alfalfa and chicken feathers.

The use of *Deinococcus* or related bacteria can also reduce the viscosity of biomass (vegetal, animal, or algal), which represents a further advantage. Indeed, the proteolytic activity of the *Deinococcus* or related bacteria reduces the viscosity of gelatin-containing biomass. Also, the pectinolytic activity of *Deinococcus* or related bacteria reduces the viscosity of pectin-containing biomass.

The inventors have discovered that *Deinococcus* or related bacteria may present a high pectinolytic activity. According to the invention, *Deinococcus* or related bacteria synthesizing pectinases may be advantageously used in combination with a biomass containing pectin to decrease its viscosity and so increase its palatableness. The bacteria hydrolyze the pectin, eliminating the viscous properties of the biomass. Such viscous biomass may be used as growing medium for *Deinococcus* or related bacterial, and the resulting mix may be used as ingredient for feedstuff.

In a particular embodiment of the invention, the *Deinococcus* or related bacterium used in the mix for feedstuff therefore presents a pectinolytic activity, particularly useful to at least partially hydrolyse pectin contained in some viscous biomass.

It is the case for example of beet pulp that contains a high level of pectin (15 to 20% by weight). The beet pulp is a feedstock that is not easily exploitable because of the pectin making this biomass viscous. It is not possible to feed animals directly with such viscous biomass and its set into pleasant feed requires costly treatments.

In one embodiment, the viscous biomass is only part of the biomass of the mix. For example, the used biomass comprises 60% of lignocellulosic biomass, such as canola seed cattle cake, and 40%> of viscous biomass, such as beet pulp.

Process for preparing the mix

The digestion of the biomass by *Deinococcus* or related bacteria may be advantageously conducted in a reactor, for managing the parameters of the reaction easily. Depending on the strain(s) of bacteria and/or on the biomass used, the reaction may be conducted in an aerobic or anaerobic reactor.

In one embodiment, the biomass is introduced in one time, together with the bacteria. In another embodiment, the reactor may be reloaded with biomass or bacteria during the process. In such case, the nature and quantity of biomass may vary to improve the final content in nutrients in the final mix. The final mix comprises the remaining biomass, which has not been hydrolyzed/digested yet, the products resulting from digestion/hydrolysis (unless consumed by the bacteria), and the bacteria which have been expanded.
The amount of liquid added inside the reactor may depend on the form of the final composition, i.e. liquid or solid form, and on the moisture content of the biomass.

In another embodiment, the preparation of the mix of the invention is conducted in open country. For example, the biomass and *Deinococcus* or related bacterial are first mixed together before to be spread on the soil. This initial mix can be covered with a canvas sheet or conversely the mix can be put in the fresh air.

According to the invention, the mix can be used directly as part of an animal feed composition. Otherwise, the mix can be processed (for example dehydrated, filtered, dried, milled, etc.) before to be used. In another embodiment, the mix may be treated to kill or inactivate the bacteria, or to eliminate remaining raw biomass. The resulting product comprises essentially bacteria and digested biomass components and may be used as straight protein and lipid raw material.

The mix of biomass and *Deinococcus* or related bacteria according to the invention may be used as an ingredient in an animal feed composition. An animal feed composition as used therein is a composition for animal nutrition, in solid or liquid form. The feed may be defined as substance with sufficient nutritional value to allow growth and maintenance of adequate body conditions on an animal. For example, an animal feed composition may be on the form of pellets, meal, grains, extruded or expanded grains, tablets powder, bolus form or mix thereof.

Preferentially, the animal feed composition of the invention, including the mix of bacteria and biomass, is in a form and/or a composition approved by a governmental institution

such as National Food Administration (for example AFSSA in France, ACIA in Canada, or FAD in the US).

In a preferred embodiment, the animal feed composition of the invention comprises at least 10% by weight of the mix of *Deinococcus* or related bacteria and at least partially digested/hydrolyzed biomass. Depending on the other ingredients used to form the animal feed composition, and their impact on both the physical quality of the feeds after forming (such as pelleting) and the nutritional quality required, the level of the mix of the invention may be increased or decreased. For example, to obtain pellets with a good quality standard in terms of hardness and durability an amount of about 40% of pre-gelatinized starch may be added. If native starch is used, the required amount may be higher. In the same way, the amount of dispersible protein may also positively affect hardness and durability properties of pelleted feeds.

In this regard, the invention also relates to a method for preparing a feedstuff comprising:

- submitting a biomass to at least partial digestion by *Deinococcus* or related bacteria, and
- formulating a mix of at least partially digested biomass and bacteria, as ingredient for feedstuff.

In a particular embodiment, the method comprises a further step of adding to the mix other ingredients commonly used for feedstuff.

In an alternative embodiment, the method comprises:

(a) combining a biomass and *Deinococcus* or related bacteria to obtain a mix,
(b) maintaining the mix under conditions allowing digestion of the biomass by said Deinococcus or related bacteria,
(c) collecting the mix of (a) or (b), and
(d) optionally mixing the mix of (c) to one or several further ingredients.

The feedstuff may be packaged in any suitable form or container.

In the mix, it is possible to use one species or strain of a Deinococcus or related bacterium, or to combine various strains, or the same or distinct species of Deinococcus bacteria. Also, in addition to Deinococcus or related bacteria, the mix or feedstuff product may comprise further bacteria or yeast cells, if appropriate. Furthermore, additional agents such as enzymes may be added to the biomass.

As indicated above, the invention may be used to produce feedstuff or feed additives suitable for use in any non-human animals. It is particularly suitable for livestock (including ruminants and non-ruminants), breeding animals, avian animals, fishes or companion animals. Specific examples include cattle, ovine, porcine, poultry, fish, schrimp and crustaceans.

Further aspects and advantages of the invention will be disclosed in the following experimental section, which should be considered as illustrative of the invention.

