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(54) Title: LACTOBACILLUS FERMENTUM BACTERIA REDUCING THE CONCENTRATION OF ACETALDEHYDE

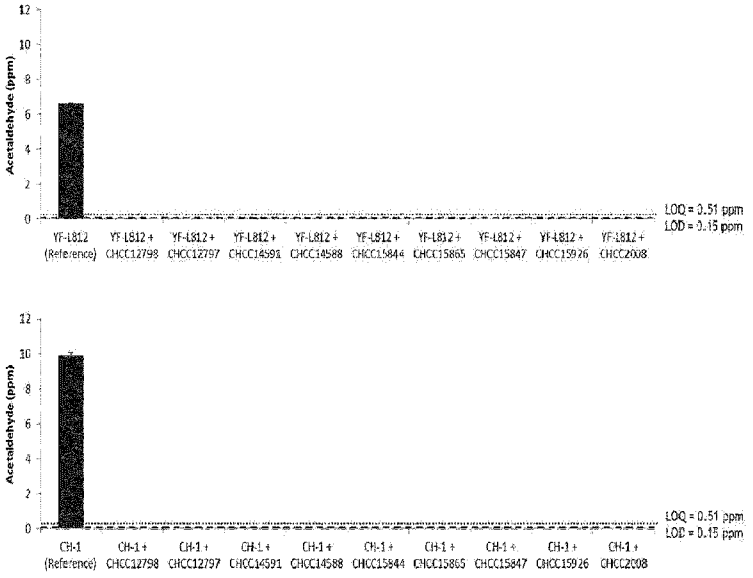

Figure 5

(57) Abstract: The present invention relates to a bacterium of the species Lactobacillus fermentum wherein the bacterium has the ability to reduce the concentration of acetaldehyde produced by a starter culture during fermentation in a fermented milk product by at least 50%. The invention further relates to compositions comprising the bacterium, methods for producing fermented milk products using the bacterium and the products thus obtained.

LACTOBACILLUS FERMENTUM BACTERIA REDUCING THE CONCENTRATION OF ACETALDEHYDE

FIELD OF THE INVENTION

The present invention relates *Lactobacillus fermentum* bacteria having the ability to reduce the concentration of acetaldehyde produced by a starter culture during fermentation in a fermented milk product, compositions comprising the bacteria, in particular adjunct cultures comprising the bacteria, methods of producing a fermented milk product using the bacteria or the cultures and the fermented milk products thus obtained, including food, feed and pharmaceutical products.

BACKGROUND OF THE INVENTION

Lactic acid bacteria (LAB) have been used over decades for increasing the shelf life of food products. During fermentation LAB produce lactic acids as well as other organic acids which cause a reduction of pH of the fermented product. Products having an acidic pH do not support further growth of most microorganisms, including pathogenic and spoilage organisms.

Traditionally, yoghurt is produced by fermentation of milk with a specific yoghurt starter culture consisting of a mixture of two species of lactic acid bacteria (LAB), *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*. The main roles of the starter in the production of yoghurt are (i) acidification through the conversion of lactose into lactic acid, (ii) creation of viscous texture e.g. by denaturation of proteins and production of exopolysaccharides, and (iii) development of the typical yoghurt flavor (1).

The typical yoghurt flavor is caused by lactic acid, which imparts an acidic and refreshing taste, and a mixture of various carbonyl compounds like acetone, diacetyl, and acetaldehyde, the latter of which is considered the major flavor component (2). The relatively high concentration of acetaldehyde found in yoghurt is suspected to be due to a low utilization rate of this metabolite since the common yoghurt bacteria lack the main enzyme for acetaldehyde conversion into ethanol, alcohol dehydrogenase (3).

During yoghurt fermentation, acetaldehyde can be produced directly from lactose metabolism as a result of pyruvate decarboxylation. It can be produced (i) directly via pyruvate decarboxylase or pyruvate oxidase or (ii) indirectly through the formation of the intermediate acetyl coenzyme A by pyruvate dehydrogenase or pyruvate formate lyase. Furthermore, acetaldehyde can be formed by the activity of deoxyriboaldolase, which degrades thymidine into acetaldehyde and glyceraldehyde-3-phosphate. Finally, while several amino acids can be converted into acetaldehyde via pyruvate as a metabolic intermediate, threonine can be

directly converted into acetaldehyde and glycine by the activity of threonine aldolase (TA). Hence the exact biochemical pathway of acetealdehyde formation may differ between bacterial species and depend on intracellular regulatory mechanisms. Further available substrates may as well influence the acetaldehyde synthesis pathway.

Historical sensory analysis has indicated that for optimal flavor in yoghurt, the acetaldehyde concentration should be between 23 and 41 mg/kg of yoghurt (1) why researchers have strived to isolate bacterial strains that produce significant amounts of the desired flavor element.

However new markets and new consumer preferences seem to indicate a growing interest in yoghurt and other fermented milk products exhibiting less acetaldehyde flavor.

There is thus a need for fermented products with reduced acetaldehyde content.

SUMMARY OF THE INVENTION

The *Lactobacillus fermentum* strains of the present invention is characterized in having the ability to reduce the concentration of acetaldehyde produced by a starter culture during fermentation in a fermented milk product by at least 50%.

The present invention provides the bacteria as described above, compositions comprising the same, methods using the bacteria for producing fermented milk products, as well as the products thus obtained.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a bacterium of the species *Lactobacillus fermentum* having the ability to reduce the concentration of acetaldehyde produced by a starter culture during fermentation in a fermented milk product by at least 50%. The reduction is determined in comparison to a fermented product produced without the *Lactobacillus fermentum* strains of the present invention. Different assays are known in the art for determining the concentration of acetaldehyde in a fermented product and can be used for that purpose in accordance with the present invention. The ability to reduce the concentration of acetaldehyde produced by a starter culture during fermentation in a fermented milk product by at least 50% is preferably determined in an assay comprising:

(1) preparing a fermented milk product by:
   (a) inoculating a milk with the *Lactobacillus fermentum* in a concentration of at least 10^7 CFU/g and with a starter culture,

(b) fermenting until a pH of 4.6 is reached, and;

(2) storing the fermented milk product at 7±1°C for 14 days;

(3) adding 200 µl of 4N H_2S0_4 to 1 g of the fermented milk product and determining the concentration of acetaldehyde by static head space gas chromatography.

Acetaldehyde is a taste component produced by lactic acid bacteria during fermentation. While the component is desirable in certain applications, it would be advantageous to reduce or avoid the presence of acetaldehyde in other applications. *Lactobacillus fermentum* bacteria reducing the concentration of acetaldehyde in a fermented milk product therefore provide advantages in specific applications, for example when preparing sweetened or mild yoghurt. The *Lactobacillus fermentum* strains of the present invention may for example reduce the concentration of acetaldehyde produced by a starter culture during fermentation in a fermented milk product by at least 75%, at least 95% or at least 98%.

The *Lactobacillus fermentum* strains of the present invention can for example be characterized in that they have the ability to reduce the concentration of acetaldehyde produced by a starter culture during fermentation in a fermented milk product by at least 50%, wherein the starter culture used for the preparation of the fermented milk product comprises LAB which are able to produce acetaldehyde in a concentration of 3 ppm or more. For example, the assay may be based using a starter culture comprising *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*. Respective mixtures are frequently used for the production of yoghurt and known to produce acetaldehyde.

