



GRAS Notice (GRN) No. 502

<http://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/default.htm>

ORIGINAL SUBMISSION

000001

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February 4, 2014

Dr. Paulette Gaynor
Office of Food Additive Safety, GRAS Notification Program (HFS-255)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835

Re: GRAS Notice-Exemption Claim for *Lactobacillus acidophilus* La-14

Dear Dr Gaynor:

On behalf of my client, Danisco USA, Inc., please accept the attached documentation, in compliance with the GRAS notification procedure set out in the April 17, 1997 Federal Register (62 FR 18937), as submission of notice of a GRAS exemption claim for the above referenced substance, i.e. use in food of *Lactobacillus acidophilus* La-14. As specified in the aforementioned proposed rule, this GRAS notice is submitted in triplicate with each document being comprised of a GRAS notice exemption claim; detailed information on the notified substance; and an appendix containing further referenced and substantiating information on the substance.

Please promptly contact me should you have any question regarding the submitted notice. I look forward to receiving acknowledgment of receipt of this notice and to a timely response regarding the noticed substance. Thank you.

Sincerely,

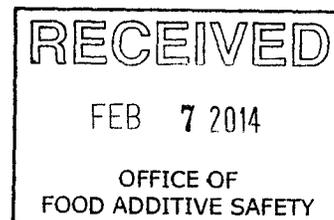
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Robert H. Sindt

Enc.

Cc : Amy Smith, Ph.D., Danisco USA, Inc.

RHS/bs



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**GENERALLY RECOGNIZED
AS SAFE NOTICE**

***Lactobacillus acidophilus* La-14**

February 2014

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February 4, 2014

Dr. Paulette Gaynor
Office of Food Additive Safety, GRAS Notification Program (HFS-255)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, Maryland 20740-3835

Re: GRAS Notice-Exemption Claim for *Lactobacillus acidophilus* La-14

Dear Dr. Gaynor:

On behalf of my client, DuPont, doing business as Danisco USA, Inc. (Danisco), and in accordance with FDA's proposed rule of April 17, 1997 (62 FR 18938) relating to the filing of generally recognized as safe (GRAS) notices, please accept this claim and the attached information, submitted in triplicate, for the purpose of complying with those requirements to establish a GRAS notice exemption claim relating to the use of *Lactobacillus acidophilus* La-14 in certain foods. Specifically, Danisco has determined that use of *Lactobacillus acidophilus* La-14 as an ingredient in foods, including ready-to-eat breakfast cereals; bars (e.g. breakfast, energy, nutrition); milk, milk drinks (e.g. flavored milks), milk products (e.g. butter), fermented milks (e.g. Kefir, sour cream, buttermilk), yogurt, cheese (incl. cheese food, cheese spreads) and ice cream; soy drinks and soy products; bottled water and teas; dry beverages including sports nutrition beverages; fruit juices, fruit nectars, fruit "ades", fruit drinks, jams and jellies; chewing gum; medical foods; nut and peanut spreads; margarines; snack foods (e.g. cookies, crackers, chips, granola); meal replacements; sauces, condiments; confections (e.g. bars, candy, coatings, drops, cookie filling), but excluding infant formula (all as specified in the detailed information submitted herewith), is GRAS based on scientific procedures and, as such is exempt from the premarket approval requirements of the Federal Food, Drug and Cosmetic Act. In conformity with the requirements outlined in the proposed rule and subsequent agency guidance, the following information is included with this exemption claim:

(i) Name and Address of the Notifier:

Danisco USA, Inc.
3329 Agricultural Drive
Madison, WI 53716

(ii) Common or Usual Name of Notified Substance: *Lactobacillus acidophilus* La-14

- (iii) **Applicable Conditions of Use:** *Lactobacillus acidophilus* La-14 is manufactured in compliance with current Good Manufacturing Practice as specified in 21 CFR Part 110. *Lactobacillus acidophilus* La-14 is manufactured through a specific time and temperature controlled fermentation of safe and suitable food grade ingredients with *Lactobacillus acidophilus* La-14. It is used as an ingredient in foods, including ready-to-eat breakfast cereals; bars (e.g. breakfast, energy, nutrition); milk, milk drinks (e.g. flavored milks), milk products (e.g. butter), fermented milks (e.g. Kefir, sour cream, buttermilk), yogurt, cheese (incl. cheese food, cheese spreads) and ice cream; soy drinks and soy products; bottled water and teas; dry beverages including sports nutrition beverages; fruit juices, fruit nectars, fruit “ades”, fruit drinks, jams and jellies; chewing gum; medical foods; nut and peanut spreads; margarines; snack foods (e.g. cookies, crackers, chips, granola); meal replacements; sauces, condiments; confections (e.g. bars, candy, coatings, drops, cookie filling); but excluding infant formula; at levels not to exceed current good manufacturing practice in accordance with 21 CFR 184.1(b). The targeted use level of foods will be to typically provide at least 1×10^9 cfu of *Lactobacillus acidophilus* La-14/250g serving of food at the time of consumption. All population age groups, except infants, are expected to consume these foods.
- (iv) **Basis for the GRAS Determination:** Scientific procedures.
- (v) **Availability to FDA of Data and Information that are Basis of Determination:** The data and information forming the basis for Danisco’s GRAS determination and the exemption claim asserted herein are available for FDA review and copying during reasonable business hours at the following address, or will be sent to FDA upon request:

Robert H. Sindt, Attorney at Law
Suite 110G
1250 Thomas Jefferson Street, NW
Washington, DC 20007
Phone: (202) 466-4500
rsindt@bobsindtlaw.com

Consequently, on the basis of the above specified information, and the additional requested information specified in the proposed rule and attached and made a part hereof and submitted with this letter, please accept this as Danisco’s GRAS notification and claim of exemption from the statutory premarket approval requirements for the use of *Lactobacillus acidophilus* La-14 as an ingredient in foods, including ready-to-eat breakfast cereals; bars (e.g. breakfast, energy, nutrition); milk, milk drinks (e.g. flavored milks), milk products (e.g. butter), fermented milks (e.g. Kefir, sour cream, buttermilk), yogurt, cheese (incl. cheese food, cheese spreads) and ice cream; soy drinks and soy products; bottled water and teas; dry beverages including sports nutrition beverages; fruit juices, fruit nectars, fruit “ades”, fruit drinks, jams and jellies; chewing gum; medical foods; nut and peanut spreads; margarines; snack foods (e.g. cookies, crackers,

Dr. Paulette Gaynor, OFAS-FDA
February 4, 2014
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chips, granola); meal replacements; sauces, condiments; confections (e.g. bars, candy, coatings, drops, cookie filling); but excluding infant formula. Should you have any questions regarding the submission of this notice, please contact me at the above number. Thank you for your prompt consideration of, and response to, this notice.

Sincerely,

(b) (6)

Robert H. Sindt

RHS:bs

Attachments

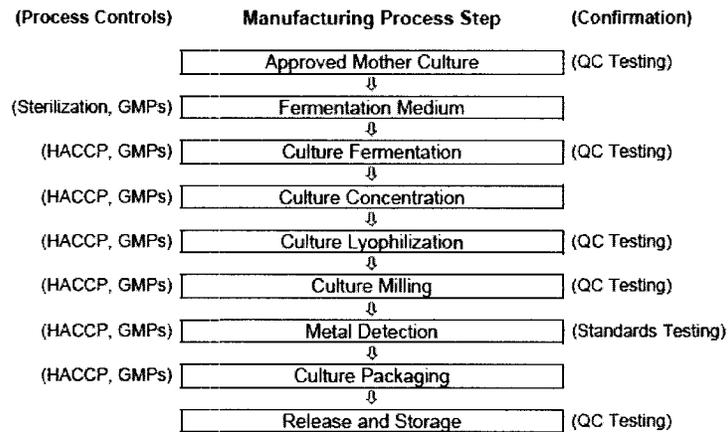
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***L. acidophilus* La-14--GRAS NOTICE INFORMATION**

(2) DETAILED INFORMATION ABOUT THE IDENTITY OF THE NOTIFIED SUBSTANCE

- Common and Usual Name of the Food Grade Substance: *Lactobacillus acidophilus*
LA-14
- Chemical Name for *Lactobacillus acidophilus* La-14: None
- Chemical Abstract Service (CAS) Registry Number for *Lactobacillus acidophilus* La-14: None
- Empirical Formula for *Lactobacillus acidophilus* La-14: None
- Structural Formula for *Lactobacillus acidophilus* La-14: None
- Quantitative Composition for *Lactobacillus acidophilus* La-14: *Lactobacillus acidophilus* La-14 is a commercially available food ingredient that is produced by culture fermentation utilizing *Lactobacillus acidophilus* La-14 as the source organism. Use in foods will be targeted to typically contain 1×10^9 cfu of *L. acidophilus* La-14/250g serving of food at time of consumption.
- Method of Manufacture for *Lactobacillus acidophilus* La-14: *Lactobacillus acidophilus* La-14 is manufactured in compliance with the U.S. Food and Drug Administration's current Good Manufacturing Practice guidelines, as specified in 21

CFR, part 110, and manufactured in FDA regulated and inspected facilities. All ingredients utilized are food grade or approved for use by the FDA. The manufacturing process is summarized below:



The source organism used is *L. acidophilus* La-14. The cultures are maintained in the culture bank of Danisco USA Inc. as frozen 1ml. vials at -80°C. Danisco USA Inc. independently verifies the identity of each organism. Each seed lot in the culture bank is fully characterized to insure the identity of the seed strains. From the seed vials, Danisco USA Inc. produces concentrated starter for the industrial fermentation.

The product is manufactured through a specific time and temperature controlled fermentation of safe and suitable food grade ingredients with *L. acidophilus* La-14. Prior to addition of *L. acidophilus* La-14, the mixture is sterilized and cooled to an incubation temperature of 37° C. The mixture is then inoculated with *L. acidophilus* La-14 and allowed to incubate to the fermentation endpoint under constant temperature.

After the required incubation period, the pH is adjusted with ammonium hydroxide, and concentrated via centrifugation. To the concentrated bacterial slurry, food-grade cryoprotectants are added, the material is frozen, and subsequently freeze-dried. The dried cultured product is packaged, stored in a cool, dry environment, and then tested for stability on a regular basis prior to use to insure deliverability of a target amount of live culture throughout shelf life of a final food product.

Release of product for sale according to established specifications is under the responsibility of Danisco Quality Control. Final product testing methods comply with standard Methods for the Examination of Dairy Products of the American Public Health Association. Examples of the Danisco Product Description and a representative Certificate of Analysis for La-14 are attached in the Appendix.

- Source Information for *L. acidophilus* La-14: The genus *Lactobacillus* is a wide and heterogeneous taxonomic unit, comprising the rod-shaped lactic acid bacteria. This genus encompasses a diverse group with a large variety of phenotypic, biochemical and physiological properties (1; 6; 16). Several species are intentionally introduced in the food chain, being involved in a range of food and feed fermentations, and also applied as dietary supplements for humans and animals. They are rod-shaped, non-motile and non-spore forming bacteria. Phylogenetic molecular taxonomy, 16S rRNA gene sequence, and comparative genomic analysis are used for assigning strains to particular species within this genus (2; 7; 9; 10).