EXAMPLES

Example 1: Identification of mesophilic and thermophilic *Deinococcus* strains with biomass degrading activities

This example discloses tests suitable to determine whether a genus, a species and/or a bacterial strain is able to function in a method for preparing feedstuff according to the invention. Non limitative examples of tests that may be performed to identify bacteria exhibiting particular enzymatic activities are described below.

Materials and Methods

Complex Medium Glucose (CMG) 1% composition

Peptone : 2 g/L
Yeast extract : 5 g/L
Autoclavation 121°C, 15 min.

Glucose : 10 g/L - Filter sterilized (0.22 μηι)
Then addition of MOPS, micronutrients, vitamins, $FeCl_3$, K_2HP0_4 (see below)

Media composition (MM)

14g/L of agar are added to 704 ml of ultrapure water. Then an autoclavation is performed. Then, after medium cooling, 80 ml of MOPS 10X, 8 ml of FeCB 100X, 8 ml of K2HP04 100X, 80μΙ of micronutrients 10 000X, and 80μΙ of vitamins 10 000X are added.

Media composition for solid screening of cellulolytic activity (5% AZO-Cellulose)

14g/L of agar are added to 704 ml of ultrapure water. Then an autoclavation is performed. Then, after medium cooling, 80 ml of MOPS 10X, 8 ml of FeCB 100X, 8 ml of K2HP04 100X, 80μΙ of micronutrients 10 000X, and 80μΙ of vitamins 10 000X are added.
Finally, AZO-Cellulose solution at 5% is added.

Media composition for solid screening of proteolytic activity (1% milk)

lOg/L of milk powder and 14g/L of agar are added to 704 ml of ultrapure water. Then an autoclavation is performed. Then, after medium cooling, 80 ml of MOPS 10X, 8 ml of FeCB 100X, 8 ml of K2HP04 100X, 80μΙ of micronutrients 10 000X, and 80μΙ of vitamins 10 000X are added.

Media composition for solid screening of amylolytic activity (0.5% starch)

5g/L of starch and 14g/L of agar are added to 704 ml of ultrapure water. Then an autoclavation is performed. Then, after medium cooling, 80 ml of MOPS 10X, 8 ml of FeCB 100X, 8 ml of K2HP04 100X, 80μΙ of micronutrients 10 000X, and 80μΙ of vitamins 10 000X are added.

Media composition for solid screening of xylanolytic activity (5% AZO-xylan)

14g/L of agar are added to 704 ml of ultrapure water. Then an autoclavation is performed. Then, after medium cooling, 80 ml of MOPS 10X, 8 ml of FeCB 100X, 8 ml of K2HP04 100X, 80μΙ of micronutrients 10 000X, and 80μΙ of vitamins 10 000X are added.
Finally, AZO-Xylan solution at 5% is added.

MOPS-Buffer mixture IPX. pH7.0

MOPS acid	400 mM
NH_4C1	200 mM
NaOH	100 mM
KOH	100 mM
$CaCl_2$	5 μM
Na_2S0_4	2.76 mM
$MgCl_2$	5.28 mM

Filter sterilized (0.22 μιη)

Micronutrients 10 000X

$(NH_4)_6(Mo_7)0_{24}$	30 μM
H_3B0_3	4 mM
$CoCl_2$	300 μM
$CuS0_4$	100 μM
$MnCl_2$	2.5 mM
$ZnS0_4$	100 μM

Adjusted to pH 5 with HC1.

Filter sterilized (0.22 μιη)

Vitamins 10 000X

10 mg/L of each : D-biotin, Niacin (nicotinic acid), Pyridoxin (pyroxidal hydrochloride) B6, Thiamin (vitamin B1 hydrochloride) - Stock pH4 - filter sterilized (0.22 μιη).

FeC13 100X

2 mM FeC13 in 2mM sodium citrate, filter sterilized (0.22 μιη)

K_2HPO_4 100X

100 g/L, autoclaved .

Detection of enzymatic activities

Detection of the cellulolytic activity

Solid screening (test on agar plate)

A preculture was carried out in CMG 1% medium in microplate using isolated clones (5 colonies in 200µL of CMG 1% medium).

From a stationary phase, 5µL of preculture were spotted on agar plates containing MM and 5% AZO-cellulose.

The cellulolytic enzymatic activity was followed by measuring the hydrolysis halo diameter after 1, 2 and 5 days (1 plate/ day was required).

Liquid screening

A preculture was carried out in CMG 1 %medium in microplate using isolated clones (5 colonies in 200µL of CMG 1% medium).

From a stationary phase, 5µL of preculture were added to 200 µï MM + 1% CMC or 1% CMC4M or 1% cellobiose in microplates.

The cellulolytic enzymatic activity was estimated by following the growth at ODeoonm (reading twice a day for 5 days).

Detection of the proteolytic activity

Solid screening (test on agar plate)

A preculture was carried out in CMG 1% medium in microplate using isolated clones (5 colonies in 200µL of CMG 1% medium).

From a stationary phase, 5µL of preculture were spotted on agar plates containing MM and 1% milk.

The proteolytic enzymatic activity was followed by measuring the hydrolysis halo diameter after 1, 2 and 5 days (1 plate/ day was required).

Liquid screening

A preculture was carried out in CMG 1 %medium in microplate using isolated clones (5 colonies in 200µL of CMG 1% medium).

From a stationary phase, 5µL of preculture were added to 200 µï MM + 1% pepton or 1% casein in microplates.

The proteolytic enzymatic activity was estimated by following the growth at OD_{600nm} (reading twice a day for 5 days).

Detection of the amylolytic activity

Solid screening (test on agar plate)

A preculture was carried out in CMG 1% medium in microplate using isolated clones (5 colonies in 200 µL of CMG 1% medium).

From a stationary phase, 5µL of preculture were spotted on agar plates containing MM and 0.5% starch.