Bacteria of the present invention may advantageously be derived from one of the following deposited strains:

(a) the *Lactobacillus fermentum* strain CHCC12798 as deposited at German Collection of Microorganisms and Cell Cultures (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH; DSMZ), Inhoffenstr. 7B, D-38124 Braunschweig under the accession No.: 32084;

(b) the *Lactobacillus fermentum* strain CHCC12797 as deposited at German Collection of Microorganisms and Cell Cultures (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH; DSMZ), Inhoffenstr. 7B, D-38124 Braunschweig under the accession No.: 32085;

(c) the *Lactobacillus fermentum* strain CHCC14591 as deposited at German Collection of Microorganisms and Cell Cultures (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH; DSMZ), Inhoffenstr. 7B, D-38124 Braunschweig under the accession No.: 32086;

(d) the *Lactobacillus fermentum* strain CHCC14588 as deposited at German Collection of Microorganisms and Cell Cultures (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH; DSMZ), Inhoffenstr. 7B, D-38124 Braunschweig under the accession No.: 32087;

(e) the *Lactobacillus fermentum* strain CHCC15844 as deposited at German Collection of Microorganisms and Cell Cultures (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH; DSMZ), Inhoffenstr. 7B, D-38124 Braunschweig under the accession No.: 32088;

(f) the *Lactobacillus fermentum* strain CHCC15865 as deposited at German Collection of Microorganisms and Cell Cultures (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH; DSMZ), Inhoffenstr. 7B, D-38124 Braunschweig under the accession No.: 32089;

(g) the *Lactobacillus fermentum* strain CHCC15847 as deposited at German Collection of Microorganisms and Cell Cultures (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH; DSMZ), Inhoffenstr. 7B, D-38124 Braunschweig under the accession No.: 32090;

(h) the *Lactobacillus fermentum* strain CHCC15848 as deposited at German Collection of Microorganisms and Cell Cultures (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH; DSMZ), Inhoffenstr. 7B, D-38124 Braunschweig under the accession No.: 32091;

(i) the *Lactobacillus fermentum* strain CHCC15926 as deposited at German Collection of Microorganisms and Cell Cultures (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH; DSMZ), Inhoffenstr. 7B, D-38124 Braunschweig under the accession No.: 32096;

(j) the *Lactobacillus fermentum* strain CHCC2008 as deposited at German Collection of Microorganisms and Cell Cultures (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH; DSMZ), Inhoffenstr. 7B, D-38124 Braunschweig under the accession No.: 22584;

(k) a mutant strain obtainable from one the deposited bacteria according to (a) to (j), wherein the mutant has the ability to reduce the concentration of acetaldehyde produced by a starter culture during fermentation in a fermented milk product by at least 50%.

In the context of the present application, the term "lactic acid bacteria" or "LAB" is used to refer to food-grade bacteria producing lactic acid as the major metabolic end-product of carbohydrate fermentation. These bacteria are related by their common metabolic and physiological characteristics and are usually Gram positive, low-GC, acid tolerant, non-sporulating, non-respiring, rod-shaped bacilli or cocci. During the fermentation stage, the

consumption of lactose by these bacteria causes the formation of lactic acid, reducing the pH and leading to the formation of a protein coagulum. These bacteria are thus responsible for the acidification of milk and for the texture of the dairy product. As used herein, the term "lactic acid bacteria" encompasses, but is not limited to, bacteria belonging to the genus of *Lactobacillus* spp., *Bifidobacterium* spp., *Streptococcus* spp., *Lactococcus* spp., such as *Lactobacillus delbrueckii subsp. bulgaricus*, *Streptococcus thermophilus*, *Lactobacillus lactis*, *Bifidobacterium animalis*, *Lactococcus lactis*, *Lactobacillus paracasei*, *Lactobacillus plantarum*, *Lactobacillus helveticus*, *Lactobacillus acidophilus*, *Bifidobacterium breve* and *Leuconostoc* spp.

Depending on the optimum temperature for propagation, LAB are characterized as mesophilic or thermophilic LAB. The term "mesophile" refers to microorganisms that thrive best at moderate temperatures. The term "mesophilic fermentation" herein refers to fermentation at a temperature between about 22°C and about 35°C. The term "mesophilic fermented milk product" refers to fermented milk products prepared by mesophilic fermentation of a mesophilic starter culture and include such fermented milk products as buttermilk, sour milk, cultured milk, smetana, sour cream and fresh cheese, such as quark, tvarog and cream cheese. The industrially most useful mesophilic bacteria include *Lactococcus* spp. and *Leuconostoc* spp.

The term "thermophile" refers to microorganisms that thrive best at high temperatures. The term "thermophilic fermentation" refers to fermentation methods carried out at a temperature between about 35°C and about 45°C. The term "thermophilic fermented milk product" refers to fermented milk products prepared by thermophilic fermentation using a thermophilic starter culture and include such fermented milk products as set-yoghurt, stirred-yoghurt, strained yoghurt and drinking yoghurt. The industrially most useful thermophilic bacteria include *Streptococcus* spp. and *Lactobacillus* spp.

As will be outlined below, the present invention encompasses methods using mesophilic and thermophilic fermentation.

The terms "inhibit" in relation to fungi, yeasts and moulds refers to a decrease in the growth or sporulation or a reduction in the number or in the concentration of fungi, yeasts and moulds, for example in food products and/or on the surface of food products comprising the bacteria of the present invention in relation to food products which do not comprise such bacteria. The extent of inhibition provided by the *Lactobacillus fermentum* bacteria of the present invention is preferably determined by growth on agar solidified fermented milk in the presence and absence of the *Lactobacillus fermentum* bacteria.

In the present context, the term "mutant" should be understood as a strain derived from a strain of the invention by means of e.g. genetic engineering, radiation and/or chemical treatment. It is preferred that the mutant is a functionally equivalent mutant, e.g. a mutant that has substantially the same, or improved, properties in particular in relation to the effect of reducing acetaldehyde, as the deposited strain. Such a mutant is a part of the present invention. Especially, the term "mutant" refers to a strain obtained by subjecting a strain of the invention to any conventionally used mutagenization treatment including treatment with a chemical mutagen such as ethane methane sulphonate (EMS) or N-methyl-N'-nitro-N-nitroguanidine (NTG), UV light or to a spontaneously occurring mutant. A mutant may have been subjected to several mutagenization treatments (a single treatment should be understood one mutagenization step followed by a screening/selection step), but it is presently preferred that no more than 20, or no more than 10, or no more than 5, treatments (or screening/selection steps) are carried out. In a presently preferred mutant, less than 5%, or less than 1% or even less than 0.1% of the nucleotides in the bacterial genome have been shifted with another nucleotide, or deleted, compared to the mother strain.

The use of the terms "a" and "an" and "the" and similar referents in the context of describing the invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context.

The present invention further provides compositions comprising at least one bacterium of the species *Lactobacillus fermentum* with the ability to reduce the concentration of acetaldehyde produced by a starter culture during fermentation in a fermented milk product by at least 50%.