Lactobacillus acidophilus is a homofermentative species, fermenting sugars into lactic acid, and occurs naturally as resident flora of the intestinal tracts of human and animals (1; 5). The taxonomy of *L. acidophilus* has undergone significant revisions over the past

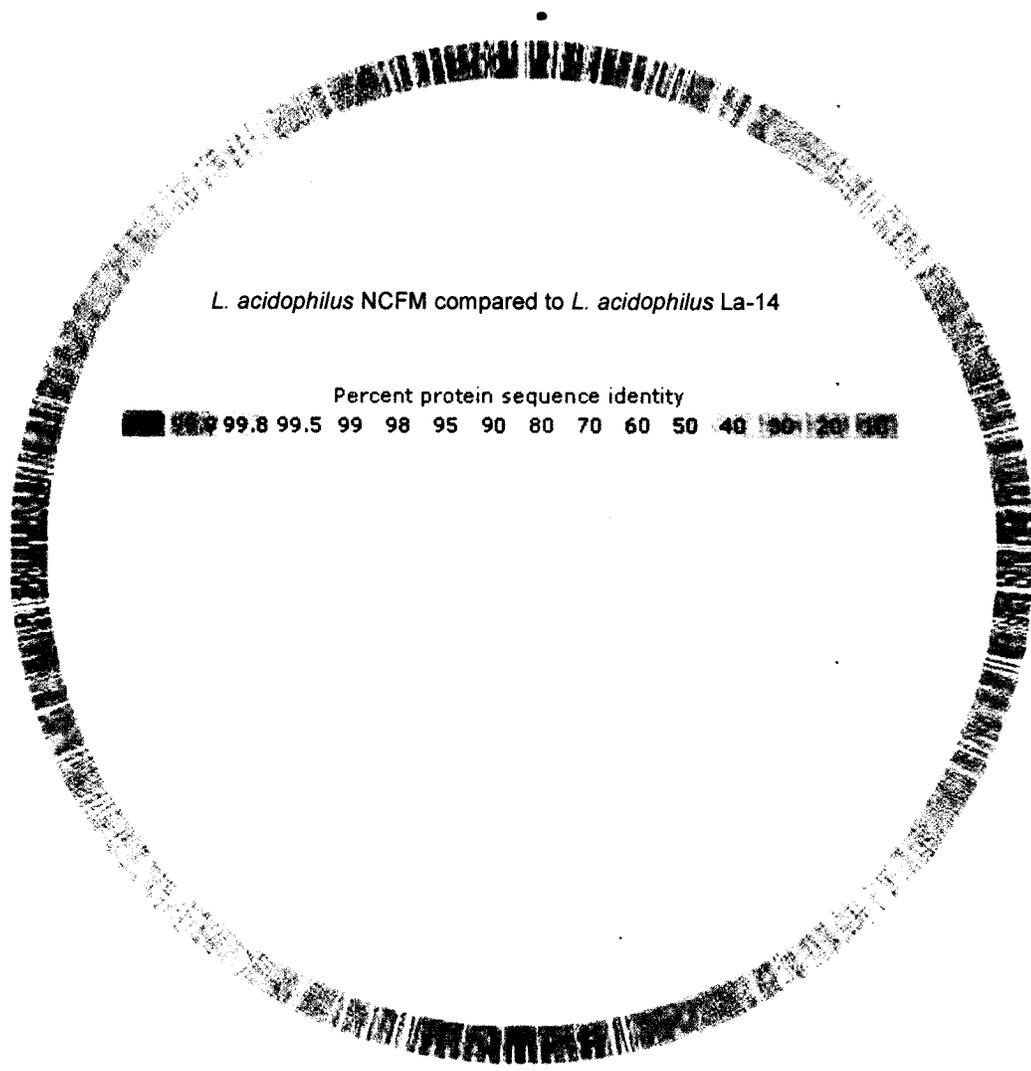
Nucleotide sequence comparison demonstrated that NCFM[®] and La-14 DNA congruently have a 34.7% GC ratio (8; 10). Barrangou et al. established that the nucleotide sequence of strain La14 contains a high level of similarity, with very few genetic differences in this *Lactobacillus acidophilus* strain compared to the NCFM[®] strain. However, the genomes are not identical, and a deletion in the La-14 genome had initially been detected when partial genome sequence information was reported. This demonstrated at least one genomic distinction between the strains. This La-14 deletion was characterized as a 416 base pair deletion in gene La14_1146, which is believed to encode for “ABC-type multidrug transport system, ATPase and permease components” (11). This study referenced that ABC transporters in *L. acidophilus* NCFM[®] are believed to be involved in the import of particular carbohydrates such as raffinose, fructooligosaccharides and maltose. Correspondingly, a deletion in this genetic region in La-14 may inhibit import function and may have an effect on carbohydrate utilization as compared to NCFM[®]. This demonstrates differences among the strains La-14 and NCFM[®] in regard to propagation and fermentation profiles, but should not affect safety.

The genome of La-14 has been completed and publically deposited at the National Center for Biotechnology Information under accession number CP_005926 (11). Direct comparison of NCFM[®] and La-14 revealed 16 single base pair INDELS, of which 14 predicatively cause frameshifts. Of the 95 SNPs discovered, 47 of them are non-

synonymous, and 29 occur in intergenic regions. A total of 52 genes are possibly affected by these minor changes, although the genomes are 99.9% similar at the sequence level.

Figure 1. Comparative analysis of *L. acidophilus* NCFM[®] and La-14 genomes

Alignment of *L. acidophilus* genomes demonstrated that La-14 and NCFM are extremely similar.



- **Characteristic Properties of *L. acidophilus* La-14:** *L. acidophilus* La-14 is a harmless lactic acid producing bacteria. Commercially, it is produced by fermentation utilizing common safe and suitable culturing food grade ingredients with *L. acidophilus* La-14, a safe and suitable bacterium. In powdered form, it has a white to cream color and is typically stored at or below 4°C.

- **Content of Potential Human Toxicants for *L. acidophilus* La-14:** None

- **Specifications for Food Grade *L. acidophilus* La-14:** *L. acidophilus* La-14 is a freeze dried powder produced by culture fermentation utilizing *L. acidophilus* La-14. *L. acidophilus* La-14 microbiological specifications /kg are:

Cell count	Greater than 1.5E+11 /g
Non-lactic Count	Less than 5000 /g
Enterococci	Less than 100 /g
Coliforms	Less than 10 /g
E. coli	Negative by test (less than .03 /g)
Salmonella	Negative (40 g enrichment)
Staphylococcus (C.P.)	Negative by test (less than 10 /g)
Listeria	Negative (25 g enrichment)

Also, note that La-14 complies with The Food Allergen Labeling and Consumer Protection Act (FALCPA) of 2004, and Section 201 of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 321). There are no allergens included in fermentation or formulation of *Lactobacillus acidophilus* strain La-14. Danisco USA, Inc. has removed all potential sources of allergens from fermentation media of all microbial cultures, including La-14. See Appendix for the La-14 Product Description, for allergen declaration table.

L. acidophilus La-14--GRAS NOTICE INFORMATION

(3) INFORMATION ON SELF-LIMITING LEVELS OF USE, IF ANY

- Uses are self-limited to those foods that can sustain living *L. acidophilus* for the shelf life of the food.

L. acidophilus LA-14--GRAS NOTICE INFORMATION

(4) DETAILED SUMMARY OF THE BASIS FOR GRAS DETERMINATION

- (i) Danisco's determination, that the notified uses of *L. acidophilus* La-14 (as an ingredient in food, including in ready-to-eat breakfast cereals; bars (e.g. breakfast, energy, nutrition); milk, milk drinks (e.g. flavored milks), milk products (e.g. butter), fermented milks (e.g. Kefir, sour cream, buttermilk), yogurt, cheese (incl. cheese food, cheese spreads) and ice cream; soy drinks and soy products; bottled water and teas; dry beverages including sports nutrition beverages; fruit juices, fruit nectars, fruit "ades", fruit drinks, jams and jellies; chewing gum; medical foods; nut and peanut spreads; margarines; snack foods (e.g. cookies, crackers, chips, granola); meal replacements; sauces, condiments; confections (e.g. bars, candy, coatings, drops, cookie filling); but excluding infant formula) are exempt from the premarket approval requirements of the Federal Food, Drug and Cosmetic Act based on its determination that such uses are GRAS, is based on scientific procedures. The determination has been confirmed by an independent panel of scientific experts convened by Danisco to conduct such a critical review. Each member of the independent expert panel was qualified by extensive scientific training and experience to evaluate the safety of substances used in food. The independent expert panel's report and determinations, dated October 2013, is included in its entirety in the Appendix attached hereto.

(A) **Safety of *L. acidophilus* La-14**

L. acidophilus La-14 is produced by a fermentation process utilizing safe and suitable culturing food grade ingredients with seed strains of independently identified *L. acidophilus* La-14 organisms, a safe and suitable lactic acid producing bacterium.

(1) Safety and Suitability of Organism. In conducting its assessment and making its determination, Danisco reviewed the regulatory status, in-vitro, animal and human studies, human use information, and other published and unpublished studies and information relating to *L. acidophilus* La-14.

FDA, EU and scientific consensus on safety of *L. acidophilus*

When considering the safety of cultures, the major issues that need to be assessed are pathogenicity, toxicity, and the presence of transferable antibiotic resistance genes. Data from in-vitro, animal and human studies were considered, although no animal models for assessment of safety of *Lactobacillus* have been validated (17). Species of the Genus *Lactobacillus* are considered to be non-pathogenic, non-toxicogenic and have generally been considered safe for use in foods (EFSA, see Appendix). Boriello, et al. (18) reviewed data pertinent to safety concerns for lactobacilli and concluded that “current evidence suggests that the risk of infection with probiotic lactobacilli or bifidobacteria is similar to that of infection with commensal strains, and that consumption of such products presents a negligible risk to consumers...”. This opinion is echoed in other publications (17; 19). Additionally, the species *L. acidophilus* has been recognized by FDA as a

harmless lactic acid producing bacteria used in certain foods, and it is also proposed for inclusion on the EU QPS list (Appendix). Specifically, FDA states: “Prior sanctions were granted for the use of harmless lactic acid producing bacteria, such as *Lactobacillus acidophilus*, as optional ingredients in specified standardized foods. These bacteria are permitted for use in cultured milk (which includes buttermilk) (§ 131.12), sour cream (§ 131.160), cottage cheese (§ 133.128), and yogurt (§ 131.200), provided that the mandatory cultures of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* are also used in the yogurt.” (Partial List Of Microorganisms And Microbial-Derived Ingredients That Are Used In Foods; U. S. Food and Drug Administration Center for Food Safety & Applied Nutrition Office of Food Additive Safety, July 2001).” Further, FDA has indicated it has had no questions to GRAS notices filed with the agency relating to the use of several *L. acidophilus* strains in various foods. See GRN 000171; GRN 000357; and GRN 000378.

Production of biogenic amines

Histamine: In lactic acid bacteria, production of histamine results from the catabolism of histidine by a histidine decarboxylase. A specific detection method for histidine decarboxylase genes has been developed internally to Danisco based on the scientific literature and on the most updated genomic databases. Applied to La-14, the method failed to detect a histidine decarboxylase gene. Consequently, La-14 is unlikely to produce histamine.

Tyramine: In lactic acid bacteria, production of tyramine results from the catabolism of tyrosine by a tyrosine decarboxylase. A specific detection method for tyrosine decarboxylase genes has been developed internally to Danisco based on the scientific literature and on the most updated genomic databases. Applied to La-14 the method failed to detect a tyrosine decarboxylase gene. Consequently, La-14 is unlikely to produce tyramine.

Safety Studies (Note that because of the similarity (~99.98%) of the nucleotide sequence of the genomes of La-14 and NCFM®, studies involving both strains of *L. acidophilus* were considered and believed to be relevant to the determination.)

In-Vitro Studies

Tunnoli *et al.*

This study (20) focus was to evaluate the oxalate-degrading activity in several *Lactobacillus* species, surveying those strains widely used in probiotic dairy and pharmaceutical products. *L. acidophilus* La-14 was found to produce significant oxalyl-CoA decarboxylase and formyl-CoA transferase, suggesting the potential use of this strain in oxalate catabolism and to provide the potential benefit to individuals suffering from an increased body burden of oxalate and oxalate associated disorders.

Lammers *et al.*

Peripheral blood mononuclear cells (PBMC) from healthy donors were incubated with pure DNA of eight probiotic strains, including *L. acidophilus* La-14. Total bacterial DNA was collected from feces pre- and post-exposure to probiotics.

The study demonstrates the immunological effect of probiotic DNA on PBMCs of

individuals with varying intestinal flora. The in-vitro effect demonstrated the immune response differences in individuals pre- and post- exposure to a probiotic mixture containing La-14. No adverse events were reported in this study, as represented in the “Human Studies” section below (21).

Animal studies

Luo et al.

This study (22) showed the effect of live La-14 and spent culture supernatant (from La-14) on mice intestinal flora pre-treated with ampicillin and antibiotic associated diarrhea. Results show that live and cell supernatant helps to regulate intestinal microflora. No adverse effects resulted.

Patruica et al.