The amylolytic enzymatic activity was followed by measuring the hydrolysis halo diameter after 1, 2 and 5 days (1 plate/ day was required).

On starch containing-agar plates, the hydrolysis halo revelation was done by addition Gram's iodine reagent (1 plate/day was also required).

Liquid screening

A preculture was carried out in CMG 1 %medium in microplate using isolated clones (5 colonies in 200µL of CMG 1% medium).

From a stationary phase, 5µL of preculture were added to 200 µï MM + 0.5% starch in microplates.

The amylolytic enzymatic activity was estimated by following the growth at ODeoonm (reading twice a day for 5 days).

Detection of the xylanolytic activity

Solid screening (test on agar plate)

A preculture was carried out in CMG 1% medium in microplate using isolated clones (5 colonies in 200µL of CMG 1% medium).

From a stationary phase, 5µL of preculture were spotted on agar plates containing MM and 5% AZO-xylan.

The xylanolytic enzymatic activity was followed by measuring the hydrolysis halo diameter after 1, 2 and 5 days (1 plate/ day was required).

Liquid screening

A preculture was carried out in CMG 1 %medium in microplate using isolated clones (5 colonies in 200µL of CMG 1% medium).

From a stationary phase, 5µL of preculture were added to 200 µï MM + 0.5% xylan in microplates.

The xylanolytic enzymatic activity was estimated by following the growth at ODeoonm (reading twice a day for 5 days).

Results

Table 1 (below) lists examples of bacteria identified with solid screening tests and having suitable biomass-digestion activity for use in feedstuff production.

The hydrolysis halo diameter has been measured after 2 days for the proteolytic and amylolytic activities, and after 5 days for the xylanolytic and cellulolytic activities.

More precisely, for proteolytic activity, a high activity corresponds to a hydrolysis halo diameter higher than 2.4 cm, a medium activity corresponds to a hydrolysis halo diameter between 2 cm and 2.35 cm, and a low activity corresponds to a hydrolysis halo diameter lower than 1.95 cm.

For amylolytic activity a high activity corresponds to a hydrolysis halo diameter higher than 2.4 cm, a medium activity corresponds to a hydrolysis halo diameter between 2.1 cm and 2.35 cm, and a low activity corresponds to a hydrolysis halo diameter lower than 1.9 cm.

For xylanolytic activity a high activity corresponds to a hydrolysis halo diameter higher than 2.8 cm, a medium activity corresponds to a hydrolysis halo diameter between 2.1 cm and 2.7 cm, and a low activity corresponds to a hydrolysis halo diameter lower than 2.05 cm.

For cellulolytic activity a high activity corresponds to a hydrolysis halo diameter higher than 1.6 cm, a medium activity corresponds to a hydrolysis halo diameter between 1.1 cm and 1.35 cm, and a low activity corresponds to a hydrolysis halo diameter lower than 0.9 cm.

Table 1: List of *Deinococcus* strains having cellulolytic, proteolytic, amylolytic and/or xylanolytic activities (solid screening)

	2 days	**2 days**	**5 days**	**5 days**
	Proteolytic activity	**Amylolytic activity**	**Xylanolytic activity**	**Cellulolytic activity**
DRH01	+++	+++	-	-
DRH02	++	-	-	-
DRH03	++	+	-	-
DRH46	++	+	-	+++
M1-5A	+++	+++	-	+
M2-8F	++	++	-	-
M2-9H	++	+	-	-
M3-5A	+	++	-	+++
M3-6B	+	++	-	+++
M3-6G	+	++	-	++
M3-7C	++	+	-	+
DRH05	+	+	-	-
DRH06	+	++	-	-
DRH07	+	+	+	
DRH38	+	+	+++	-
DRH39	+	-	+	-
M 11-12B	+	+	-	-
M13-1A	+	-	-	-
M13-8D	+	+	-	-
M23-1G	++	++	-	-
M23-2A	+++	++	-	-
M23-2E	+++	++	-	-
M23-2F	++	++	-	-
M23-3A	++	+++	+++	-

M31-1H	++	++	-	-
M31-2A	+++	+++	-	-
M31-2B	+++	++	-	-
M31-8F	++	++	-	-
M35-1F	++	-	+	-
DG01	++	-	++	-
MC2-2A	++	-	+++	-
MC3-4A	++	++	-	-
MC3-4B	+++	-	+++	-
MD2-3B	++	-	++	-
MD2-3B bis	+	-	+	-
MX4-2B	+	+	-	-
MX4-2D	+	+	-	-
MX4-4A	++	-	+	-
DG01_04	++	++	++	-
MC5-12E	+++	++	-	-

(+++) : high activity

(++) : medium activity

(+) : low activity

(-) no activity

The Table 2 (below) lists examples of bacteria identified with liquid screening tests and having variable enzymatic activities for production of feedstuff. Growth of the bacteria is followed by measuring the OD at 600 nm.

Table 2: List of *Deinococcus* strains having cellulolytic, proteolytic, amylolytic and/or xylanolytic activities (liquid screening)

	1% Casein	0.5% starch	0.5% xylan	1% CMC4M
DRH01	+	+	+	-
DRH22	+	-	-	-
DRH25	+	-	-	-
DRH46	+	+	+	+
M3-5A	+	+	-	+
M3-6B	+	+	-	+
M3-6G	+	+	-	+
M4-9B	+	+	-	+
M5-1D	+	+	-	-
M5-5A	+	+	-	+
DRH05	+	+	-	-
DRH06	+	+	-	-
DRH37	+	+	+	-
DRH38	+	+	+	-
DRH39	+	+	+	-
M11-12B	+	+	+	-
M13-1A	+	+	-	-
M23-2A	+	+	+	-
M23-3A	+	-	+	-
M31-3C	+	+	+	-
M31-3D	+	+	+	-
M31-8F	+	+	+	-
M35-1F	+	+	-	-
MC2-2A	+	-	+	-
MC2-2C	+	-	+	-

MC3-4A	+	+	+	-
MX4-2B	+	+	-	-
MX4-2D	+	+	+	-

(+):good growth ($\mathbf{OD_{600nm}} \geq 0.5$)
(-):low or no growth ($\mathbf{OD_{600nm}} < 0.5$).