Respective compositions may comprise numerous further bacteria including LABs. A preferred composition of the present invention is therefore characterized in that the composition further comprises at least one further bacterium selected from one or more of the following genera and species *Lactobacillus* spp., *Bifidobacterium* spp., *Streptococcus* spp., *Lactococcus* spp., such as *Lactobacillus delbrueckii subsp. bulgaricus*, *Streptococcus thermophilus*, *Lactobacillus lactis*, *Bifidobacterium animalis*, *Lactococcus lactis*, *Lactobacillus paracasei*, *Lactobacillus plantarum*, *Lactobacillus helveticus*, *Lactobacillus acidophilus*, *Bifidobacterium breve* and *Leuconostoc* spp.

In a particularly preferred embodiment, the compositions of the present invention comprise at least one bacterium of the species *Lactobacillus fermentum* with the ability to reduce the

concentration of acetaldehyde produced by a starter culture during fermentation in a fermented milk product by at least 50%. In one embodiment, several different strains of the *Lactobacillus fermentum* bacteria with the ability to reduce the concentration of acetaldehyde produced by a starter culture during fermentation in a fermented milk product by at least 50% are combined. Alternatively, these further bacteria can for example be selected from:

(a) *Lactobacillus rhamnosus* bacterium of strain CHCC15860 as deposited with the German Collection of Microorganisms and Cell Cultures (DSMZ) under accession No. DSM32092;

(b) *Lactobacillus rhamnosus* bacterium of strain CHCC5366 as deposited with the German Collection of Microorganisms and Cell Cultures (DSMZ) under accession No. DSM23035;

(c) *Lactobacillus rhamnosus* bacterium of strain CHCC12697 as deposited with the German Collection of Microorganisms and Cell Cultures (DSMZ) under accession No. DSM24616;

(d) *Lactobacillus paracasei* bacterium of strain CHCC12777 as deposited with the German Collection of Microorganisms and Cell Cultures (DSMZ) under accession No. DSM24651; and

(e) *Lactobacillus paracasei* bacterium of strain CHCC14676 as deposited with the German Collection of Microorganisms and Cell Cultures (DSMZ) under accession No. DSM25612.

The compositions of the present invention may in addition comprise numerous further components, including one or more cryoprotective compounds as well as flavoring compounds.

LAB are most commonly added in the form of a starter culture to milk. The term "starter" or "starter culture" as used in the present context refers to a culture of one or more food-grade micro-organisms, in particular lactic acid bacteria, which are responsible for the acidification of the milk base. Starter cultures may be fresh, but are most frequently frozen or freeze-dried. These products are also known as "Direct Vat Set" (DVS) cultures and are produced for direct inoculation of a fermentation vessel or vat for the production of a dairy product, such as a fermented milk product or a cheese. Respective starter cultures are commercially available from numerous sources and include F-DVS YoFlex Mild 2.0, F-DVS YF-L901, FD-DVS YF-812 and F-DVS CH-1, three cultures commercially available from Chr. Hansen containing mixtures of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*.

In one aspect the present invention therefore provides compositions in the form of a solid frozen or freeze-dried starter culture comprising lactic acid bacteria in a concentration of at least 10^9 colony forming units per g of frozen material or in a concentration of at least 10^{10} colony forming units per g of frozen material or in a concentration of at least 10^{11} colony forming units per g of frozen material which compositions include a bacterium of the species *Lactobacillus fermentum* with the ability to reduce the concentration of acetaldehyde produced by a starter culture during fermentation in a fermented milk product by at least 50% as described above.

In a further embodiment the present invention provides methods of producing a fermented milk product which comprise adding the *Lactobacillus fermentum* bacterium with the ability to reduce the concentration of acetaldehyde produced by a starter culture during fermentation in a fermented milk product by at least 50% as described above or the composition comprising the same to milk or to a milk product and fermenting the mixture at a temperature between about 22°C and about 43°C until a pH of less than 4.6 is reached.

In the context of the present application, the term "milk" is broadly used in its common meaning to refer to liquids produced by the mammary glands of animals or by plants. In accordance with the present invention the milk may have been processed and the term "milk" includes whole milk, skim milk, fat-free milk, low fat milk, full fat milk, lactose-reduced milk, or concentrated milk. Fat-free milk is non-fat or skim milk product. Low-fat milk is typically defined as milk that contains from about 1% to about 2% fat. Full fat milk often contains 2% fat or more. The term "milk" is intended to encompass milks from different mammals and plant sources. Mammal sources of milk include, but are not limited to cow, sheep, goat, buffalo, camel, llama, mare and deer. Plant sources of milk include, but are not limited to, milk extracted from soy bean, pea, peanut, barley, rice, oat, quinoa, almond, cashew, coconut, hazelnut, hemp, sesame seed and sunflower seed. In the methods and products of the present invention, milk derived from cows is most preferably used as a starting material for the fermentation.

The term "milk" also includes fat-reduced and/or lactose-reduced milk products. Respective products can be prepared using methods well known in the art and are commercially available (for example from Select Milk Producers Inc., Texas, USA). Lactose-reduced milk can be produced according to any method known in the art, including hydrolyzing the lactose by lactase enzyme to glucose and galactose, or by nanofiltration, electrodialysis, ion exchange chromatography and centrifugation.

The term "milk product" or "milk base" is broadly used in the present application to refer to a composition based on milk or milk components which can be used as a medium for growth and fermentation of LAB. The milk product or base comprises components derived from milk and any other component that can be used for the purpose of growing or fermenting LAB.

The fermentation step of the process for manufacturing fermented dairy products comprises the addition of LAB to milk. Fermentation processes used in production of dairy products are well known and a person of ordinary skill can select fermentation process conditions, including temperature, oxygen, amount and characteristics of microorganism(s) and fermentation time.

Prior to fermentation, the milk substrate may be homogenized and pasteurized according to methods known in the art. "Homogenizing" as used herein means intensive mixing to obtain a soluble suspension or emulsion. If homogenization is performed prior to fermentation, it may be performed so as to break up the milk fat into smaller sizes so that it no longer separates from the milk. This may be accomplished by forcing the milk at high pressure through small orifices. "Pasteurizing" as used herein means treatment of the milk substrate to reduce or eliminate the presence of live organisms, such as microorganisms. Preferably, pasteurization is attained by maintaining a specified temperature for a specified period of time. The specified temperature is usually attained by heating. The temperature and duration may be selected in order to kill or inactivate certain bacteria, such as harmful bacteria. A rapid cooling step may follow.

In a particularly advantageous method of the present invention the *Lactobacillus fermentum* bacterium with the ability to reduce the concentration of acetaldehyde produced by a starter culture during fermentation in a fermented milk product by at least 50% as described above or the composition comprising the same is added to milk or to a milk product and the mixture is fermented in such a manner that;

(a) the concentration of the *Lactobacillus fermentum* bacteria reducing the concentration of acetaldehyde is at least $1x10^6$ cfu/g or at least $1x10^7$ cfu/g at the termination of fermentation in the fermented milk product; and/or

(b) such that the concentration of the *Lactobacillus fermentum* bacteria reducing the concentration of acetaldehyde is at least $1x10^5$ cfu/cm^2 on the surface of the fermented milk product.

This way of proceeding has the advantage that the effect of the *Lactobacillus fermentum* bacterium on acetaldehyde reduction can be fully used.