Study (23) shows the effect of feeding bee colonies with a prebiotic and probiotic honey syrup mixture containing La-14. While this is not a study directly applicable to animal studies in the traditional sense, it does demonstrate the effect of the microflora and the health of the bee population in addition to showing lack of toxicity to this important class of insects.

Wagner *et al.*

This study (24) was conducted with two types of gnotobiotic, immunocompromised mice: bg/bg-nu/nu/+ (produce thymus-matured T-cells, euthymic) and bg/bg-nu/nu (athymic). This beige nude mouse model has defects in phagocytic cells and NK cell activity and lacks a functional thymus. In this study, mice, (male and female, adult and neonatal) were inoculated with one of the following strains; *L. acidophilus* NCFM[®], *L. reuteri*, *L. rhamnosus* GG or

Bifidobacterium animalis by swabbing oral cavity and anal area with culture of 10^8 /ml to create monoassociated mice (germ-free animals, colonized with only one strain). This resulted in colonization of the stomach, small and large intestine. Subsequent generations of mice colonized via exposure to monoassociated mothers and feces. Result of this study showed:

- no morbidity or mortality in adult and neonatal mice:
- no adverse effects on growth parameters:
- no growth or microscopic pathological changes:
- no abscesses in the stomach or small intestine:
- no evidence of inflammation or other pathologic findings:
- no deaths in gnotobiotic, immunocompromised mice:
- evidence of induction of immunoglobulins, IgM and IgG.

Daniel *et al.*

The safety of the *L. acidophilus* NCFM[®] was also evaluated in a colitis mouse using Trinitrobenzenesulphonic acid (TNBS) to induce colitis. In healthy mice, intra-gastric administration of *L. acidophilus* NCFM[®] did not show any potential adverse effect on mouse activity, weight and colon inflammation. In TNBS-treated mice (mice with very strong colitis), no significant improvement was observed in the group fed *L. acidophilus* NCFM[®]. High doses (10^{10} cfu) of *L. acidophilus* NCFM[®] led to no translocation of the organism or abnormal translocation of the intestinal microflora (25).

Human studies

No human studies have been reported with the express purpose of conducting a safety assessment. However, NCFM[®] and/or La-14 have been administered to healthy and diseased patients in the following studies, with no reported adverse incidents related to the treatment protocol:

1. Chronic kidney failure patients with small bowel bacterial overgrowth.

Administered to 22 patients (N=43 total in two arms) at 2×10^{10} /d in the form of enteric coated capsules (26). In some subjects, *L. acidophilus* NCFM[®] was co-administered with *L. gasseri* BGF204. For both treatments, dimethylamine (DMA) production and the generation of the corresponding carcinogen, nitrosodimethyl-amine (NDMA), was reduced. Changes in patients' weight and % weight gain suggested that *L. acidophilus* can improve nutrition. Hydrogen generation in the small bowel was reduced suggesting that *L. acidophilus* had some effect on altering the resident flora. Twice daily treatment with NCFM[®] and/or BGF204 produced no significant side effects in any of the patients over their course of treatment.

2. Mild to moderate symptom irritable bowel syndrome patients. *L. acidophilus* NCFM[®] was administered as part of a 2-strain blend along with *Bifidobacterium lactis* Bi-07 in patients with non-constipation IBS, functional diarrhea, or functional bloating. A total of 30 subjects were in the treatment group and consumed 10^{11} cfu of each strain daily. Significant improvement of symptoms of bloating and distention was achieved (27).

3. Feeding of 10 healthy subjects NCFM[®] 10¹⁰/d in non-fat dry milk. NCFM[®] was isolated from the feces during feeding (28).
4. Ten healthy subjects were fed 10¹⁰/d in milk or water. No difference in fecal isolation of viable NCFM[®] was observed (29).
5. The ability of NCFM[®] to alleviate symptoms of 20 lactose maldigesting children (5-16 yr) was tested in a single blinded study. Symptoms and breath hydrogen excretion were evaluated after consumption of milk with or without 10¹⁰ cfu total NCFM[®] (11.6g lactose) (30). No difference in breath hydrogen was observed, but NCFM[®] did improve symptoms.
6. Three additional studies were conducted with NCFM[®] to evaluate its impact on improvement of lactose digestion in lactose maldigesters. Kim and Gilliland (31) reported three feeding trials. In the first, subjects were fed milk (5 ml/kg body weight/bid for 6 days) with (2.5x10⁸/ml) or without NCFM[®] (N=6 healthy subjects/group). (Since milk was consumed on a per body weight basis, exact total daily dose could not be calculated from data provided in the paper; however, for a 68 kg person, the dose would have been 1.7x10¹¹ cfu/d.)

A similar second feeding trial tested lower doses of NCFM (2.5x10⁷/ml or 2.5x10⁶/ml for 6 days).

A third trial evaluated 2.5x10⁶/ml for 6 days on 5 new subjects. Improved breath hydrogen excretion was observed for the low and high, but not the middle, doses of NCFM[®] tested. A total of 17 subjects consumed NCFM[®] in the three trials reported in this study. Lin et al. (32) evaluated the impact of

NCFM* (10^7 or 10^8 cfu/ml) – along with other *L. acidophilus* strains and *S. thermophilus* plus *L. bulgaricus* - on breath hydrogen. Ten healthy lactose maldigesters were each fed 9 test meals on sequential days with 1 day between meals. Therefore, NCFM* was only fed for two days to each of 10 subjects. NCFM* did not positively impact breath hydrogen at either dose. Lastly, a study by Savaiano et al. (33) conducted a study with 9 lactose maldigesters fed 420g milk + NCFM* (1.1×10^7 /ml; 420 ml milk consumed) on one day. Consistent with the Lin et al. (32) study, this product did not improve lactose digestion.

7. Healthy volunteers (N=20 in the treatment group) taking antibiotics were fed blend of 5 strains for 20 days with no adverse incidents reported (34). NCFM* was fed at levels of 5×10^9 .
8. Healthy male volunteers (N=21, 22 or 23; publication states “4 or 5 per group”) were fed milk containing different levels of NCFM* and levels of lactobacilli were evaluated in fecal samples obtained before, during and after feeding in three separate feeding trials (35). The dose fed ranged from 2.4×10^9 /d to 3.8×10^{11} /d. No adverse incidents were reported from feeding. Increased levels of facultative lactobacilli were observed during feeding, especially in subjects with low baseline levels. [Gilliland et al. (5) evaluated methods to detect NCFM* in human feces, but this study did not report a separate feeding trial.]
9. Healthy volunteers (Goldin and Gorbach (36) found that daily consumption of milk containing NCFM* (10^9 /d; N=21 healthy volunteers) for 4 weeks resulted

in a 2- to 4-fold reduction in the activity β -glucuronidase, nitroreductase, and azoreductase in the feces. Similar results on fecal activity of β -glucuronidase and nitroreductase were observed when NCFM was added to the omnivorous diet of human subjects (37). Goldin and Gorbach (38) fed 7 healthy subjects on a standard “Western diet” ~ 10^{10} /d NCFM* for 1 month. A one-month run in and wash out period were conducted as well. Reductions in fecal activities of β -glucuronidase and nitroreductase were observed.

10. Newcomer et al. (39) tested the ability of NCFM* to alleviate symptoms of irritable bowel syndrome and lactose maldigestion. Although no benefit was observed, this crossover study provides additional evidence for safe consumption of NCFM* by 89 test subjects (61 for IBS trial, 18 for lactose malabsorption trial and 10 healthy controls). IBS subjects consumed 2.9×10^9 cfu NCFM* in 720 ml milk/d for 10 weeks. Lactose maldigesters consumed on average 1.5 glasses of milk (4×10^6 cfu/ml) per day for 2 one-week periods.
11. The ability of *L. acidophilus* NCFM® and *L. acidophilus* La-14 to stimulate specific immunity has been evaluated in a human study measuring primary immune reaction following vaccination (40). One week prior to oral vaccination with cholera vaccine, healthy volunteers received either a placebo (maltodextrin, n=20) or *L. acidophilus* NCFM® (n=9) or *L. acidophilus* La-14 (n=9). Supplementation with *L. acidophilus* NCFM® or La-14 or the placebo started on day 0 and continued for 21 days. The subjects consumed two capsules a day with 10^{10} cfu *L. acidophilus* NCFM® or two capsules a day with 10^{10} cfu *L. acidophilus* La-14 or two capsules a day with maltodextrin

(control). Supplementation with *L. acidophilus* NCFM® tended to increase the specific serum IgA for the period D21-D28 (P=0.09) compared to the placebo group and supplementation with La-14 significantly increased the specific serum IgG for the period D21-D28 (P=0.01).

12. The efficacy of *L. acidophilus* NCFM® was also tested either alone, or as part of a two-strain blend along with *B. animalis* subsp. *lactis* Bi-07, in a study of 3-5 year old children measuring the impact on cold/flu symptom reduction. Children receiving only NCFM® (n=110) or NCFM®/Bi-07 (n=112) had significant reductions in the incidence and duration of cold/flu symptoms with no adverse events reported (41).
13. In a study as reported by Fisberg et al (42), *L. acidophilus* NCFM® was part of a synbiotic group (also combined with *B. lactis* Bi-07 and fructooligosaccharides at 0.5 g/L after reconstitution) that evaluated the incidence, duration of illness, and anthropometrics in children who received a nutritional supplement with or without synbiotics. In this double-blind, randomized study of 616 children aged 1-6 years old, both study feedings were well tolerated and the overall incidence of adverse events was very low, with none of the adverse events considered as study-related. The dose in this study was not well communicated in the manuscript but is estimated to be greater than one billion CFUs per day.
14. *Lactobacillus acidophilus* NCFM® has also been tested in another synbiotic study where the combination of *B. lactis* Bi-07, *Lactobacillus acidophilus* NCFM®, and fructooligosaccharides was used as the symbiotic arm (43). In

this study, 129 children aged 1-6 who were acutely ill and receiving antibiotic therapy were randomized to receive a nutritional supplement with or without synbiotics or a fruit-flavored drink. The dose was the same as in the aforementioned study. In terms of tolerance, the authors determined that the supplements were well tolerated with no significant change from baseline in terms of symptomology or adverse events.

It may be concluded from the above that ingestion of *Lactobacillus acidophilus* at daily doses up to 3.8×10^{11} CFU/day for six days (high dosage for short duration), as well as 1×10^{10} CFU/day over six months (lower dosage for longer duration), failed to induce any adverse signs or symptoms.

The highest level of intake of NCFM[®] that has been demonstrated to be safe in humans and published in the peer-reviewed literature is 3×10^{11} cfu/day.

Transferable antibiotic resistance

Although there is negligible concern for translocation, toxigenicity, or any adverse nutritional activity from consumption of *L. acidophilus* NCFM[®], the presence of transferable antibiotic resistance genes must also be assessed in La-14. Although the presence of such genes does not in itself comprise a risk (an antibiotic resistant *Lactobacillus* is not a pathogen), there is concern that cultures which carry transferable antibiotic resistance genes may transfer these genes to less innocuous members of the commensal microbiota in vivo. Genomic sequencing did not detect any known antibiotic resistance genes (10) in NCFM[®], and due to the 99.98% genomic similarity of La-14, this indicates a lack of

transferable antibiotic resistance as well. The La-14 antibiogram is detailed in the Technical Memorandum contained in the Appendix.

It is also noted that *L. acidophilus* is not listed higher than Biosafety Level 1 organisms by the American Biological Safety Association, indicating that they are unlikely or not associated to cause disease in healthy human adults.

(<http://www.absa.org/>).

(2) Supporting Recent Safe History of Use in Food.

Lactobacillus species have a long history of safe use when consumed as part of dairy food and supplement products with thirty-six (36) *Lactobacillus* species listed in IDF Bulletin No. 377: *Inventory of Microorganisms with a Documented History of Use in Food* (12). A more recent IDF Bulletin, No. 455 *Safety Demonstration of Microbial Food Cultures in Fermented Food Products* (13), provides an update to the aforementioned inventory of microbial species, taking a global perspective versus the original focus of European dairy products. The updated inventory lists 82 species of *Lactobacillus*, and references *L. acidophilus* as originally documented in 1950 (13).

More specifically, *Lactobacillus acidophilus* has been added to human food since at least 1950 and is very common in dairy products worldwide, including the US where the species is the most common *Lactobacillus* in yogurt products (12).