This example shows that Deinoccocus bacteria having suitable biological activity may be selected from public collections, and used in the invention to produce feedstuff.

Example 2: Production of a Rapeseed straw-Deinococcus Mix

A mix of rapeseed straw and Deinococcus bacteria has been prepared. More particularly, 1% pretreated rapeseed straw has been contacted with a Deinococcus bacterium (e.g., strain DGOl). The capacity of the mixture to produce a valuable mix for feedstuuf has been determined by verifying the capacity of Deinocossus to expand and digest rapeseed straw, either alone or after enzymatic supplementation, and to produce high nutritive content.

Materials and Methods

Rapeseed straw

Rapeseed straw was obtained from Sofiproteol and was ground in a blender and then passed through a sieve to obtain fine sections, less than 1mm in length.

Cellulase and β-glucosidase

The commercial enzymes used were a cellulase from Trichoderma reesei (SIGMA ref.C8546-5KU) and a beta-glucosidase from almonds (SIGMA ref.49290-lG).

¾SQ $_4$ hydrothermal pretreatment

Pretreatment was performed into an erlen flask with 20% w/v rapeseed straw and 0.5% w/w H_2S0_4 in tap water. This mixture was autoclaved 10 min at 120°C (time of cycle : 1.5h) and then diluted with sterile tap water to obtain the final rapeseed straw

concentration. pH was adjusted to 7 with 20M NaOH solution (checked with pH paper). Mineral solutions: 20mM NH_4Cl and 5.7mM K_2HPO_4 was added before inoculation.

Counting protocol

- Take lmL of homogeneous culture (CMG, technical substrate) in Ependorff 2 ml
- Vortex 10 s and then apply ultrason in an ultrasonic bath for 10 minutes and vortex again 10 s.

In 96-wells microplate :

- Dispense 180µL of sterile MilliQ water in 9 wells in triplicate
- In triplicate: Perform serial dilutions of 1/10th from the well No. 1 (corresponding to pure sample) to the well No. 10 (corresponding to dilution 10^{-9}): take 20µL and put it in 180µL sterile milliQ water from the following well, mix & reverse pipetting three times. Change cone between each well.
- With the multi-channel pipette, spot on PGY-agar plate, 5µL of each dilution in duplicate.
- Incubate 2 days at 45 ^{0} C (for thermophile Deinococcus)
- Count the number of colonies on the first countable dilution:

Average the six spots corrected by the dilution factor and multiply by 200 to get the number of CFU / mL

TLC protocol

- Spot 5µ1of sample in TLC silica gel.
- Dry spoted samples with hot air gun.
- Migration of TLC in solvent Butanol/ acetone/ H_20 - 4/5/1 solvent.
- At the end of migration, dry TLC with Hot air gun.
- Reveal TLC using a solution containing 12g ammonium molybdate + 0.5g ammonium cerium nitrate in 80ml of H2SO4 10%.

Enzymatic hydrolysis and culture

For DGOl, enzymes were added to the culture medium to hydrolyze polymers into sugar monomers.

Rapeseed straw contains 40% cellulose. The enzyme loading is O.l l g cellulase/g cellulose and 0.05g beta-glucosidase/g cellulose. The enzyme solutions were filtered through 0.22 microns before introduction into the culture.

Preculture was done for 3 days in CMG 1%. Cell pellet was washed three times in sterile water and then used to inoculate culture medium at DOi of 0.2 ie ~10^7CFU/mL.

Growth was performed for 9 days at 30°C for DRH46 and 45°C for DGOl.

Growth is controlled by a counting of bacteria according to the protocol below.

Results

The growth of DGOl was followed by counting (UFC/ml) (figure 1) and the consumption of sugars was estimated using TLC analysis (figure 2).

After 9 days, all free glucose and xylose were consumed by DGOlwt. When this consumption was reported to total sugars, it appeared that a major part of glucose and xylose was consumed. These results therefore show that the mix comprises partially digested biomass, with reduced xylose content.

Similar results are obtained with other Deinococcus bacteria as listed in Table 1, such as DRH01, DRH02, DRH03, or DRH46.

Cultures were lyophilized and amino-acid composition, soluble nitrogen and NDF/ADF/ADL fibers determination was carried out on dry mass.

Table 3 below compares the amino-acid composition of rapeseed straw (g/kg dry mass) to the amino-acid composition of mix of the invention (g/kg dry mass).

The results show that the mix of the invention comprises at least 20 times more amino-acids than the initial rapeseed straw.

Table 3: Amino acids composition of the mix and rapeseed straw

	Mix of the invention (g/kg dry mass)	Rapeseed straw (g/kg dry mass)
aspartic acid	51,3	2,6
Threonine	31,9	1,4
Serine	18,9	1,4
glutamic acid	65,1	3,1
Proline	26	1,3
Glycine	37,1	1,8
Alanine	46,8	1,8
Valine	36,2	1,8
Cysteine	1,6	0,6
Methionine	10,4	0,4
Isoleucine	20,6	1,2
Leucine	42,5	2,1
Thyrosine	16,1	0,7
Phenylalanine	19,5	1,4
Lysine	29,4	1,2
Histidine	9,7	0,4
arginine	40	1,2

The mix of the invention therefore transforms a poor biological material into a rich and digestible mix for feedstuff.

Example 3: Production of a Wheat-Deinococcus mix.

A mix of Deinoccocus and Wheat biomass was prepared by inoculation of *Deinococcus* strain DGOl on a medium made of whole wheat (1% with or without Termamyl addition, and 6%) or fermentation residues (1% or 6 %) supplemented with NH_4C1 20 mM and K2HPO4 5.7 mM.