One way of achieving the concentration is using a method of producing a fermented milk product, wherein the parameters for fermentation are maintained such that the concentration of the *Lactobacillus fermentum* bacteria described above increases during fermentation. Using conventional starter cultures and conditions for fermentation (as described in the Examples) will generally increase the concentration of the *Lactobacillus fermentum* bacteria described above during fermentation by at least 0.5 log. Alternatively, the parameters for fermentation are maintained such that the concentration of the *Lactobacillus fermentum* bacteria described above does not significantly decrease, for example does not decrease by more than 30%, not more than 25%, or not more than 20% during fermentation and storage.

The invention further provides methods of producing a food, feed or pharmaceutical product obtainable by a method of producing a fermented milk product as described above and the food, feed or pharmaceutical product obtainable by this method.

Fermentation is carried out to produce food products, feed products or pharmaceuticals. The terms "fermented milk product", "food" or "feed" product refer to products obtainable by the fermentation methods of the present invention and include cheese, yoghurt, fruit yoghurt, yoghurt beverage, strained yoghurt (Greek yoghurt, Labneh), quark, fromage frais and cream cheese. The term food further encompasses other fermented food products, including fermented meat, such as fermented sausages, and fermented fish products.

The term "cheese" is understood to encompass any cheese, including hard, semi-hard and soft cheeses, such as cheeses of the following types: Cottage, Feta, Cheddar, Parmesan, Mozzarella, Emmentaler, Danbo, Gouda, Edam, Feta-type, blue cheeses, brine cheeses, Camembert and Brie. The person skilled in the art knows how to convert the coagulum into cheese, methods can be found in the literature, see e.g. Kosikowski, F. V., and V. V. Mistry, "Cheese and Fermented Milk Foods", 1997, 3rd Ed. F. V. Kosikowski, L. L. C. Westport, CT. As used herein, a cheese which has a NaCl concentration below 1.7% (w/w) is referred to as a "low-salt cheese".

In the context of the present application, the term "yoghurt" refers to products comprising *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus* and optionally other microorganisms such as *Lactobacillus delbrueckii subsp. lactis*, *Bifidobacterium animalis subsp. lactis*, *Lactococcus lactis*, *Lactobacillus acidophilus* and *Lactobacillus paracasei*, or any microorganism derived therefrom. The lactic acid strains other than *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus*, are included to give the finished product various properties, such as the property of promoting the equilibrium of the flora. As used herein, the term "yoghurt" encompasses set yoghurt, stirred yoghurt, drinking yoghurt,

Petit Suisse, heat treated yoghurt, strained or Greek style yoghurt characterized by a high protein level and yoghurt-like products.

In particular, term "yoghurt" encompasses, but is not limited to, yoghurt as defined according to French and European regulations, e.g. coagulated dairy products obtained by lactic acid fermentation by means of specific thermophilic lactic acid bacteria only (i.e. *Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus thermophilus*) which are cultured simultaneously and are found to be live in the final product in an amount of at least 10 million CFU (colony-forming unit) / g. Yoghurts may optionally contain added dairy raw materials (e.g. cream) or other ingredients such as sugar or sweetening agents, one or more flavouring(s), fruit, cereals, or nutritional substances, especially vitamins, minerals and fibers, as well as stabilizers and thickeners. Optionally the yoghurt meets the specifications for fermented milks and yoghurts of the AFNOR NF 04-600 standard and/or the codex StanA-lla-1975 standard. In order to satisfy the AFNOR NF 04-600 standard, the product must not have been heated after fermentation and the dairy raw materials must represent a minimum of 70% (m/m) of the finished product.

In a further embodiment the present invention provides food, feed or pharmaceutical products comprising one or more bacteria of the species *Lactobacillus fermentum* with the ability to reduce the concentration of acetaldehyde produced by a starter culture during fermentation in a fermented milk product by at least 50% as described above and one or more of:

(a) least one further bacterium selected from one or more of the following genera *Lactococcus* spp., *Streptococcus* spp., *Lactobacillus* spp., *Leuconostoc* spp., *Pseudoleuconostoc* spp., *Pediococcus* spp., *Brevibacterium* spp. and *Enterococcus* spp.;

(b) *Lactobacillus rhamnosus* bacterium of strain CHCC15860 as deposited with the German Collection of Microorganisms and Cell Cultures (DSMZ) under accession No. DSM32092;

(c) *Lactobacillus rhamnosus* bacterium of strain CHCC5366 as deposited with the German Collection of Microorganisms and Cell Cultures (DSMZ) under accession No. DSM23035;

(d) *Lactobacillus rhamnosus* bacterium of strain CHCC12697 as deposited with the German Collection of Microorganisms and Cell Cultures (DSMZ) under accession No. DSM24616;

(e) *Lactobacillus paracasei* bacterium of strain CHCC12777 as deposited with the German Collection of Microorganisms and Cell Cultures (DSMZ) under accession No. DSM24651; and

(f) *Lactobacillus paracasei* bacterium of strain CHCC14676 as deposited with the German Collection of Microorganisms and Cell Cultures (DSMZ) under accession No. DSM25612.

DESCRIPTION OF THE FIGURES

Figure 1: Acetaldehyde levels after storage at 7±1°C for 14 days in fermented milk products fermented with starter culture alone (Reference), or starter cultures in combination with Lb. fermentum strains. LOD: Limit of detection. LOQ: Limit of quantification.

Figure 2: Acetaldehyde levels after storage at 7±1°C for 14 days in fermented milk products fermented with starter culture alone (Reference), or starter cultures in combination with Lb. fermentum CHCC14591. LOD: Limit of detection. LOQ: Limit of quantification.

Figure 3: Acidification curves of four commercial starter cultures, FD-DVS YF-L812, F-DVS YF-L901, F-DVS YoFlex Mild 2.0 and F-DVS CH-1, grown in milk (1% fat and 4.5% protein) at 43°C.

Figure 4: Post-acidification curves of yoghurt fermented with one of four commercial starter cultures, FD-DVS YF-L812, F-DVS YF-L901, F-DVS YoFlex Mild 2.0 and F-DVS CH-1 after storage at 6°C for up to 43 days.

Figure 5: Acetaldehyde levels after storage at 7±1°C for 14 days in fermented milk products fermented with starter culture, FD DVS YF-L812 or F-DVS CH-1, alone (Reference), or starter cultures in combination one of the nine *Lb. fermentum* strains. LOD: Limit of detection. LOQ: Limit of quantification.

Example 1:

Effect of the ten *Lb. fermentum* strains on acetaldehyde content

Ten *Lb. fermentum* strains were tested for their ability to lower acetaldehyde content.

Reduced-fat (1.5% w/v) homogenized milk was heat-treated at 90±1°C for 20 min and cooled immediately. A commercial starter culture (F-DVS YF-L901 Yo-Flex®) was inoculated at 0.02% (v/w), and the inoculated milk was distributed into 200 ml bottles. Ten bottles were inoculated with the *Lb. fermentum* strains in concentrations of 1 x 10^7 CFU/g and one bottle was used as a reference and only inoculated with the starter culture. All bottles were

incubated in a water bath at 43±1°C and fermented at these conditions until pH of 4.60±0.1 was reached. After fermentation, the bottles were vigorously shaken to break the coagulum and cooled on ice. The bottles were stored at 7±1°C for 14 days.