Lactobacillus acidophilus La-14 is a strain utilized in conventional foods (e.g., milk, yogurt) and in dietary supplements. It has been commercially available in

the United States for more than a decade with no adverse incidents reported (34, 41). The strain has been characterized extensively and its characteristics including safety have been evaluated in vitro and in vivo (animal and human studies – see Safety Testing herein).

(3) Probable Consumption/Effect of *L. acidophilus* La-14 in Diet. Uses will be limited to foods that can sustain living *L. acidophilus* La-14 during shelf life. *Lactobacillus acidophilus* La-14 is intended to be added to a variety of foods at concentrations needed to provide at least 10^9 cfu/250g serving throughout the shelf life of the product. Based on the highest dosage of La-14 administered in a clinical trial where no adverse events were observed or reported, foods will be targeted to typically contain between 1×10^9 cfu/serving up to 2×10^{10} cfu/serving of *L. acidophilus* La-14. There are currently published papers on *L. acidophilus* efficacy for gut and immune health at this concentration (40), and no adverse events have been observed. It is recognized, however, that the cell count may decrease over the shelf life of some foods, such as fruit juices with a low pH. The initial addition level may be as high as 5×10^{11} cfu/250g serving (i.e. 2×10^9 cfu/g) in order to insure at least 10^9 cfu/250g serving remains viable over the product shelf life.

Realistically, there will be limited types of foods that will be available containing the strain and thus the safety margin developed above is highly conservative. Also, consumers are very likely only to consume the food such that they achieve the daily benefit of products containing *L. acidophilus* La-14. Furthermore,

because of anticipated marketing, few products in a given category will likely contain the strain. Based on these assumptions, consumers will most probably consume a single 250 g serving to achieve the benefit, thus ingesting an average of 5×10^9 cfu per day.

At an average level of 5×10^{10} cfu/250g serving, a consumer could consume six servings of products containing *L. acidophilus* La-14 and would be below the level shown to be tolerated by humans in safety studies.

(B) Information That May Appear Inconsistent With GRAS Determination:

Danisco is not aware of information that appears to be inconsistent with the determination of safety or general recognition of safety for the present or proposed uses of *L. acidophilus* La-14. It should be noted, however, that *Lactobacillus* species have been isolated on very rare occasions from patients with endocarditis, in the absence of supplementation (14). One such study accounts such extreme clinical cases, where an immunocompromised individual with documented steroid use for over a year, discontinued dental treatment. Infectious endocarditis was the result, and *L. acidophilus* was isolated (44). And, *Lactobacillus* sp. are widely considered safe for use, but are believed to be associated with illness as the result of an opportunistic infection, where clinical isolates are often distinct from those strains commercially available in food and supplements (15). Also, in the situation where the consumer has gut barrier dysfunction or is immunocompromised in any way, caution should be exercised, as translocation of commensal or consumed microbes is an increased risk. Recent studies of immunocompromised individuals contracting infections caused by

Lactobacillus spp. demonstrate susceptibility of the immunocompromised community (44, 45).

(C) Expert Consensus for GRAS Determination for *L. acidophilus* LA-14:

To further its internal safety and GRAS determinations of the subject food uses of *Lactobacillus acidophilus* La-14, Danisco convened a panel of independent scientists (“Expert Panel”), qualified by their relevant national and international experience and scientific training, to evaluate the safety of food and food ingredients, to conduct a critical and comprehensive evaluation of the available pertinent published literature and other information on *Lactobacillus acidophilus*. Danisco asked the Panel to determine, based on its review, the safety and the GRAS status of the intended uses of *Lactobacillus acidophilus* La-14 in various foods. The Expert Panel consisted of Joseph F. Borzelleca, Ph.D. (Professor Emeritus, Virginia Commonwealth University School of Medicine), and Michael W. Pariza, Ph.D. (Professor Emeritus, University of Wisconsin- Madison). The panel of scientific experts confirmed Danisco’s determination of the safety and general recognition of safety of the present and proposed uses of *Lactobacillus acidophilus* La-14. (See Appendix for Expert Panel Report).

In making its determination the Expert Panel stated that it had “independently and collectively, critically evaluated a GRAS dossier prepared by Danisco USA (GRAS DOSSIER, *Lactobacillus acidophilus*, La-14, September 2013), as well as published information about the genus *Lactobacillus*, the species *Lactobacillus acidophilus*, and other materials deemed appropriate or necessary by the Expert Panel. The GRAS Dossier included information derived from a comprehensive

search of the scientific literature conducted by Danisco USA through July 2013. The GRAS Dossier also included the product identity of *L. acidophilus* La-14, method of manufacture including specifications and stability data, conditions of intended use including use levels and functionality, estimated dietary exposures, and biological studies pertinent to the safety of *L. acidophilus* La-14 under the conditions of intended use.”

In its report, the Expert Panel noted with regard to *Lactobacillus* that the genus “is considered to be non-pathogenic and non-toxicogenic, and many of its species are natural and typical residents within the normal microbial flora of the intestinal tracts of humans and animals. *Lactobacillus* sp. have a long history of safe use in food fermentations, and more recently, safe use as dietary supplements.” With specific regard to La-14, the Expert Panel stated that the “genome of Danisco USA's *L. acidophilus* La-14 has been completely sequenced and deposited at the National Center for Biotechnology Information, where it is publically available under accession number CP_005926. It is very closely related, both genetically and phenotypically, to *L. acidophilus* NCFM[®], which is also produced by Danisco USA.” After noting minor differences in the strains, the Expert Panel went on to state that “the genomes of *L. acidophilus* La-14 and *L. acidophilus* NCFM[®] do not contain elements associated with pathogenicity in humans or animals, i.e., virulence factors, genes coding for resistance to antibiotics that are used in medical or veterinary applications, or genes coding for toxins that are active against humans or animals. In addition, the *L. acidophilus* La-14 gene does not

contain genes for either histidine or tyrosine decarboxylase, indicating that the strain is highly unlikely to produce either histamine or tyramine.”

The Expert Panel also noted that “*Lactobacillus acidophilus* has been in commercial use for food fermentations since at least 1950, and it is the most common species of *Lactobacillus* isolated from yogurt in the US. Danisco USA's *L. acidophilus* La-14 was isolated from a human source and has been commercially available in the US for more than a decade. It is used in the manufacture of fermented milk, yogurt, and dietary supplements, with no reports of adverse incidents.”

With regard to safety studies, the Expert Panel observed that “Although Danisco USA's *L. acidophilus* La-14, and the virtually identical *L. acidophilus* NCFM[®], have both been studied extensively in experimental animals and in human clinical trials, none of the studies were specifically designed to assess safety. An appropriate animal model to assess the safety/toxicity of microorganisms has not been defined. Nevertheless, safety can be inferred from the reported lack of adverse effects following exposure to the microorganisms.” The Expert Panel also went on to state that “*L. acidophilus* La-14 and *L. acidophilus* NCFM were evaluated in healthy (including lactose mal-digesters) and diseased (e.g. chronic kidney failure, irritable bowel syndrome) subjects. The highest oral doses of *L. acidophilus* La-14 and *L. acidophilus* NCFM[®], respectively, that were administered in the published human clinical trials, were 2×10^{10} cfu/day and 3×10^{11} cfu/day, respectively. Given the close genetic and phenotypic similarities of *L. acidophilus* NCFM[®] and *L. acidophilus* La-14, the Expert Panel considers

3x10¹¹ cfu/day to be a reasonably safe upper limit that is applicable to both strains.”

In reviewing consumer exposure for the potential variety of food uses proposed by Danisco, the Expert Panel stated that “Typical formulations will specify that finished product should contain between 1x10⁹-2x10¹⁰ cfu/serving of *L. acidophilus* La-14. Publications in the peer-reviewed scientific literature support the efficacy of *L. acidophilus* for gut and immune health at this concentration, with no adverse events having been reported.” The Expert Panel also noted that Danisco expects that consumers will most likely consume a single 250 g serving per day, thus ingesting an average of 5x10⁹ cfu of *L. acidophilus* La-14 per day. Based on Danisco’s estimate, the Expert Panel observed that “If the number of viable cells were to be increased to 5x10¹⁰ cfu/250g serving, a consumer could consume six servings of such products without exceeding 3 x10¹¹ cfu which, in the opinion of the Expert Panel, is a safe upper limit of exposure.”

Finally, the Expert Panel reached its conclusion of safety and general recognition of safety for the proposed food uses of *Lactobacillus acidophilus* La-14 by stating “We, the members of the Expert Panel, have independently and collectively, critically evaluated the published and unpublished data and information summarized above, and any other data and information that we deemed pertinent to the safety of the intended uses of Danisco USA's *Lactobacillus acidophilus* La-14.

We conclude that the intended uses for addition to foods in which the viable cells will survive processing and persist for the duration of shelf life of Danisco USA's *L. acidophilus* La-14, manufactured consistent with cGMP and meeting appropriate food grade specifications, are safe and suitable.

We further conclude that the intended uses for addition to foods in which the viable cells will survive processing and persist for the duration of shelf life of Danisco USA's *L. acidophilus* La-14, manufactured consistent with cGMP and meeting appropriate food grade specifications, are Generally Recognized As Safe (GRAS) based on scientific procedures and supported by a history of safe use in food manufacture.

It is our opinion that other experts, qualified by scientific training and experience, and evaluating the same data and information, would concur with these conclusions.”

Based on the information contained in the exemption claim, the above additional and supplementary information, and the information contained in the Appendix attached hereto, a clear and ample basis exists to support Danisco's determination, confirmed by the Expert Panel, of general recognition of safety for the food uses, present and proposed herein, of *Lactobacillus acidophilus* La-14, and for their exemption from the premarket approval requirements of the Federal food, Drug and Cosmetic Act.

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L. acidophilus La-14 GRAS NOTICE
APPENDIX

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Expert Panel Report on the Generally Recognized As Safe (GRAS) Status of the Intended Uses of Danisco USA's *Lactobacillus acidophilus* La-14 for Addition to Foods in Which the Viable Cells Will Survive Processing and Persist for the Duration of Shelf Life

Danisco USA convened a panel of experts ("Expert Panel"), qualified by their scientific training and experience to evaluate the safety of food and food ingredients, to determine whether the intended uses of *Lactobacillus acidophilus* La-14 for addition to foods in which the viable cells will survive processing and persist for the duration of shelf life is Generally Recognized As Safe ("GRAS") based on scientific procedures. The Expert Panel Members were Michael W. Pariza Ph.D. (Professor Emeritus, University of Wisconsin-Madison), and Joseph F. Borzelleca, Ph.D. (Professor Emeritus, Virginia Commonwealth University School of Medicine).

The Expert Panel, independently and collectively, critically evaluated a GRAS dossier prepared by Danisco USA (GRAS DOSSIER, *Lactobacillus acidophilus*, La-14, September 2013), as well as published information about the genus *Lactobacillus*, the species *Lactobacillus acidophilus*, and other materials deemed appropriate or necessary by the Expert Panel. The GRAS Dossier included information derived from a comprehensive search of the scientific literature conducted by Danisco USA through July 2013. The GRAS Dossier also included the product identity of *L. acidophilus* La-14, method of manufacture including specifications and stability data, conditions of intended use including use levels and functionality, estimated dietary exposures, and biological studies pertinent to the safety of *L. acidophilus* La-14 under the conditions of intended use.

Following its independent and collective critical evaluation of the available published and unpublished data and other information pertinent to safety of the intended use, the Expert Panel convened in a teleconference with Dr. Amy Smith (Danisco USA) on August 26, 2013. The Expert Panel independently and jointly concluded that the intended uses of *L. acidophilus* La-14 for addition to foods in which the viable cells will survive processing and persist for the duration of shelf life, are GRAS based on scientific procedures. A summary of the basis for the Expert Panel's conclusion follows.

Background

The genus *Lactobacillus* is comprised of Gram-positive, homofermentative, rod-shaped bacteria that are microaerophilic or facultative anaerobes. The genus is considered to be non-pathogenic and non-toxicogenic, and many of its species are natural and typical residents within the normal microbial flora of the intestinal tracts of humans and animals. *Lactobacillus* sp. have a long history of safe use in food fermentations, and more recently, safe use as dietary supplements.