The capacity of the mixture to produce a valuable mix for feedstuuf has been determined by verifying the capacity of Deinocossus to expand on wheat and to reduce the gluten content of wheat.

Gluten consumption was evaluated by using Enzyme Immunoassay for the quantitative determination of Gliadin (soluble fraction of gluten)/Gluten. This test is based on the principle of the enzyme-linked immunoabsorbent assay.

Protocol

Protein consumption is measured using a commercial kit from Libios (Ref GLI-E02).

Samples preparation: 100 mg of finely ground lyophilized technical substrate is re-suspended in 1ml EtOH40%, mixed during 5min and finally centrifuge for 10 min at 3800 rpm. The supernatant is diluted (1/500000) in sample dilution bufferlX.

Elisa TEST:

Samples and standards are tested in duplicate.

-IOOμI of standards and samples are added in 96-wells microplate coated with antigliadin antibody.
- Incubate 20 min at room temperature.
- Wash wells three times with 300μ1washing solution IX.
- Add IOOμI of secondary conjugated antibody (Antigliadin peroxidase) in empty wells.
- Wash wells three times with 300μ1washing solution IX.
- Add IOOμI of TMB solution (substrate).
- Incubate 20min at room temperature, in the dark.
- Add IOOμI of stop solution (color shift from blue to yellow)
- Homogenize and read with a spectrophotometer at $OD_{450nm.}$

Result

The concentration of gliadin is directly proportional to the colour intensity of the test sample. Because of the equal amount of gliadin and gluten in wheat, the gluten concentration of the sample is calculated by multiplication with the factor 2.

As shown figure 3, Deinococcus is able to hydrolyze gluten from whole wheat or fermentation residues thereof, and to consume it after 2 days of growth at 45°C. After 48h, all proteins contained in the vegetal biomasses are consumed by the Deinococcus strain.

Similar results are obtained with other Deinococcus bacteria as listed in Table 1, such as DRH01, DRH03, or DRH46.

These results therefore clearly demonstrate Deinococcus strains can exhibit a strong proteolytic activity resulting in a more digestible mix from wheat suitable for use in animal consumption.

Example 4: Analysis of the amino acid composition of *Deinococcus seothermalis*

The amino acid composition of *Deinococcus geothermalis* (strain M36-7D_21) has been determined after growing the cells in a medium containing peptone and glucose as sole carbon sources. The composition was then compared to the amino acid composition of yeasts that are commonly used as a complementary protein source in fish diet and/or as a supplement in animals feed to compensate amino acids and/or vitamin deficiencies of cereals.

Materials and Methods

Complex Medium Glucose (CMG) 1% composition

Peptone 2 g/L ;

Yeast Extract 5 g/L ;

Glucose 55 mM (10 g/L) ;
MOPS acid 40 mM ;
NH_4C 120 mM ;
NaOH lOmM ;
KOH lOmM ;
$CaCl_2.2H_20$ 0,5 µM ;
$Na_2SO4.10H_2O$ 0,276 mM ;
$MgCl_2.6H_20$ 0,528 mM ;
$(NH_4)6(M\theta 7)0_{24}.4H_20$ 3 nM ;
H_3BO_3 0,4 µM ;
$CoCl_2.6H_20$ 30 nM ;
$CuS0_4.5H_20$ 10 nM ;
$ZnS0_4.7H_20$ 10 nM ;
D-Biotin 1 µg/L ;
Niacin (nicotinic acid) 1 µg/L ;
Pyridoxin (pyridoxal HC1 ou vitamine B6) 1 µg/L ;
Thiamin HC1 (vitamine Bl) ;
$FeCl_3$ 20 µM ;
Sodium Citrate. $2H_20$ 20 µM ;
K_2HP0_4 5,7 mM.
The final concentration of $MnCl_2$ was 5,25 µM.

Cells were grown in CMG 1% medium containing 6µg/ml of Bleomycin in a fermentor of 3,5 L at 45°C and under agitation of 400 rpm.
About 100 to 200 ml of the culture medium were harvested by centrifugation (4000 rpm, 20 min at 4°C) at exponential and stationary phase of growth and washed prior to lyophilisation.
The cultures were then lyophilized and amino acid composition was carried out on dry mass.

The amino acids composition and quantification have been determined by HPLC.

The values of the amino acid composition of yeast extract is derived from *S. Cortassa et al,* 2002 (S. Cortassa *et al,* 2002, "An introduction to metabolic and cellular engineering" World scientific Publishing).

Results

Table 4 below compares the amino acid composition of *Deinococcus geothermalis* to the amino acid composition of Yeast extract. Quantities are given in g/lOOg of dried matter.

Table 4: Amino acid compositions of *Deinococcus* compared to yeast extract.

	Yeast extract[1]	**D geothermalis Exponential Growth phase**	**D. geothermalis Stationary growth phase**
Amino acids :			
Aspartic acid	5,10	4,65	5,46
Glutamic acid	6,50	5,98	6,90
Alanine	nd	4,67	4,99
Arginine§	**0,78**	**3,92**	**4,57**
Cysteine	nd	0,31	0,08
Glycine	2,40	3,05	3,47
Histidine*§	0,94	0,93	1,08
Isoleucine*§	2,90	1,94	2,23
Leucine*§	**3,60**	**4,38**	**5,23**
Lysine *§	4,00	2,34	2,70
Methionine*§	**0,79**	**1,51**	**0,44**
Phenylalanine *§	2,20	1,88	2,09
Proline	nd	2,22	2,55
Serine	nd	1,75	2,15
Threonine *§	nd	2,82	3,39
Tryptophane *§	0,88	0,76	0,79