On day 14 samples were analyzed for acetaldehyde by static head space gas chromatography (HSGC), a sensitive method for analyzing volatiles in complex matrices. The setup consisted of a Static Head Space sampler connected to Gas Chromatograph with Flame Ionization Detector (FID). For that purpose the following equipment was used:

HS-autosampler: HS40XI, TurboMatrix 110, Perkin Elmer.
HS-software: HSControl v.2.00, Perkin Elmer.
GC: Autosystem XL, Perkin Elmer.
GC-software: Turbochrom navigator, Perkin Elmer.
Column: HP-FFAP 25 m x 0.20 mm x 0.33 µιη, Agilent Technologies

Standards of known concentration were used to determine response factors (calibration), controls were used to control that the used response factors were stable within an analytical series as well as in-between series and over time (months). Concentration of volatiles (ppm) in samples and controls was determined using response factors coming from standards. Samples were prepared by adding 200 µl of 4N H_2S0_4 to 1 g yoghurt sample and immediately analyzed by HSGC.

The results are illustrated in Figure 1 and show that each of the strains *Lb. fermentum* CHCC12798, *Lb. fermentum* CHCC12797, *Lb. fermentum* CHCC14591, *Lb. fermentum* CHCC14588, *Lb. fermentum* CHCC15844, *Lb. fermentum* CHCC15865, *Lb. fermentum* CHCC15847, *Lb. fermentum* CHCC15848, *Lb. fermentum* CHCC15926, and *Lb. fermentum* CHCC2008 has the ability to reduce the concentration of acetaldehyde produced by a starter culture during fermentation in a fermented milk product.

Example 2:

Effect of one *Lb. fermentum* strain on acetaldehyde content

One *Lb. fermentum* strain was tested for the ability to lower acetaldehyde content.

Reduced-fat (1.5% w/v) homogenized milk was heat-treated at 90±1°C for 20 min and cooled immediately. A commercial starter culture (F-DVS YoFlex Mild 2.0) was inoculated at 0.02% (v/w), and the inoculated milk was distributed into two 200 ml bottles. One bottle was inoculated with the *Lb. fermentum* strains in concentrations of 1 x 10^7 CFU/g and one bottle

was used as a reference and only inoculated with the starter culture. Both bottles were incubated in a water bath at 43±1°C and fermented at these conditions until pH of 4.60±0.1 was reached. After fermentation, the bottles were vigorously shaken to break the coagulum and cooled on ice. The bottles were stored at 7±1°C for 14 days.

On day 14 samples were analyzed for acetaldehyde by static head space gas chromatography (HSGC), a sensitive method for analyzing volatiles in complex matrices. The setup consisted of a Static Head Space sampler connected to Gas Chromatograph with Flame Ionization Detector (FID). For that purpose the following equipment was used:

HS-autosampler: HS40XI, TurboMatrix 110, Perkin Elmer.
HS-software: HSControl v.2.00, Perkin Elmer.
GC: Autosystem XL, Perkin Elmer.
GC-software: Turbochrom navigator, Perkin Elmer.
Column: HP-FFAP 25 m x 0.20 mm x 0.33 µιη, Agilent Technologies

Standards of known concentration were used to determine response factors (calibration), controls were used to control that the used response factors were stable within an analytical series as well as in-between series and over time (months). Concentration of volatiles (ppm) in samples and controls was determined using response factors coming from standards. Samples were prepared by adding 200 µl of 4N H_2S0_4 to 1 g yoghurt sample and immediately analyzed by HSGC.

The results are illustrated in Figure 2 and show that *Lb. fermentum 14591* has the ability to reduce the concentration of acetaldehyde produced by a starter culture during fermentation in a fermented milk product.

Example 3:

Functional analysis of commercial starter starter cultures

The three commercial starter cultures included herein were chosen based on their different acidification profiles. Three were frozen, F-DVS CH-1, F-DVS YoFlex Mild 2.0 and F-DVS YF-L901, and one was freeze dried, FD-DVS YF-L812. To test the difference in acidification profiles, semi fat milk was standardized to 1% fat and 4.5% protein with skim milk powder and heat-treated at 85±1°C for 30 min and cooled immediately. One of four different commercial starter cultures (F-DVS CH-1, F-DVS YoFlex Mild 2.0, F-DVS YF-L901 or FD-DVS YF-L812) was inoculated at 0.02% (v/w), and the inoculated milk was distributed into 200 ml bottles. The bottles were incubated in a water bath at 43±1°C and fermented under these

conditions until pH 4.5 was reached. The pH was measured continually throughout the fermentation. Subsequently, the bottles were stored at 6°C for 43 for days and pH was measured with intervals of 7 days to determine the level of post-acidification.

The acidification profiles of the three commercial starter cultures, F-DVS CH-1, F-DVS YoFlex Mild 2.0, F-DVS YF-L901 and FD-DVS YF-L812, are shown in Figure 3. F-DVS CH-1 showed fast fermentation time reaching pH 4.55 in 4.87 hours. F-DVS YoFlex Mild 2.0 showed intermediate fermentation time reaching pH 4.55 in 5.29 hours. FD-DVS YF-L812 and F-DVS YF-L901 showed slower fermentation reaching pH 4.55 in 6.45 and 5.87 hours, respectively. Post-acidification profiles showed very low levels of post-acidification for FD-DVS YF-L812 and F-DVS YoFlex Mild 2.0 (ΔpH=0.12 and ΔpH=0.11 after storage at 6°C for 43 days, respectively), intermediate levels of post-acidification for F-DVS YF-L901 (ΔpH=0.26 after storage at 6°C for 43 days and high degree of post-acidification for F-DVS CH-1 (ΔpH=0.55 after storage at 6°C for 43 days) (Figure 4).

Example 4:

Effect of the nine *Lb. fermentum* strains on acetaldehyde content when fermented with two different starter cultures

Nine *Lb. fermentum* strains were tested for their ability to lower acetaldehyde content.

Reduced-fat (1.5% w/v) homogenized milk was heat-treated at 90±1°C for 20 min and cooled immediately. Milk was inoculated with one of two commercial starter cultures (F-DVS CH-1 or FD-DVS YF-L812) at 0.02% (v/w), and the inoculated milk was distributed into 200 ml bottles. Nine bottles were inoculated with the *Lb. fermentum* strains in concentrations of 1 x 10^7 CFU/g and one bottle inoculated with each starter culture was used as a reference and only inoculated with the starter culture. All bottles were incubated in a water bath at 43±1°C and fermented at these conditions until pH of 4.55±0.1 was reached. After fermentation, the bottles were vigorously shaken to break the coagulum and cooled on ice. The bottles were stored at 7±1°C for 14 days.

The tested *Lb. fermentum* strains were: *Lb. fermentum* CHCC12798, *Lb. fermentum* CHCC12797, *Lb. fermentum* CHCC14591, *Lb. fermentum* CHCC14588, *Lb. fermentum* CHCC15844, *Lb. fermentum* CHCC15865, *Lb. fermentum* CHCC15847, *Lb. fermentum* CHCC15926, and *Lb. fermentum* CHCC2008.

On day 14 samples were analyzed for acetaldehyde by static head space gas chromatography (HSGC), a sensitive method for analyzing volatiles in complex matrices. The setup consisted

of a Static Head Space sampler connected to Gas Chromatograph with Flame Ionization Detector (FID). For that purpose the following equipment was used:

HS-autosampler: HS40XI, TurboMatrix 110, Perkin Elmer.
HS-software: HSControl v.2.00, Perkin Elmer.
GC: Autosystem XL, Perkin Elmer.
GC-software: Turbochrom navigator, Perkin Elmer.
Column: HP-FFAP 25 m x 0.20 mm x 0.33 µιη, Agilent Technologies

Standards of known concentration were used to determine response factors (calibration), controls were used to control that the used response factors were stable within an analytical series as well as in-between series and over time (months). Concentration of volatiles (ppm) in samples and controls was determined using response factors coming from standards. Samples were prepared by adding 200 µl of 4N H_2SO_4 to 1 g yoghurt sample and immediately analyzed by HSGC.