The taxonomy of *L. acidophilus* has undergone significant revisions over the past decade. Strains previously considered to be of the species "*acidophilus*" have been subdivided into 6

new species: *acidophilus*, *amylovarus*, *crispatus*, *gallinarum*, *gasseri* and *johnsonii*. *Lactobacillus acidophilus* ATCC 4356 is the type strain for the *L. acidophilus* species.

The genome of Danisco USA's *L. acidophilus* La-14 has been completely sequenced and deposited at the National Center for Biotechnology Information, where it is publically available under accession number CP_005926. It is very closely related, both genetically and phenotypically, to *L. acidophilus* NCFM[®], which is also produced by Danisco USA. For example, the fermentation and growth characteristics of *L. acidophilus* La-14 are virtually identical to those of *L. acidophilus* NCFM[®]. Direct comparison of their respective genomes indicates 16 single base-pair INDELS, of which 14 predicatively cause frameshifts. Of the 95 SNPs discovered, 47 of them are non-synonymous, and 29 occur in intergenic regions. A total of 52 genes are possibly affected by these minor changes, and otherwise the genomes are 99.9% identical. Most importantly, and as predicted, the genomes of *L. acidophilus* La-14 and *L. acidophilus* NCFM[®] do not contain elements associated with pathogenicity in humans or animals, i.e., virulence factors, genes coding for resistance to antibiotics that are used in medical or veterinary applications, or genes coding for toxins that are active against humans or animals. In addition, the *L. acidophilus* La-14 genome does not contain genes for either histidine or tyrosine decarboxylase, indicating that the strain is highly unlikely to produce either histamine or tyramine.

History of safe use

Lactobacillus acidophilus has been in commercial use for food fermentations since at least 1950, and it is the most common species of *Lactobacillus* isolated from yogurt in the US. Danisco USA's *L. acidophilus* La-14 was isolated from a human source and has been commercially available in the US for more than a decade. It is used in the manufacture of fermented milk, yogurt, and dietary supplements, with no reports of adverse incidents. There are rare cases of opportunistic infections associated with *Lactobacillus* sp., including *L. acidophilus*, in immunocompromised patients. However, there is no evidence that any species of *Lactobacillus* expresses a pathogenic phenotype, i.e., that it can cross intact host barriers and induce illness in otherwise healthy human subjects, so the genus is classified as non-pathogenic. Accordingly, as expected and discussed above, the genome of Danisco USA's *L. acidophilus* La-14 does not contain virulence factors, genes coding for resistance to antibiotics that are used in medicinal or veterinary applications, or genes coding for toxins that are active against humans or animals.

Safety studies

Although Danisco USA's *L. acidophilus* La-14, and the virtually identical *L. acidophilus* NCFM[®], have both been studied extensively in experimental animals and in human clinical trials, none of the studies were specifically designed to assess safety. An appropriate animal model to assess the safety/toxicity of microorganisms has not been defined. Nevertheless, safety can be inferred from the reported lack of adverse effects following exposure to the microorganisms.

La-14 was evaluated in bee colonies, normal mice and gnotobiotic immunocompromised mice. No adverse effects were reported.

L. acidophilus La-14 and *L. acidophilus* NCFM were evaluated in healthy (including lactose mal-digesters) and diseased (e.g. chronic kidney failure, irritable bowel syndrome) subjects. The highest oral doses of *L. acidophilus* La-14 and *L. acidophilus* NCFM[®], respectively, that were administered in the published human clinical trials, were 2×10^{10} cfu/day and 3×10^{11} cfu/day, respectively. Given the close genetic and phenotypic similarities of *L. acidophilus* NCFM[®] and *L. acidophilus* La-14, the Expert Panel considers 3×10^{11} cfu/day to be a reasonably safe upper limit that is applicable to both strains.

Intended uses, use levels, and exposure estimates

There is interest among food manufacturers in the US for using *Lactobacillus acidophilus* strains in foods that have not historically contained the organisms. Accordingly, it is Danisco's intent to market *L. acidophilus* La-14 for use in a variety of foods in which this species has not been previously used. The new uses must accommodate conditions under which the viable cells will survive processing and persist for the duration of shelf life, specifically, ready-to-eat breakfast cereals; bars (e.g. breakfast, energy, nutrition); milk, milk drinks (e.g. flavored milks), milk products (e.g. butter), fermented milks (e.g. Kefir, sour cream, buttermilk), yogurt, cheese (incl. cheese food, cheese spreads) and ice cream; soy drinks and soy products; bottled water and teas; dry beverages including sports nutrition beverages; fruit juices, fruit nectars, fruit "ades", fruit drinks, jams and jellies; chewing gum; medical foods; nut and peanut spreads; margarines; snack foods (e.g. cookies, crackers, chips, granola); meal replacements; sauces, condiments; and confections (e.g. bars, candy, coatings, drops, cookie filling) but not infant formulas.

Typical formulations will specify that finished product should contain between 1×10^9 - 2×10^{10} cfu/serving of *L. acidophilus* La-14. Publications in the peer-reviewed scientific literature support the efficacy of *L. acidophilus* for gut and immune health at this concentration, with no adverse events having been reported. It is recognized, however, that the viable cell count may decrease over the shelf life of some foods, such as fruit juices with a low pH. Therefore, a initial use level of up to about 5×10^{11} cfu/serving may be utilized for products likely to experience a drop in viable cell counts as a result of low pH.

Danisco USA estimates that relatively few food and beverage types within each of these categories will be developed with *L. acidophilus* La-14 as an ingredient. Danisco USA concludes that it is reasonable to assume that consumption will be for the express purpose of ingesting an amount of the organisms required to achieve the claimed benefit, in general a single serving per day. Accordingly, consumers will most likely consume a single 250 g serving to achieve the benefit, thus ingesting an average of 5×10^9 cfu per day. If the number of viable cells were to be increased to 5×10^{10} cfu/250g serving, a consumer could consume six servings of such products without exceeding 3×10^{11} cfu which, in the opinion of the Expert Panel, is a safe upper limit of exposure.

Manufacturing and Specifications

Danisco USA's *L. acidophilus* La-14 is manufactured in accordance with the U.S. Food & Drug Administration's current Good Manufacturing Practice (cGMP) guidelines in an FDA regulated and inspected facility. The manufacturing procedure and product specifications that are detailed in the GRAS dossier are appropriate for a food grade ingredient.

Conclusion

We, the members of the Expert Panel, have independently and collectively, critically evaluated the published and unpublished data and information summarized above, and any other data and information that we deemed pertinent to the safety of the intended uses of Danisco USA's *Lactobacillus acidophilus* La-14.

We conclude that the intended uses for addition to foods in which the viable cells will survive processing and persist for the duration of shelf life of Danisco USA's *L. acidophilus* La-14, manufactured consistent with cGMP and meeting appropriate food grade specifications, are safe and suitable.

We further conclude that the intended uses for addition to foods in which the viable cells will survive processing and persist for the duration of shelf life of Danisco USA's *L. acidophilus* La-14, manufactured consistent with cGMP and meeting appropriate food grade specifications, are Generally Recognized As Safe (GRAS) based on scientific procedures and supported by a history of safe use in food manufacture.

It is our opinion that other experts, qualified by scientific training and experience, and evaluating the same data and information, would concur with these conclusions.

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Date: _____

Home Food Ingredients, Packaging & Labeling Generally Recognized as Safe (GRAS)

Food

Microorganisms & Microbial-Derived Ingredients Used in Food (Partial List)

Food ingredients may be "food additives" that are approved by FDA for specific uses or GRAS (generally recognized as safe) substances. A substance may be GRAS only if its general recognition of safety is based on the views of experts qualified to evaluate the safety of the substance. GRAS status may be based either on a history of safe use in food prior to 1958 or on scientific procedures, which require the same quantity and quality of evidence as would be required to obtain a food additive regulation. Because GRAS status may be either affirmed by FDA or determined independently by qualified experts, FDA's regulations do not include all GRAS ingredients and the specific uses described in the GRAS regulations may not be comprehensive for the listed ingredients.

The list below includes some ingredients that are not listed in 21 CFR but have been the subject of opinion letters from FDA to individuals who asked whether FDA would object to the use of the ingredient in food on the basis of an independent GRAS determination. Because the list is not updated on a regular basis, questions about the regulatory status of microorganisms or microbial-derived ingredients that are not on this list may be directed to us via electronic mail at Premarkt@fda.hhs.gov

The following list, which derives partially from FDA's regulations in Title 21 of the Code of Federal Regulations (21 CFR), includes approved food additives, substances whose GRAS status has been affirmed by FDA and substances that FDA listed as GRAS based on a history of safe use in food. In addition, microorganisms and microbial-derived ingredients may be the subject of a GRAS notice. For further information, consult the summary listing of GRAS ingredients.

The following is a compilation of food additives listed in Title 21 of the Code of Federal Regulations (21 CFR) Part 172 and 173, which are derived from microorganisms. This list also includes seaweed sources. Conditions for their use are prescribed in the referent regulations and are predicated on the use of good manufacturing practices.

To access the specific regulations listed below, type in the title number, use the links below to access the Government Printing Office web site.

Table 1. Food Additives Derived from Microorganisms listed in 21 CFR 172 and 173

Regulation in 21 CFR	Ingredient
§172.155	Natamycin derived from <i>Streptomyces natalensis</i> and <i>Streptomyces chattanoogensis</i>
§172.325	Bakers yeast protein derived from <i>Saccharomyces cerevisiae</i>
§172.590	Yeast-malt sprout extract, derived from <i>Saccharomyces cerevisiae</i> , <i>Saccharomyces fragilis</i> , <i>Candida utilis</i>
§172.620	Carrageenan, a hydrocolloid extracted from the following members of the families <i>Gigartinaceae</i> and <i>Soliericeae</i> of the class <i>Rhodophyceae</i> (red seaweed): <i>Chondrus crispus</i> , <i>Chondrus ocellatus</i> , <i>Euचेuma cottonii</i> , <i>Euचेuma</i>

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- spinosum*, *Gigartina acicularis*, *Gigartina pistillata*, *Gigartina radula*, *Gigartina stellata*
- §172.655 Furcelleran, the refined hydrocolloid extracted from *Furcellaria fastigiata* of the class *Rhodophyceae* (red seaweed)
- §172.695 Xanthan Gum derived from *Xanthomonas campestris*
- §172.725 Gibberellic acid derived by fermentation from *Fusarium moniliforme*
- §172.896 Dried yeasts, *Saccharomyces cerevisiae*, *Saccharomyces fragilis*, and dried torula yeast, *Candida utilis*
- §172.898 Bakers yeast glycan from *Saccharomyces cerevisiae*
- §173.110 Amyloglucosidase derived from *Rhizopus niveus* for use in degrading gelatinized starch into constituent sugars
- §173.120 Carbohydrase and cellulase derived from *Aspergillus niger* for use in clam and shrimp processing
- §173.130 Carbohydrase derived from *Rhizopus oryzae* for use in the production of dextrose from starch
- §173.135 Catalase derived from *Micrococcus lysodeikticus* for use in the manufacture of cheese
- §173.140 Esterase-lipase derived from *Mucor miehei* var. *Cooney et Emerson* as a flavor enhancer in cheeses, fats and oils, and milk products
- §173.145 *Alpha*-galactosidase derived from *Mortierella vinaceae* var. *raffinoseutilizer* for use in the production of sucrose from sugar beets
- §173.150 Milk-clotting enzymes, microbial for use in the production of cheese (Milk-clotting enzymes are derived from *Endothia parasitica*, *Bacillus cereus*, *Mucor pusillus* Lindt and *Mucor miehei* and *Aspergillus oryzae* modified to contain the gene for aspartic proteinase from *Rhizomucor miehei* var *Cooney et Emerson*)
- §173.160 *Candida guilliermondii* as the organism for fermentation production of citric acid
- §173.165 *Candida lipolytica* for fermentation production of citric acid.
- §173.280 A solvent extraction process for recovery of citric acid from *Aspergillus niger* fermentation liquor

The following is a compilation of GRAS affirmed substances listed in 21 CFR part 184 which are derived from microorganisms. This list also includes seaweed sources. Conditions for their use are prescribed in the referent regulations and are predicated on the use of nonpathogenic and nontoxicogenic strains of the respective organisms and on the use of current good manufacturing practice (184.1(b)). Please be aware that not all GRAS substances have been recorded as such and so this does not represent a complete list of all microbial derived GRAS food ingredients.