Tyrosine	**0,60**	**1,55**	**1,91**
Valine*§	3,40	3,31	3,82
Total amino acids :	**34,00**	**48,00**	**54,00**
Proteins :	**29,45**	**41,05**	**46,19**

"§": Amino acids whose carbon skeletons are not synthesized by animal cells and which are essential for fish growth *(H. George Ketola 1982, Comp biochem physiol 73B, N°l, p 17-24);*

"*": Amino acids whose carbon skeletons are not synthesized by animal cells and which are essential for monograstric mammals;

"nd": not determined

These data show that the total amino acids content of *Deinococcus* cells reaches 48% and 54% (g/100 g of dried matter) in the exponential and stationary growth phase, respectively. These values are higher than those obtained with yeast extract (34%) whatever the growth phase. The total *Deinococcus* protein content is also higher than that of the yeast extract. In addition, compared to the yeast extract, *Deinococcus* provides significant amount of arginine, which is an essential amino acid for fish *(H. George Ketola 1982, Comp biochem physiol 73B, N°l, p i 7-24). Deinococcus* biomass provides also more tyrosine and leucine compared to yeast extract, leucine being an essential amino acid for both monogastric mammals and fish.

Therefore, *Deinococcus* bacteria may be used as a source of highly valuable proteins and amino acids for feedstuff, and may even replace yeast extracts in feedstuff compositions.

Example 5: Analysis of particular culture conditions

Culture conditions allowing carotenoid production

Deinococcus geothermalis strain MX6-1E was grown in 1 L medium containing 20 g/L peptone and 10 g/L yeast extract in 1 L fermentor at 45°C under 0.35 L/min air.

DO regulated at 20 % by cascade control on aeration and agitation rate.

After 20 hours, the culture medium displayed a strong red color, indicating presence of carotenoids in said culture medium.

This experimentation confirms that *Deinococcus* bacteria may produce significant amount of carotenoids. In addition, Deinococcus biomass provides more methionine than yeast extract when the cells are in exponential phase of growth: Aand methionine is an essential amino acid for animal cells and is also required for fish growth.

Culture conditions allowing substantial expansion

Deinococcus geothermalis strain MX6-1E-14 was grown in 1 L CMG 10% medium (as described in example 4) containing 20 g/L glucose in 1 L fermentor at 45°C.

In that culture condition, the optical density at 600 nm (OD_{600nm}) reaches the value 20 in 40 hours (specific growth rate being 0,5 h^{-1}) indicating a strong expansion of the cells.

CLAIMS

1- An ingredient for feedstuff comprising a mix of biomass and a *Deinococcus* or *Deinococcus-related* bacterium, said biomass being at least partially digested by said *Deinococcus* or related bacterium.

2- The ingredient for feedstuff of claim 1, wherein the biomass comprises lignocellulosic biomass.

3- The ingredient for feedstuff of claim 2, wherein the lignocellulosic biomass is selected from the group comprising cereal bean, such as wheat bean, vegetal cattle cake, such as rapeseed and soybean cattle cakes, sugar cane and derivatives thereof, corn, sugarbeet, miscanthus, switch grass, hemp, poplar, willow, sorghum, and tree species, ranging from eucalyptus to oil palm.

4- The ingredient for feedstuff of any one of the preceding claims, wherein the biomass comprises proteins which are at least partially hydrolyzed by said *Deinococcus* or related bacterium.

5- The ingredient for feedstuff of claim 4, wherein the biomass comprising proteins is selected from beet pulp, soybeans, alfalfa and chicken feathers.

6- A feedstuff composition comprising the ingredient of any one of the preceding claims.

7- Use of a mix of biomass and *Deinococcus* or related bacteria, said biomass being at least partially digested by said *Deinococcus* or related bacteria, as protein supply for feedstuff.

8- Use of claim 7, wherein the biomass comprises lignocellulosic biomass.

9- Use of claim 7 or 8, wherein the biomass comprises proteins.

10- A method for preparing a feedstuff comprising submitting a biomass to at least partial digestion by *Deinococcus* or related bacteria to obtain a mix, and formulating said mix as ingredient for feedstuff.

11- The method of claim 10, wherein the biomass comprises lignocellulosic biomass selected from the group comprising cereal bean, such as wheat bean, vegetal cattle cake, such as rapeseed cattle cake and soybean cattle cake, sugar cane and derivatives thereof, corn, sugarbeet, miscanthus, switch grass, hemp, poplar, willow, sorghum, tree species, ranging from eucalyptus to oil palm.

12- The method of claim 10 or 11, wherein the biomass comprises proteins and is selected preferably from beet pulp, soybeans, alfalfa and chicken feathers.

13- A method for improving the nutritive value of a biomass, comprising subjecting said biomass to at least partial digestion by a *Deinococcus* or related bacterium.

14- The method of claim 13, further comprising allowing the growth of *Deinococcus* or related bacteria in said biomass.

15- A method for improving the palatableness and/or digestibility of a biomass, comprising subjecting said biomass to at least partial digestion by a *Deinococcus* or related bacterium, in order to at least partially degrade complex sugar of said biomass.

16- The use of a *Deinococcus* bacterium for animal feeding.

17- The use of a Deinococcus bacterium as a source of amino acids for animal feeding.

18- The use of a mix of *Deinococcus* bacteria and a biomass for animal feeding.