The results are illustrated in Figure 5 and show that each of the strains *Lb. fermentum* CHCC12798, *Lb. fermentum* CHCC12797, *Lb. fermentum* CHCC14591, *Lb. fermentum* CHCC14588, *Lb. fermentum* CHCC15844, *Lb. fermentum* CHCC15865, *Lb. fermentum* CHCC15847, *Lb. fermentum* CHCC15926, and *Lb. fermentum* CHCC2008 has the ability to reduce the concentration of acetaldehyde produced by a starter culture during fermentation in a fermented milk product.

REFERENCES

1. **Tamime, A. Y., and H. C. Deeth.** 1980. Yoghurt: technology and biochemistry. J. Food Prot. 43:939-977.

2. A. C. S. D. Chaves, M. Fernandez, A. L. S. Lerayer, I. Mierau, M. Kleerebezem, and J. Hugenholtz, 2002, Metabolic Engineering of Acetaldehyde Production by *Streptococcus thermophilus*

3. **Lees, G. J., and G. R. Jago.** 1976. Formation of acetaldehyde from threonine by lactic acid bacteria. J. Dairy Res. 43:75-83.

DEPOSITS and EXPERT SOLUTION

The applicant requests that a sample of the deposited micro-organisms stated below may only be made available to an expert, until the date on which the patent is granted.

The *Lactobacillus fermentum* strain CHCC12798 was deposited at German Collection of Microorganisms and Cell Cultures (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH; DSMZ), Inhoffenstr. 7B, D-38124 Braunschweig deposited on 2015-07-16 under the accession No.: 32084.

The *Lactobacillus fermentum* strain CHCC12797 was deposited at German Collection of Microorganisms and Cell Cultures (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH; DSMZ), Inhoffenstr. 7B, D-38124 Braunschweig deposited on 2015-07-16 under the accession No.: 32085.

The *Lactobacillus fermentum* strain CHCC14591 was deposited at German Collection of Microorganisms and Cell Cultures (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH; DSMZ), Inhoffenstr. 7B, D-38124 Braunschweig deposited on 2015-07-16 under the accession No.: 32086.

The *Lactobacillus fermentum* strain CHCC14588 was deposited at German Collection of Microorganisms and Cell Cultures (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH; DSMZ), Inhoffenstr. 7B, D-38124 Braunschweig deposited on 2015-07-16 under the accession No.: 32087.

The *Lactobacillus fermentum* strain CHCC15844 was deposited at German Collection of Microorganisms and Cell Cultures (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH; DSMZ), Inhoffenstr. 7B, D-38124 Braunschweig deposited on 2015-07-16 under the accession No.: 32088.

The *Lactobacillus fermentum* strain CHCC15865 was deposited at German Collection of Microorganisms and Cell Cultures (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH; DSMZ), Inhoffenstr. 7B, D-38124 Braunschweig deposited on 2015-07-16 under the accession No.: 32089.

The *Lactobacillus fermentum* strain CHCC15847 was deposited at German Collection of Microorganisms and Cell Cultures (Deutsche Sammlung von Mikroorganismen und

Zellkulturen GmbH; DSMZ), Inhoffenstr. 7B, D-38124 Braunschweig deposited on 2015-07-16 under the accession No.: 32090.

The *Lactobacillus fermentum* strain CHCC15848 was deposited at German Collection of Microorganisms and Cell Cultures (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH; DSMZ), Inhoffenstr. 7B, D-38124 Braunschweig deposited on 2015-07-16 under the accession No.: 32091.

The *Lactobacillus fermentum* strain CHCC15926 was deposited at German Collection of Microorganisms and Cell Cultures (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH; DSMZ), Inhoffenstr. 7B, D-38124 Braunschweig deposited on 2015-07-22 under the accession No.: 32096.

The *Lactobacillus fermentum* strain CHCC2008 was deposited at German Collection of Microorganisms and Cell Cultures (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH; DSMZ), Inhoffenstr. 7B, D-38124 Braunschweig deposited on 2009-05-19 under the accession No.: 22584.

The *Lactobacillus rhamnosus* strain CHCC15860 was deposited at German Collection of Microorganisms and Cell Cultures (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH; DSMZ), Inhoffenstr. 7B, D-38124 Braunschweig deposited on 2015-07-16 under the accession No.: 32092.

The deposits were made according to the Budapest treaty on the international recognition of the deposit of microorganisms for the purposes of patent procedure.

CLAIMS

1. Bacterium of the species *Lactobacillus fermentum* according, wherein the bacterium has the ability to reduce the concentration of acetaldehyde produced by a starter culture during fermentation in a fermented milk product by at least 50%.

2. Bacterium of the species *Lactobacillus fermentum* according to claim 1, wherein the bacterium has the ability to reduce the concentration of acetaldehyde produced by a starter culture during fermentation in a fermented milk product by at least 75%, at least 95% or at least 98%, and wherein the concentration of acetaldehyde is determined in an assay comprising:

   (1) preparing a fermented milk product by:
      (a) inoculating a milk with the *Lactobacillus fermentum* in a concentration of at least 10^7 CFU/g and with a starter culture,
      (b) fermenting until a pH of 4.6 is reached, and;
   (2) storing the fermented milk product at 7±1°C for 14 days;
   (3) adding 200 µl of 4N H_2S0_4 to 1 g of the fermented milk product and determining the concentration of acetaldehyde by static head space gas chromatography.

3. Bacterium of the species *Lactobacillus fermentum* according to claims 1 or 2, wherein the starter culture used for the preparation of the fermented milk product comprises LAB which are able to produce acetaldehyde in a concentration of 3 ppm or more.

4. Bacterium of the species *Lactobacillus fermentum* according to any one of claims 1 to 3, wherein the starter culture comprises *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*.

5. Bacterium of the species *Lactobacillus fermentum* according to any one of claims 1 to 4, wherein the bacterium is selected from the group consisting of:

   (a) the *Lactobacillus fermentum* strain deposited as DSM32084;
   (b) the *Lactobacillus fermentum* strain deposited as DSM32085;
   (c) the *Lactobacillus fermentum* strain deposited as DSM32086;
   (d) the *Lactobacillus fermentum* strain deposited as DSM32087;
   (e) the *Lactobacillus fermentum* strain deposited as DSM32088;
   (f) the *Lactobacillus fermentum* strain deposited as DSM32089;

(g) the *Lactobacillus fermentum* strain deposited as DSM32090;

(h) the *Lactobacillus fermentum* strain deposited as DSM32091;

(i) the *Lactobacillus fermentum* strain deposited as DSM32096;

(j) the *Lactobacillus fermentum* strain deposited as DSM22584; or

(k) a mutant strain obtainable from one the deposited bacteria according to (a) to (j), wherein the mutant has the ability to reduce the concentration of acetaldehyde produced by a starter culture during fermentation in a fermented milk product by at least 50%.