Table 2. Substances Derived from Microorganisms Affirmed by FDA as Generally Recognized as Safe in 21 CFR184

Section in 21 CFR	Ingredient or Substance
§184.1005	Acetic acid may be produced by fermentation
§184.1011	Alginic acid made from certain brown algae
§184.1012	<i>Alpha</i> -amylase enzyme preparation from <i>Bacillus stearothermophilus</i> used to hydrolyze edible starch to produce maltodextrin and nutritive carbohydrate sweeteners.
§184.1027	Mixed carbohydrase and protease enzyme product derived from <i>Bacillus licheniformis</i> for use in hydrolyzing proteins and

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- carbohydrates in the preparation of alcoholic beverages, candy, nutritive sweeteners and protein hydrolysates
- §184.1061 Lactic acid may be produced by fermentation
- §184.1081 Propionic acid from bacterial fermentation
- §184.1115 Agar-agar, extracted from a number of related species of red algae class *Rhodophyceae*
Brown algae, to be used dried as a flavor enhancer, are seaweeds of the species: *Anelipes japonicus*, *Eisenia bicyclis*, *Hizikia fusiforme*, *Kjellmaniella gyrata*, *Laminaria angustata*,
- §184.1120 *Laminaria longiruris*, *Laminaria Longissima*, *Laminaria ochotensis*, *Laminaria claustronia*, *Laminaria saccharina*, *Laminaria digitata*, *Laminaria japonica*, *Macrocystis pyrifera*, *Petalonia fascia*, *Scytosiphon lome*
Red algae, to be used dried as a flavor enhancer, are seaweeds of the species: *Gloiopeltis furcata*, *Porphyra crispata*, *Porphyra deutata*, *Porphyra perforata*, *Porphyra suborbiculata*, *Porphyra tenera*, *Rhodymenis palmata*
- §184.1121
- §184.1133 Ammonium alginate from certain brown algae
- §184.1187 Calcium alginate from certain brown algae
Glucono delta-lactone, by oxidation of D-glucose by microorganisms that are nonpathogenic and nontoxicogenic to man or other animals. These include but are not restricted to *Aspergillus niger* and *Acetobactor suboxydans*
- §184.1318 Insoluble glucose isomerase enzyme preparations are derived from recognized species of precisely classified, nonpathogenic, and nontoxicogenic microorganisms, including
- §184.1372 *Streptomyces rubiginosus*, *Actinoplane missouriensis*, *Streptomyces olivaceus*, *Streptomyces olivochromogenes*, and *Bacillus coagulans* grown in a pure culture fermentation that produces no antibiotic
- §184.1387 Lactase enzyme preparation from *Candida pseudotropicalis* for use in hydrolyzing lactose to glucose and galactose
- §184.1388 Lactase enzyme preparation from *Kluyveromyces lactis* (previously called *Saccharomyces lactis*) for use in hydrolyzing lactose in milk
- §184.1420 Lipase enzyme preparation from *Rhizopus niveus* used in the interesterification of fats and oils.
- §184.1538 Nisin preparation from *Lactococcus lactis* Lancefield Group N for use as an antimicrobial agent to inhibit the outgrowth of *Clostridium botulinum* spores and toxin formation in pasteurized cheese spreads.
- §184.1610 Potassium alginate, the potassium salt of alginic acid, derived from certain brown algae
- §184.1685 Rennet (animal derived) and chymosin preparation from *Escherichia coli* K-12, *Kluyveromyces marxianus* var. *lactis* or *Aspergillus niger* var. *awamori* to coagulate milk in cheeses and other dairy products
- §184.1695 Riboflavin biosynthesized by *Eremothecium ashbyii*
- §184.1724 Sodium alginate, the sodium salt of alginic acid, derived from certain brown algae
- §184.1848 Butter starter distillate from milk cultures of *Streptococcus lactis*, *Streptococcus cremoris*. *Streptococcus lactis* subspecies *diacetylactis*, *Leuconostoc citovororum*, *Leuconostoc dextranicum*
- §184.1924 Urease enzyme preparation from *Lactobacillus fermentum* for use in the production of wine

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- §184.1945 Vitamin B12 from *Streptomyces griseus*
 §184.1950 Vitamin D, produced by ultraviolet irradiation of ergosterol isolated from yeast and related fungi
 §184.1983 Bakers Yeast extract from *Saccharomyces cerevisiae*
 Aminopeptidase enzyme preparation from *Lactococcus lactis*
 §184.1985 used as an optional ingredient for flavor development in the manufacture of cheddar cheese.

The following GRAS affirmed substances are listed in 21 CFR Part 186 and are affirmed for use as substances added indirectly to food. Conditions for their use are prescribed in the referent regulations and are predicated on the use of nonpathogenic and nontoxicogenic strains of the respective organisms and on the use of current good manufacturing practice (186.1(b)).

Table 3. Substances Derived from Microorganisms Affirmed by FDA as Generally Recognized as Safe for Indirect Uses in 21 CFR186

Section in 21 CFR	Substance
§186.1275	Dextrans, made by fermentation of sucrose by <i>Leuconostoc mesenteroides</i> strain NRRL B-512(F)
§186.1839	Sorbose, made by oxidation of sorbitol by <i>Acetobacter xylinum</i> or by <i>Acetobacter suboxydans</i>

The following is a compilation of microbial derived enzymes which the FDA recognized as GRAS in opinion letters issued in the early 1960's. The opinions are predicated on the use of nonpathogenic and nontoxicogenic strains of the respective organisms and on the use of current good manufacturing practice.

Table 4. Substances Derived from Microorganisms Recognized by FDA as Generally Recognized as Safe in Opinion Letters

Enzyme
Carbohydrase, cellulase, glucose oxidase-catalase, pectinase, and lipase from <i>Aspergillus niger</i>
Carbohydrase and protease from <i>Aspergillus oryzae</i>
Carbohydrase and protease from <i>Bacillus subtilis</i>
Invertase from edible baker's yeast or brewer's yeast (<i>Saccharomyces cerevisiae</i>)

The following is a compilation of foods for human consumption listed in 21 CFR Parts 131, 133, 136 and 137 that may contain or be derived from microorganisms.

Table 5. Foods for human consumption that may contain or be derived from microorganisms listed in 21 CFR Parts 131, 133, 136, and 137

Section in 21 CFR	Standardized Food
§131.111	Acidified milk, with or without the addition of characterizing microbial organisms, and aroma - and flavor - producing microbial culture. Conditions for their use are prescribed in the referent regulations
§131.200	Yogurt made by the lactic acid-producing bacteria <i>Lactobacillus bulgaricus</i> and <i>Streptococcus thermophilus</i>
§131.106	Blue cheese, characterized by the presence of the mold <i>Penicillium roquefortii</i>
§133.113	Cheddar cheese, subjected to the action of a lactic acid producing bacterial culture and clotting enzymes of animal, plant or microbial origin used in curing or flavor development
§136.110	

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Bread, rolls, and buns may contain as optional ingredients lactic-acid producing bacteria

§137.105 Flour may contain alpha-amylase obtained from the fungus *Aspergillus oryzae*

Prior sanctions were granted for the use of harmless lactic acid producing bacteria, such as *Lactobacillus acidophilus*, as optional ingredients in specified standardized foods. These bacteria are permitted for use in cultured milk (which includes buttermilk) (§ 131.112), sour cream (§ 131.160), cottage cheese (§ 133.128), and yogurt (§ 131.200), provided that the mandatory cultures of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* are also used in the yogurt.

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U.S. Department of **Health & Human Services**

Links on this page:

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Appendix A: *L. acidophilus* species is on EFSA's QPS list

From QPS document, EFSA, **Appendix A - Assessment of gram-positive non-sporulating bacteria** *The EFSA Journal* (2007) 587, Qualified Presumption of Safety

http://www.efsa.europa.eu/cs/BlobServer/Scientific_Opinion/sc_appendixa_qps_en.pdf?ssbinary=true

Lactobacillus

The genus *Lactobacillus* is a wide and heterogeneous taxonomic unit, comprising the rod-shaped lactic acid bacteria. This genus encompasses more than 100 different species with a large variety of phenotypic, biochemical and physiological properties. Many of the species are significant constituents of the normal gut flora of humans and livestock although their occurrence and numbers are host dependent. Several species of the genus are intentionally introduced in the food chains, being involved in a range of food and feed fermentations and applied as probiotics for humans and animals.

Taxonomic unit defined

As for other lactic acid bacteria, lactobacilli belong to the phylum *Firmicutes*. They are rod shaped, non-motile and non-sporeformers. Classically, the *Lactobacillus* genus is divided into three groups: group 1, obligate homofermentative, group 2, facultative heterofermentative and group 3 obligate heterofermentative (for a review, see Axelsson 2004). The application of phylogenetic molecular taxonomy and 16S rRNA gene sequence analysis resulted in several changes within the taxonomy of this genus, with an increase in the number of species. At present 112 species belong to the genus *Lactobacillus*. Several molecular methods are available for the identification of lactobacilli to species level.

Is the body of knowledge sufficient?

The characteristics and habitat of most of *Lactobacillus* species are well known. Some of the species of this genus have a long history of apparent safe use in industrial and agricultural applications. Lactobacilli are used as starter cultures in a variety of food fermentation, such as dairy products, fermented and cured meats, fermented vegetables, sourdough and silage. Moreover, they are among the dominant populations in microbial communities of traditional fermented foods, being part of the natural starter cultures. Increased information on this genus is being derived from the sequence analysis of several genomes of *Lactobacillus* species.

Are there safety concerns?

Members of the *Lactobacillus* genus are daily consumed in large quantities in a variety of fermented foods by people of all ages, ethnic groups and health status with apparently no ill effects. Apart from their possible involvement in the development of dental caries, lactobacilli have generally been considered to be non-pathogenic. However, there have been an increasing number of reports that these organisms might occasionally be involved in human disease (Sharpe, Hill *et al.* 1973; Gasser 1994; Salminen, Rautelin *et al.* 2006). A variety of different *Lactobacillus* species has been recovered from human clinical specimens. These include *L. rhamnosus*, *L. fermentum*, *L. plantarum*, *L. casei*, *L. jensenii*, *L. salivarius*, *L. gasseri*, *L. salivarius*, and *L. acidophilus*. Clinical conditions from which these species were derived were chiefly subacute endocarditis and bacteremia or systemic septicemia, but also included abscesses, chorioamnionitis, and urosepsis (Lorenz, Appelbaum *et al.* 1982; Dickgiesser, Weiss *et al.* 1984; Salminen, Tynkkynen *et al.* 2002; Salminen, Rautelin *et al.* 2004; Salminen, Rautelin *et al.* 2006). Even the strain *L. rhamnosus* ATCC 53103,

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used as human probiotic, has occasionally been encountered in clinical specimens such as blood or pus samples (Rautio, Jousimies-Somer *et al.* 1999; Salminen, Tynkkynen *et al.* 2002; Salminen, Rautelin *et al.* 2004; De Groot, Frank *et al.* 2005; Salminen, Rautelin *et al.* 2006). However, Salminen and co-workers (Salminen, Rautelin *et al.* 2006) demonstrated that increased probiotic use of *L. rhamnosus* ATCC 53103 had not led to an increase in *Lactobacillus* bacteraemia. Furthermore, it has been demonstrated that strains isolated from clinical samples, show phenotypic, differences from probiotic *L. rhamnosus* strains (Klein, Hack *et al.* 1995; Ouwehand, Saxelin *et al.* 2004). Many of the patients with apparent *Lactobacillus* infection were immunocompromised or had other severe underlying illnesses. As far as endocarditis due to lactobacilli is concerned, this infection usually develops on the basis of preceding anatomical alterations of the heart valves. There are indications, however, that good adhesion properties of lactobacilli and, thus, of probiotic strains, might be a potential risk for bacteremia (Apostolou, Kirjavainen *et al.* 2001). In conclusion, most of the *Lactobacillus* species described to date can rightly be considered to be non-pathogenic to humans (Bernardeau, Guguen *et al.* 2006). Only certain strains of *L. rhamnosus* may be considered to be potential human opportunistic pathogens because they not only affect severely immunocompromised, but also immunologically healthy individuals with a history of rheumatic endocarditis or heart valve replacement.