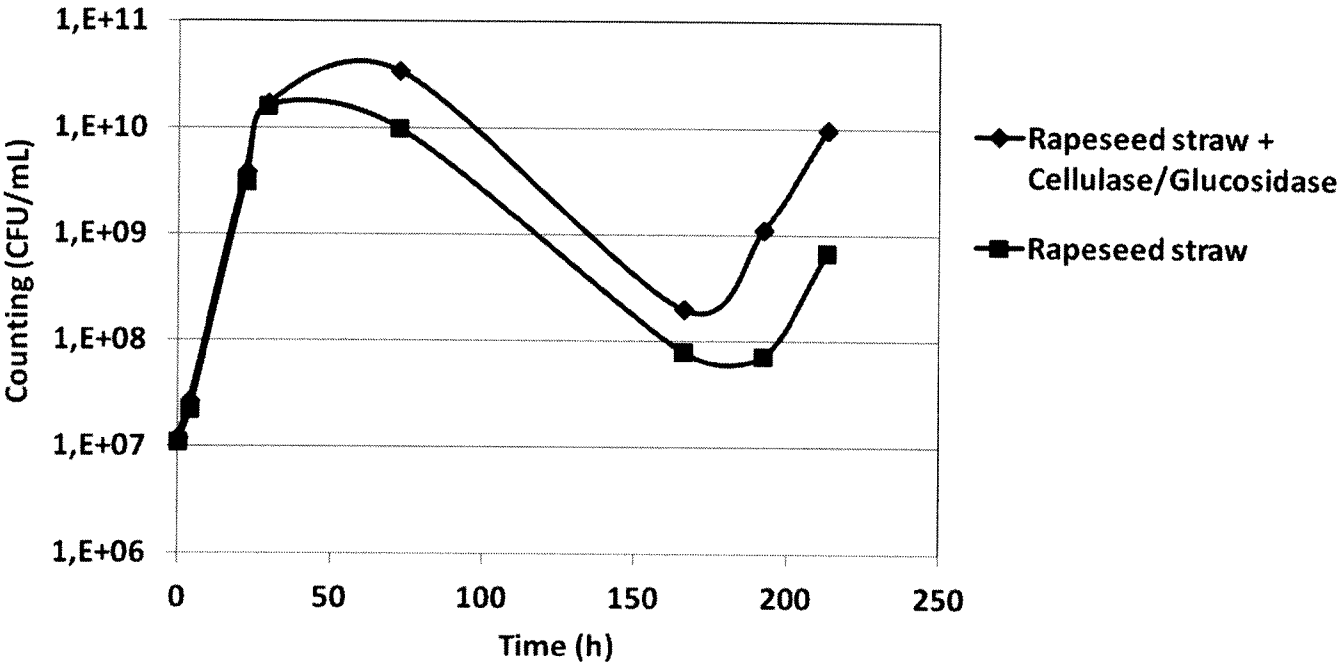
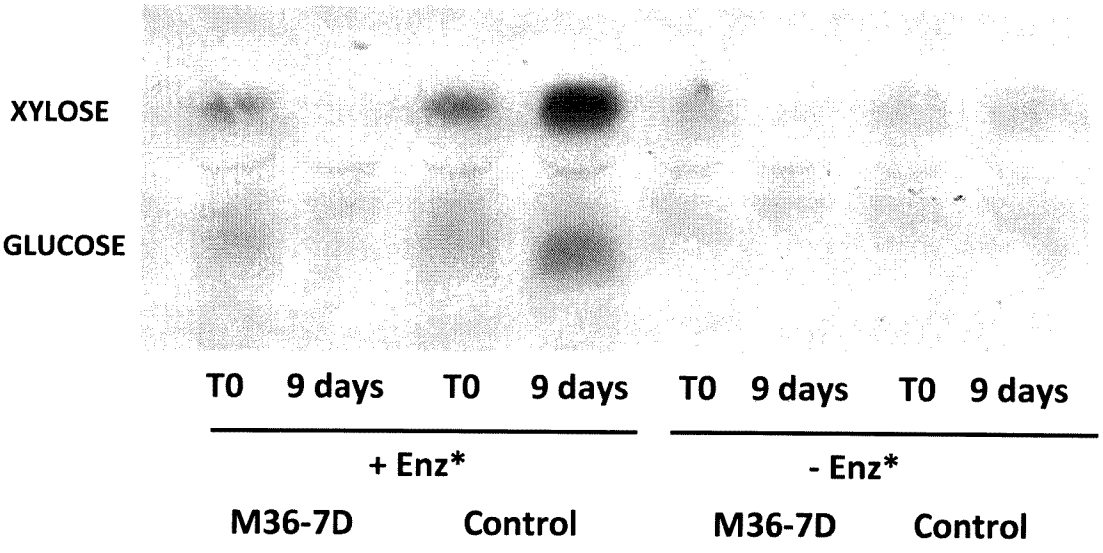


Figure 1



*Enz = Cellulase+β-Glucosidase added in the culture at T0
0,11g of cellulase/g of cellulose
0,05g of β-Glucosidase/g of cellulose