6. Composition comprising at least one *Lactobacillus fermentum* strain according to any one of claims 1 to 5.

7. The composition according to claim 6, wherein the composition further comprises at least one further bacterium selected from:

(a) *Lactobacillus rhamnosus* bacterium of strain CHCC15860 as deposited with the German Collection of Microorganisms and Cell Cultures (DSMZ) under accession No. DSM32092;

(b) *Lactobacillus rhamnosus* bacterium of strain CHCC5366 as deposited with the German Collection of Microorganisms and Cell Cultures (DSMZ) under accession No. DSM23035;

(c) *Lactobacillus rhamnosus* bacterium of strain CHCC12697 as deposited with the German Collection of Microorganisms and Cell Cultures (DSMZ) under accession No. DSM24616;

(d) *Lactobacillus paracasei* bacterium of strain CHCC12777 as deposited with the German Collection of Microorganisms and Cell Cultures (DSMZ) under accession No. DSM24651; and

(e) *Lactobacillus paracasei* bacterium of strain CHCC14676 as deposited with the German Collection of Microorganisms and Cell Cultures (DSMZ) under accession No. DSM25612.

8. Composition according to claim 6 or 7, further comprising at least one cryoprotective compound.

9. Composition according to any one of claims 6 to 8, wherein the composition is a solid frozen or freeze dried starter culture comprising lactic acid bacteria in a concentration of at least 10^9 colony forming units per g of frozen material or in a concentration of at

least 10^{10} colony forming units per g of frozen material or in a concentration of at least 10^{11} colony forming units per g of frozen material.

10. Method of producing a fermented milk product comprising adding a *Lactobacillus fermentum* bacterium according to any one of claims 1 to 5 or the composition according to any one of claims 6 to 9 to milk or to a milk product and fermenting the mixture at a temperature between about 22°C and about 43°C until a pH of less than 4.6 is reached.

11. The method according to claim 10, comprising adding a *Lactobacillus fermentum* bacterium according to any one of claims 1 to 5 or the composition according to any one of claims 6 to 9 to milk or to a milk product and fermenting the mixture

(a) such that the concentration of the *Lactobacillus fermentum* bacteria of claim 1 is at least $1x10^6$ cfu/g or at least $1x10^7$ cfu/g at the termination of fermentation in the fermented milk product; and/or

(b) such that the concentration of the *Lactobacillus fermentum* bacteria of claim 1 is at least $1x10^5$ cfu/cm^2 on the surface of the fermented milk product.

12. Method of producing a food, feed or pharmaceutical product comprising a method of producing a fermented milk product according to claim 10 or 11.

13. Food, feed or pharmaceutical product obtainable by a method of claim 12.

14. Food, feed or pharmaceutical product according to claim 12, comprising *Lactobacillus fermentum* bacteria according to any one of claims 1 to 9 in a concentration of at least 10^7 CFU/g, including a concentration of 10^7 CFU/g to 10^{11} CFU/g, 10^7 CFU/g to 10^{10} CFU/g and 10^7 CFU/g to 10^9 CFU/g.

15. Food, feed or pharmaceutical product comprising a bacterium of the species *Lactobacillus fermentum* selected according to one of claims 1 to 6 and one or more of:

(a) least one further bacterium selected from one or more of the following genera *Lactococcus* spp., *Streptococcus* spp., *Lactobacillus* spp., *Leuconostoc* spp., *Pseudoleuconostoc* spp., *Pediococcus* spp., *Brevibacterium* spp. and *Enterococcus* spp.;

(b) *Lactobacillus rhamnosus* bacterium of strain CHCC15860 as deposited with the German Collection of Microorganisms and Cell Cultures (DSMZ) under accession No. DSM32092;

(c) *Lactobacillus rhamnosus* bacterium of strain CHCC5366 as deposited with the German Collection of Microorganisms and Cell Cultures (DSMZ) under accession No. DSM23035;

(d) *Lactobacillus rhamnosus* bacterium of strain CHCC12697 as deposited with the German Collection of Microorganisms and Cell Cultures (DSMZ) under accession No. DSM24616;

(e) *Lactobacillus paracasei* bacterium of strain CHCC12777 as deposited with the German Collection of Microorganisms and Cell Cultures (DSMZ) under accession No. DSM24651; and

(f) *Lactobacillus paracasei* bacterium of strain CHCC14676 as deposited with the German Collection of Microorganisms and Cell Cultures (DSMZ) under accession No. DSM25612.

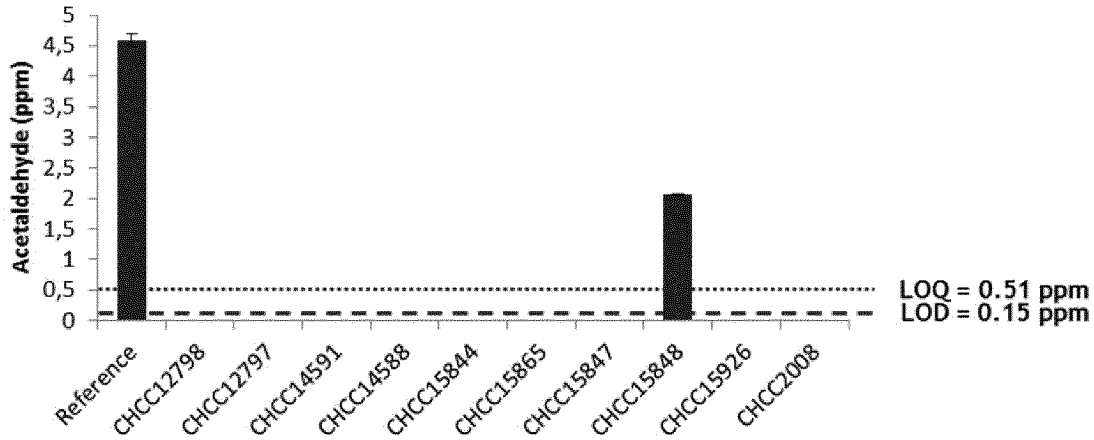
Acetaldehyde (ppm)
5
4,5
4
3,5
3
2,5
2
1,5
1
0,5
0
LOQ = 0.51 ppm
LOD = 0.15 ppm
Reference
CHCC12798
CHCC12797
CHCC14591
CHCC14588
CHCC15844
CHCC15865
CHCC15847
CHCC15848
CHCC15926
CHCC2008

Figure 1

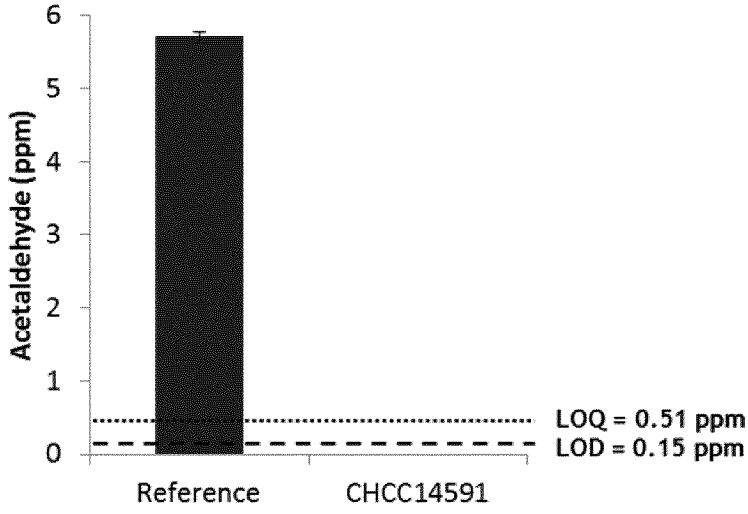
Acetaldehyde (ppm)
6
5
4
3
2
1
0
LOQ = 0.51 ppm
LOD = 0.15 ppm
Reference
CHCC14591