Several examples of antibiotic resistant lactobacilli isolated from food or from the gut of animals exist. Acquired genes for antibiotic resistance have been detected in *Lactobacillus* species: *tet*(M) has been found in *L. plantarum*, *L. brevis*, *L. sakei* and *L. curvatus* (Danielsen 2002; Gevers, Danielsen *et al.* 2003) and *tet*(S) in *L. plantarum* (Huys, D'Haene *et al.* 2006). Erythromycin resistance determinants *erm*(B) has been found in *L. plantarum*, *L. salivarius*, *L. animalis*, *L. fermentum*, *L. reuteri* (Axelsson, Ahrne *et al.* 1988; Fons, Hege *et al.* 1997; Gevers, Danielsen *et al.* 2003; Martel, Meulenaere *et al.* 2003). Moreover, the gene coding for the bifunctional aminoglycoside-modifying enzyme AAC(6')-APH(2'') was detected in *L. salivarius* and *L. acidophilus* (Tenorio, Zarazaga *et al.* 2001) and chloramphenicol resistance gene *cat* was identified in *L. reuteri* (Lin, Fung *et al.* 1996). Obligate and facultative heterofermentative lactobacilli, and *L. salivarius*, are intrinsically resistant to vancomycin and other glycopeptide antibiotics.

Several genetic determinants for antibiotic resistance in *Lactobacillus* are harboured by extrachromosomal elements (Lin, Fung *et al.* 1996; Danielsen 2002; Gevers, Danielsen *et al.* 2003; Gfeller, Roth *et al.* 2003; Huys, D'Haene *et al.* 2006). However, transferable elements encoding resistances of clinical relevance, such as to the glycopeptides have been excluded for some probiotic *L. reuteri* and *L. rhamnosus* strains (Klein, Hallmann *et al.* 2000).

Livestock. No report can be found on safety concerns related to lactobacilli in animals

Can the safety concerns be excluded?

There are apparently no specific safety concerns regarding a number of *Lactobacillus* species which have a long history of apparent safe use in the food chain. Susceptibility to antibiotics should be assessed as defined by the EFSA opinion for each strain (EFSA 2005).

4.5 Units proposed for QPS status

Due to the long history of safe use the following species are proposed for QPS status:

L. acidophilus, *L. amylolyticus*, *L. amylovorus*, *L. alimentarius*, *L. aviaries*, *L. brevis*, *L. buchneri*, *L. casei*, *L. crispatus*, *L. curvatus*, *L. delbrueckii*, *L. farciminis*, *L. fermentum*, *L. gallinarum*, *L. gasseri*, *L. helveticus*, *L. hilgardii*, *L. johnsonii*, *L. kefirifaciens*, *L. kefirii*, *L. mucosae*, *L. panis*, *L.*

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paracasei, *L. paraplantarum*, *L. pentosus*, *L. plantarum*, *L. pontis*, *L. reuteri*, *L. rhamnosus*, *L. sakei*, *L. salivarius*, *L. sanfranciscensis* and *L. zeae*.

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PRODUCT DESCRIPTION - PD 204250-8.1EN

Material no. M85014E

La-14 200B - 1 KG

Description

Freeze-dried probiotic powder. White to cream-color in appearance.

Directions for use

See Danisco Probiotic Usage & Handling Guide

Composition

Lactobacillus acidophilus (La-14)

Microbiological specifications

Cell count	> 2.00E+11 / g
Non-Lactic Count	< 5000 / g
Enterococci	< 100 / g
Coliforms	< 10 / g
E. coli	neg. by test (< 0.3 / g)
Staphylococcus (coag. pos.)	neg. by test < 10 / g
Salmonella	neg. (40 g enrichment)
Listeria	neg. (25 g enrichment)

Storage

Shelf life is 24 months when stored in the original, sealed package at or below 4°C. Frozen storage will extend shelf life.

Packaging

High barrier foil laminate bags

Quantity

1 kg

Purity and legal status

Local regulations should always be consulted concerning the status of this product, as legislation regarding its intended use may vary from country to country.

Safety and handling

MSDS is available on request.

Kosher status

Circle K certification

Halal status

IFANCA certification

PRODUCT DESCRIPTION - PD 204250-8.1EN

Material no. M85014E

La-14 200B - 1 KG

Allergens

Below table indicates the presence (as added component) of the following allergens and products thereof:

Yes	No	Allergens	Description of components
	X	wheat	
	X	other cereals containing gluten	
	X	crustacean shellfish	
	X	eggs	
	X	fish	
	X	peanuts	
	X	soybeans	
	X	milk (including lactose)	
	X	nuts	
	X	celery	
	X	mustard	
	X	sesame seeds	
	X	sulphur dioxide and sulphites (> 10 mg/kg)	
	X	lupin	
	X	molluscs	

Local regulation has always to be consulted as allergen labelling requirements may vary from country to country.

Additional information

Country of Origin: USA

GMO status

La-14 200B - 1 KG does not consist of, nor contains, nor is produced from genetically modified organisms according to the definitions of Regulation (EC) 1829/2003 and Regulation (EC) 1830/2003 of the European Parliament and of the Council of 22 September 2003.

The information contained in this publication is based on our own research and development work and is to the best of our knowledge reliable. Users should, however, conduct their own tests to determine the suitability of our products for their own specific purposes and the legal status for their intended use of the product. Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for the infringement of any patents

000057

Certificate of Analysis

Date: 24 Jun 2013
Our ref. no.: 0
Your ref.

Material:	M85014E	LA-14 200B - 1 KG
Batch No.:	(b) (6)	Best before date: 29 May 2015
		Production date: 29 May 2013

Test	Result	Specification	Unit	Reference
Viable Cell Count	4.40E+11	> 2.00E+11	/g	ISO 7889/IDF 117
Enterococcus	< 100	< 100	/g	CMMEF, 4TH EDITION
Non Lactics	< 5000	< 5000	/g	ISO 13559
Coliforms	< 10.0	< 10.0	/g	AOAC
E. coli, neg. by test (<0.3/g)	Negative	Negative		AOAC
Staph. aureus, neg. by test (<10/g)	Negative	Negative		AOAC
Salmonella, negative in 40 g	Negative	Negative		AOAC
Listeria, negative in 25 g	Negative	Negative		AOAC

Comments

Exceeds 200 billion CFU/gm of freeze-dried Lb. acidophilus.

The above product has been analyzed by Danisco and/or its contract testing laboratory. Analytical results on a representative sample from this batch show that this product meets the above criteria.

Best if used before the date listed above when stored at or below 4°C.

AOAC references above reflect the current edition of AOAC.

Culture identity is confirmed at the genus/species level based on 16S rRNA sequencing.

Certificate of Analysis

Date: 24 Jun 2013
Our ref. no.: 0
Your ref.

Material:	M85014E	LA-14 200B - 1 KG
Batch No.:	1102085982	Best before date: 29 May 2015
		Production date: 29 May 2013

This certificate is generated automatically

(b) (6)

Phil Ihrke

Quality Control Department

Lactobacillus acidophilus La-14

DANISCO

CHARACTERISTICS OF THE GENUS

Lactobacillus acidophilus is a Gram-positive, non-spore forming, homo-fermentative, catalase-negative rod. It is a common inhabitant of the human intestinal tract, the human mouth and vagina. It is also found in some traditional fermented milks (e.g. kefir) and is today widely used in probiotic foods and supplements. Numerous studies have demonstrated the diverse beneficial effects of different strains of *L. acidophilus* validating its use as a probiotic.

SELECTION AND TAXONOMY

The group of organisms previously known as *L. acidophilus* was shown to be highly heterogeneous (1). The results of DNA-DNA hybridisation studies have suggested that the previous *L. acidophilus* species was composed of six different species (2), which were divided into different DNA homology groups.

Homology group A1 was designated as *L. acidophilus*.

These species are quite difficult and sometimes impossible to differentiate by phenotypic methods, so they are still considered the "*L. acidophilus* group".

L. acidophilus La-14 has been confirmed as a type A1 *L. acidophilus* using phenotypic and genotypic methods, including

16S rRNA gene sequencing and hybridisation to a species-specific probe.

L. acidophilus La-14 is of human origin and has been deposited in the American Type Culture Collection as SD5212.

SAFE FOR CONSUMPTION

Lactic acid bacteria have long been considered safe and suitable for human consumption. Very few instances of infection have been associated with these bacteria and several published studies have addressed their safety (3-6). Moreover, no *L. acidophilus* bacteraemia were identified in a 10-year survey in Finland (7).

More specifically, *L. acidophilus* has been consumed in fermented milks and other food products for decades and is listed in the *Inventory of Microorganisms With Documented History of Use in Human Food* (8). The European Food Safety Authority has also added the species to the Qualified Presumption of Safety list (9).

In addition to a long history of safe human consumption of the species, no acquired antibiotic resistance was detected in *L. acidophilus* La-14 during screening by the EU-funded PROSAFE project.

GASTROINTESTINAL PERFORMANCE

Resistance to acid and bile

According to the generally accepted definition of a probiotic, the probiotic microorganism should be viable at the time of ingestion to confer a health benefit. Although not explicitly stated, this definition implies that a probiotic should survive GI tract passage and, according to some, colonize the host epithelium.

A variety of traits are believed to be relevant for surviving GI tract passage, the most important of which is tolerance both to the highly acidic conditions present in the stomach and to concentrations of bile salts found in the small intestine.

In vitro studies have shown that *L. acidophilus* La-14 is very resistant to low pH conditions and survive the presence of bile at concentrations present in the duodenum.

Adhesion to intestinal mucosa

Interaction with the intestinal mucosa is considered important for a number of reasons. Binding to the intestinal mucosa may prolong the time a probiotic strain can reside in the intestine. This interaction with the mucosa brings

Acid tolerance	++++ (>90% survival in hydrochloric acid and pepsin, 1%) at pH 3 for 1h at 37°C)
Bile salt tolerance	++++ (>90% survival in 0.3% bile salt containing medium)
Pepsin resistance	+++ (>60% in 0.3% pepsin containing medium at pH 2 for 1h)
Pancreatin resistance	+++ (>60% survival in 0.1% pancreatin containing medium at pH 8 for 2h)

Selected characteristics of *L. acidophilus* La-14 (internally generated data):
 +++ Excellent; +++ Very good; ++ Good; + Fair

the probiotic in close contact with the intestinal immune system, giving it a better opportunity to modulate the immune response. It may also protect against enteric pathogens by limiting their ability to colonize the intestine.

Currently, adherence is measured using two *in vitro* cell lines, Caco-2 and HT-29. While this is not a thorough test of the ability of probiotics to adhere to intestinal mucosa in the body, attachment to these cell lines is considered a good indicator of their potential to attach.

L. acidophilus La-14 has demonstrated excellent adhesion to human epithelial cell lines (HT-29) applied in *in vitro* studies.

Adherence to human intestinal cells <i>in vitro</i>	HT-29: +++++ Caco-2: +++++
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Selected characteristics of *L. acidophilus* La-14 (internally generated data): +++++ Excellent; +++ Very good; ++ Good; + Fair

Inhibition of pathogens

The protective role of probiotic bacteria against gastrointestinal pathogens is highly important to therapeutic modulation of the enteric microbiota. Probiotics are able to inhibit, displace and compete with pathogens, although these abilities are strain-dependent.

The probiotic strains' putative mechanisms of action against pathogenic microorganisms include the production of inhibitory compounds, competition with pathogens for adhesion sites or nutritional sources, inhibition of the production or action of bacterial toxins, ability to coaggregate with pathogens, and the stimulation of immunoglobulin A.

In vitro inhibition is usually investigated using an agar inhibition assay, where soft agar containing the pathogen is laid over colonies of probiotic cultures, causing the development of inhibition zones around the colonies. This effect may be due to the production of acids, hydrogen peroxide, bacteriocins and other substances that act as antibiotic agents as well as

competition for nutrients. It should be pointed out, however, that extending of such results to the *in vivo* situation is not straightforward. The assessment in the table below is based on an *in vitro* assay.