Figure 2

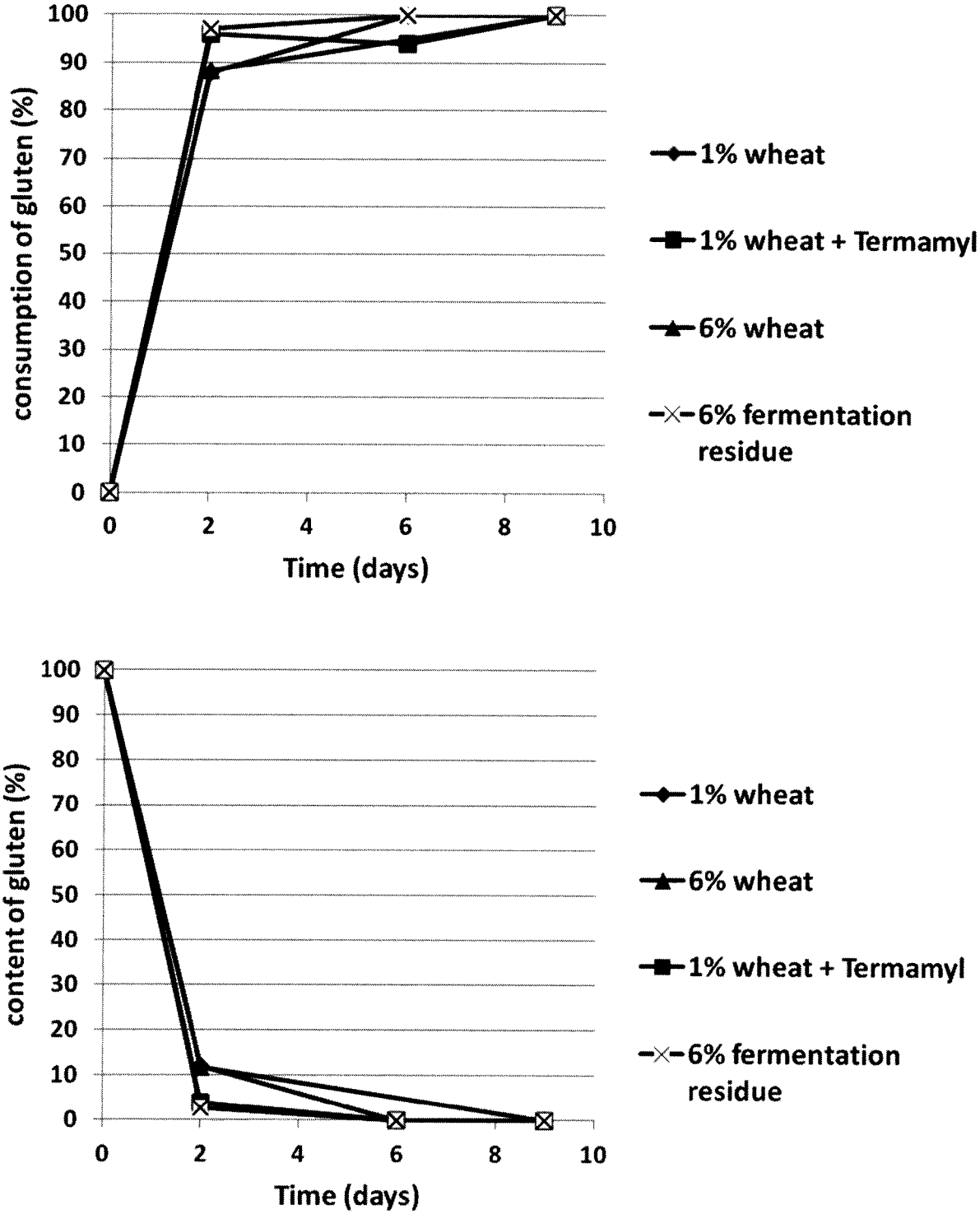

consumption of gluten (%)
100
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
Time (days)
1% wheat
1% wheat + Termamyl
6% wheat
6% fermentation residue
content of gluten (%)
100
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
Time (days)
1% wheat
6% wheat
1% wheat + Termamyl
6% fermentation residue

Figure 3

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2012/076046

A. CLASSIFICATION OF SUBJECT MATTER
INV. A23K1/00 A23K1/10 A23K1/12 A23K1/16
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A23K

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal , FSTA, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 2 218 773 Al (DEINOVE [FR] ; CENTRE NAT RECH SCI ENT [FR]) 18 August 2010 (2010-08-18)	1-6, 13-15
A	claims 1, 6; example 4	7-12 , 16-18

X	Wo 2011/107506 Al (DEINOVE [FR] ; CLAVERI E JEAN-MICHEL [FR] ; BIT0N JACQUES [FR] ; LE0NETTI) 9 September 2011 (2011-09-09)	1-6, 13-15
A	page 28, lines 10-16; claims 10, 11	7-12 , 16-18

X	wo 2009/063079 Al (DEINOVE [FR] ; CENTRE NAT RECH SCI ENT [FR] ; LE0NETTI JEAN-PAUL [FR] ; MA) 22 May 2009 (2009-05-22)	1-6, 13-15
A	claim 1; example 9	7-12 , 16-18

	-/-- ·	

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" documentwhich may throw doubts on priority claim(s) orwhich is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
3 June 2013	11/06/2013

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Di l ler, Rei nhard

1

Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2012/076046

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WEON HANG-YEON ET AL: "Dei nococcus eel lulosi lyti cus sp nov. , i sol ated from air", INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY, SOCI ETY FOR GENERAL MICROBIOLOGY, READING, GB, vol . 57, no. Part 8, 1 August 2007 (2007-08-01) , pages 1685-1688, XP002534882 , ISSN: 1466-5026, D0I : 10. 1099/IJS. 0. 64951-0 the whol e document --- wo 97/10352 Al (LOCKHEED MARTIN ENERGY SYS INC [US]) 20 March 1997 (1997-03-20) the whol e document ---	1-18 1-18

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2012/076046

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 2218773 A1	18-08-2010	AU 2010215597 A1	25-08-2011
		CA 2751472 A1	26-08-2010
		CN 102369272 A	07-03-2012
		EA 201101213 A1	30-03-2012
		EP 2218773 A1	18-08-2010
		EP 2398891 A2	28-12-2011
		JP 2012517817 A	09-08-2012
		US 2011306085 A1	15-12-2011
		WO 2010094665 A2	26-08-2010
WO 2011107506 A1	09-09-2011	CN 102884175 A	16-01-2013
		EA 201201206 A1	30-04-2013
		EP 2542664 A1	09-01-2013
		US 2013045482 A1	21-02-2013
		WO 2011107506 A1	09-09-2011
WO 2009063079 A1	22-05-2009	AU 2008322867 A1	22-05-2009
		CA 2704429 A1	22-05-2009
		CN 101861395 A	13-10-2010
		EA 201070605 A1	28-02-2011
		EP 2209900 A1	28-07-2010
		FR 2923491 A1	15-05-2009
		US 2011104766 A1	05-05-2011
		WO 2009063079 A1	22-05-2009
W0 9710352 A1	20-03-1997	AU 3630895 A	01-04-1997
		WO 9710352 A1	20-03-1997