Figure 2

Figure 3

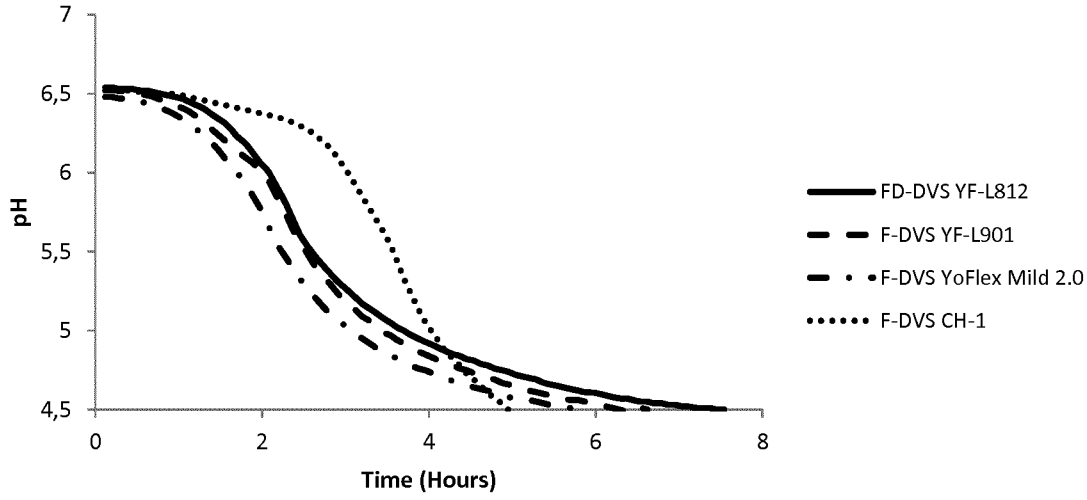
7
6,5
6
5,5
5
4,5
pH
0
2
4
6
8
Time (Hours)
FD-DVS YF-L812
F-DVS YF-L901
F-DVS YoFlex Mild 2.0
F-DVS CH-1

Figure 4

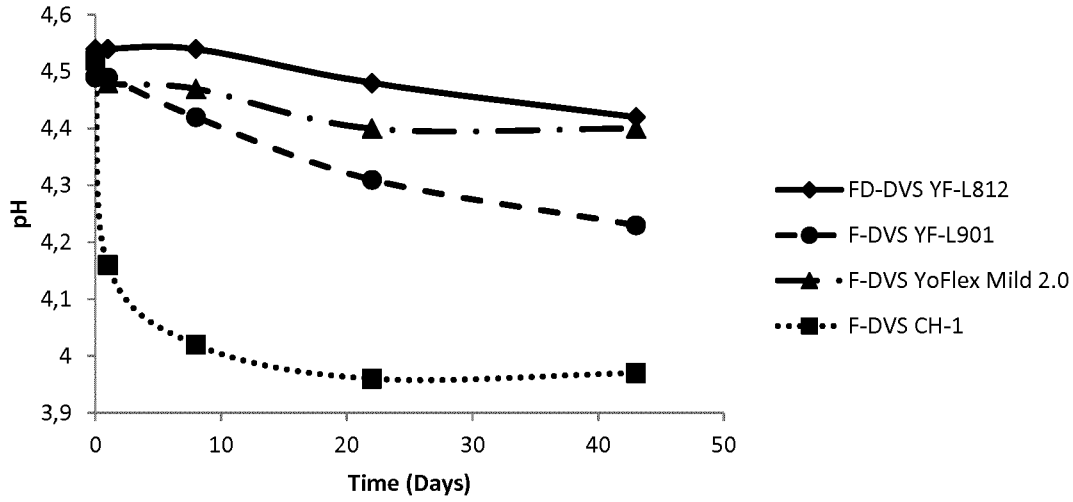
4,6
4,5
4,4
4,3
4,2
4,1
4
3,9
pH
0
10
20
30
40
50
Time (Days)
FD-DVS YF-L812
F-DVS YF-L901
F-DVS YoFlex Mild 2.0
F-DVS CH-1

Figure 5


Acetaldehyde (ppm)
0
2
4
6
8
10
12
CH-1 (Reference)
CH-1 + CHCC12798
CH-1 + CHCC12797
CH-1 + CHCC14591
CH-1 + CHCC14588
CH-1 + CHCC15844
CH-1 + CHCC15865
CH-1 + CHCC15847
CH-1 + CHCC15926
CH-1 + CHCC2008
LOQ = 0.51 ppm
LOD = 0.15 ppm
Acetaldehyde (ppm)
0
2
4
6
8
10
12
YF-L812 (Reference)
YF-L812 + CHCC12798
YF-L812 + CHCC12797
YF-L812 + CHCC14591
YF-L812 + CHCC14588
YF-L812 + CHCC15844
YF-L812 + CHCC15865
YF-L812 + CHCC15847
YF-L812 + CHCC15926
YF-L812 + CHCC2008
LOQ = 0.51 ppm
LOD = 0.15 ppm

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2016/070408

A. CLASSIFICATION OF SUBJECT MATTER
INV. A23C9/123 C12R1/225
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)
A23C C12R

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, BIOSIS, EMBASE, WPI Data, FSTA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2011/000879 A2 (CHR HANSEN AS [DK]; FOLKENBERG DITTE MARIE [DK]; POULSEN LONE [DK]) 6 January 2011 (2011-01-06)	1-6,8-15
Y	claim 21; examples 1, 2; tables 1, 2	7

Y	WO 2012/136830 A1 (CHR HANSEN AS [DK]; HORNBAEK TINA [DK]; LISBERG MAIKE [DK]; DIEMER SIL) 11 October 2012 (2012-10-11) claims	7

☐ Further documents are listed in the continuation of Box C.

☒ 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" document which may throw doubts on priority claim(s) or which 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
21 October 2016	31/10/2016

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	Smeets, Dieter

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2016/070408

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2011000879 A2	06-01-2011	BR PI1015366 A2	01-09-2015
		CN 102469804 A	23-05-2012
		EA 201270095 A1	29-06-2012
		EP 2448420 A2	09-05-2012
		JP 2012531189 A	10-12-2012
		KR 20120107451 A	02-10-2012
		UA 106381 C2	26-08-2014
		US 2012107451 A1	03-05-2012
		US 2014348980 A1	27-11-2014
		WO 2011000879 A2	06-01-2011
WO 2012136830 A1	11-10-2012	AR 085943 A1	06-11-2013
		BR 112013025912 A2	20-09-2016
		CN 103596435 A	19-02-2014
		EA 201391500 A1	28-02-2014
		EP 2693885 A1	12-02-2014
		JP 2014512178 A	22-05-2014
		KR 20140026468 A	05-03-2014
		US 2014093487 A1	03-04-2014
		WO 2012136830 A1	11-10-2012