L. acidophilus La-14 displayed *in vitro* inhibition of selected pathogens.

Pathogen inhibition <i>in vitro</i>	<i>Salmonella typhimurium</i> : +++++ <i>Staphylococcus aureus</i> : +++++ <i>Escherichia coli</i> : + <i>Listeria monocytogenes</i> : +
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Selected characteristics of *L. acidophilus* La-14 (internally generated data): +++++ Excellent; +++ Very good; ++ Good; + Fair

L/D- lactic acid production

Lactic acid is the most important metabolic end product of fermentation processes by lactic acid bacteria and other microorganisms.

Due to the molecular structure, lactic acid has two optical isomers. One is known as L(+)-lactic acid and the other, its mirror image, is D(-)-lactic acid. L(+)-lactic acid is the normal metabolic intermediary in mammalian tissues. D(-)-lactic acid is normally present in the blood of mammals at nanomolar concentrations.

In the past, D(-)-lactic acid was thought to be "non-physiological" and, due to the slower metabolism in the human body, the possible cause for lactate acidosis (10, 11). In 1967, this led to a recommendation from WHO/FAO for a maximum D(-)-lactic acid intake of 100mg per kg body weight. More recent studies using modern methods have shown that, in fact, the metabolism of D(-)-lactic acid in healthy humans is comparable with L-lactate. Due to the scientific evidence, WHO/FAO withdrew this intake recommendation in 1974, but still with the restriction not to use D(-)-lactic acid in food for infants (12).

Special attention has been paid to children below the age of 12 months, because their metabolism is premature. The CODEX Standard for Infant Formula for the age group below 12 months (STAN 72-1981 revision 2007) contains the

restriction under "Optional ingredients": "Only L(+)-lactic acid producing cultures may be used" as well as for the use as acidity regulator.

This recommendation is based on three studies (13, 14, 15) in which DL-lactic acid was added to infant formulas at concentrations of 0.35 to 0.5%. Some infants in the study could not tolerate lactic acid supplementation. The effects were reversed on withdrawing these high doses of lactic acid from the diet.

In another recent study (16), healthy infants fed a D(-)-lactic acid producing *Lactobacillus* sp. at 10⁸ CFU/day from birth to 12 months demonstrated no change in serum D(-)-lactic acid levels compared to placebo-fed control, this study concluded that probiotics producing D(-)-lactic acid can be safely fed to infants.

Considering all these results, the use of D(-)-lactic acid in infant nutrition is still questioned today.

Anyhow, these concerns should not be applied directly to the use of probiotics as nutritional ingredients that do not produce lactic acid in the infant formula.

In conclusion, despite the fact that there is no real scientific consensus to suggest that healthy infants or any healthy human would be affected detrimentally by the addition of lactobacilli that produce D(-)-lactic acid, Danisco follows the CODEX recommendation not to use D(-)-lactic acid producing cultures in food for infants below the age of 12 months.

L/D-lactic acid production	60/40
Molar ratio	Boehringer Mannheim/ R-Biopharm D-lactic acid/ L-lactic acid UV-method

internally generated data

OXALATE-DEGRADING ACTIVITY

A study was undertaken to evaluate the oxalate-degrading activity of 60 *Lactobacillus* strains, including *L. acidophilus* La-14. In humans, an accumulation of oxalic acid can result in a number

of pathological conditions, including hyperoxaluria, kidney stones, renal failure, cardiomyopathy and cardiac conduction disorders.

The oxalate-degrading activity of *L. acidophilus* La-14 was found to be 100%, which was as high as the positive control *Oxalobacter formigenes* DSM 4420. The activity of other strains of *L. acidophilus* ranged from 35 – 100%. The use of probiotic strains with oxalate-degrading activity may offer the opportunity to provide this capacity to individuals suffering from oxalate associated disorders (17).

Another study, including *L. acidophilus* La-14 and *O. formigenes* DSM 4420 evaluated the kinetics of Oxalyl-coenzyme A decarboxylase (OXC), which is a key enzyme in the catabolism of the highly toxic oxalate. Steady state kinetic constants of OXC were estimated for both the bacteria. Although *L. acidophilus* La-14 provided a lower oxalate breakdown than *O. formigenes* DSM 4420, it could be a potentially useful probiotic in the prevention of diseases related to oxalate (18).

Animal studies

A study has investigated the effects of the spent culture supernatant (SCS) of *L. acidophilus* La-14 on the intestinal flora of mice with antibiotic-associated diarrhoea (19).

The microbiota imbalance in this animal model was induced by intraperitoneal administration of ampicillin 2000mg/ (kg•d) for 3 days.

In the mice administered with ampicillin, there was an obvious change in the numbers of four main groups of the microbiota. Compared with those in the control group, the numbers of *Escherichia coli* and *Enterococcus* spp. were significantly increased.

After the mice were administered with SCS, living bacteria, and SCS plus living bacteria, the numbers (compared with those in the spontaneous recovery group) of *Lactobacillus* and

Bifidobacterium had increased remarkably while the numbers of *Escherichia coli* and *Enterococcus* were significantly reduced. These results indicate that both living bacteria and SCS of *L. acidophilus* La-14 can regulate the microbiota imbalance in mice with antibiotic-associated diarrhoea, and help to recover the numbers of *Lactobacillus* and *Bifidobacterium* in the intestinal tract (19).

IMMUNOMODULATION

An immune system that functions optimally is an important safeguard against infectious and non-infectious diseases. The intestinal microbiota represent one of the key elements in the body's immune defence system. Probiotic bacteria with the ability to modulate certain immune functions may improve the response to oral vaccination, shorten the duration or reduce the risk of certain types of infection, or reduce the risk of or alleviate the symptoms of allergy and other immune-based conditions.

Modulation of the immune system is an area of intense study in relation to the Danisco probiotic range. The goal is to understand how each strain contributes to the maintenance and balance of optimal immune function. The immune system is controlled by compounds known as cytokines. Cytokines are hormone-like proteins made by cells that affect the behaviour of other cells and, thereby, play an important role in the regulation of immune system functions.

In vitro studies

In vitro assays are widely used to define the cytokine expression profiles of probiotics and, thereby determine their immunological effects. By measuring the impact of probiotic bacteria during interaction with cytokine-expressing peripheral blood mononucleocytes (PBMCs), information is generated that can help determine the ability of each strain to contribute to balanced immune health.

L. acidophilus La-14 was investigated *in vitro* for its ability to induce the PBMC secretion of selected cytokines: interleukin (IL)-10, IL-12, tumour necrosis factor (TNF)- α and interferon (IFN)- γ . The results were compared with a strain of *L. plantarum* (8826). *L. acidophilus* La-14 was found to induce IL-10, TNF- α and IFN- γ to a lesser degree than *L. plantarum*. However, *L. acidophilus* La-14 induced significantly higher PBMC excretion of IL-12 (figure 1). This is known to shift the immune system towards a so-called Th1 type of response; which plays a key role in, for example, warding off tumours and viruses and the anti-allergy response. This is also in line with the moderate induction of TNF- α and IFN- γ (results not published).

In another study including *L. acidophilus* La-14, it was investigated whether bacterial DNA is involved in the beneficial effects obtained by probiotic treatment. Peripheral blood mononuclear cells (PBMC) from healthy donors were incubated with pure DNA of probiotic strains before and after probiotic ingestion. Cytokine production was analysed in culture supernatants. It was shown that the DNA of *L. acidophilus* La-14 stimulated the secretion of IL-10 only to the same level as that induced by lipopolysaccharides (LPS) (20).

These results are in accordance with those obtained with living cells (see above). They also indicate the possible role of pure bacterial DNA in probiotic efficacy.

Animal studies

Using the cytokine expression data as a predictive test, *L. acidophilus* La-14 would not be expected to be a strong inflammation reducer due to its relatively low induction of IL-10. This has been confirmed in a chemically-induced inflammation animal model. The graph below (figure 2) depicts an inflammation score (Wallace) in which *L. acidophilus* La-14 does not significantly reduce the intestinal inflammation in this model, compared to

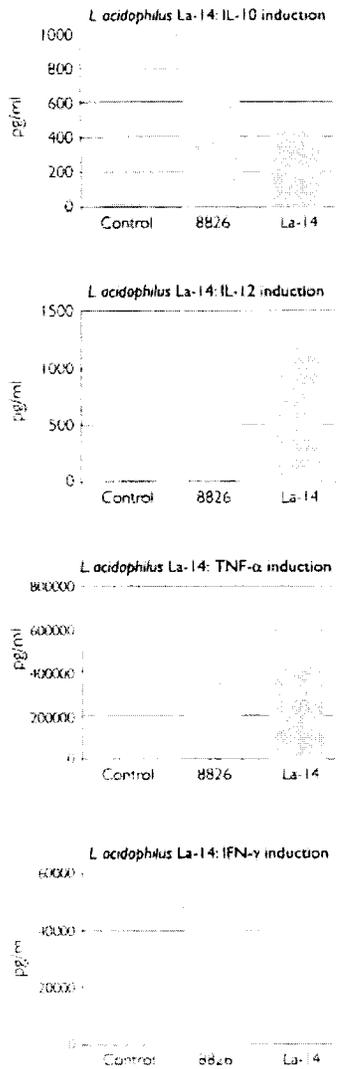


Figure 1. In vitro cytokine expression of *L. acidophilus* La-14 (internally generated data)

a control. Although *L. acidophilus* La-14 has been shown to modulate several immune response markers, according to Danisco's condition-specific approach to health benefits, the strain would not be recommended for conditions requiring downward modulation of an inflammation response. The strain is more suitable for an anti-infection and anti-allergy response (results not published).

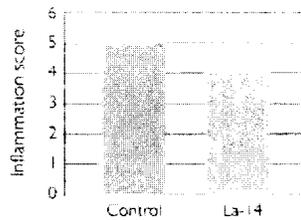


Figure 2. Influence of *L. acidophilus* La-14 on inflammation score (internally generated data).

Human studies

The ability of *L. acidophilus* La-14 to stimulate specific immunity has been evaluated in a human study measuring primary immune reaction following vaccination.

Human volunteers were orally vaccinated (using cholera vaccine as the vaccination model) and then received either a placebo (maltodextrin, n=20) or *L. acidophilus* La-14 (n=9). Supplementation with *L. acidophilus* La-14 or the placebo started on day 0 and continued for 21 days. The subjects consumed two capsules a day with 10^{10} CFU *L. acidophilus* La-14 or two capsules a day with maltodextrin (control). On day 7 and 14, the subjects received the oral vaccine. Blood samples were collected on day 0, 21 and 28, and antigen-specific antibodies (immunoglobulins, IgA, IgG, IgM) were determined. These immunoglobulins play different roles in the body's immune defence strategy.

Supplementation with *L. acidophilus* La-14 resulted in a faster and higher induction of specific IgA and IgG than in the control group. This indicates the stimulation of specific immunity by *L. acidophilus* La-14 (manuscript submitted, figure 3).

ANTIBIOTIC RESISTANCE PATTERNS

Antibiotic susceptibility patterns are an important means of demonstrating the potential of an organism to be readily inactivated by the antibiotics used in human therapy.

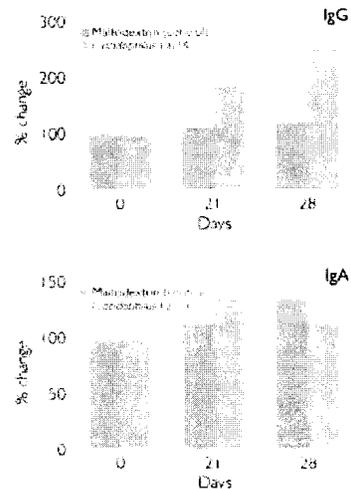


Figure 3. Relative change in specific IgG and IgA titre in orally vaccinated humans after supplementation with *L. acidophilus* La-14

Antibiotic resistance is a natural property of microorganisms and existed before antibiotics became used by humans. In many cases, resistance is due to the absence of the specific antibiotic target or is a consequence of natural selection.

Antibiotic resistance can be defined as the ability of some bacteria to survive or even grow in the presence of certain substances that usually inhibit or kill other bacteria. This resistance may be:

Inherent or intrinsic: most, if not all, strains of a certain bacterial species are not normally susceptible to a certain antibiotic. The antibiotic has no effect on these cells, being unable to kill or inhibit the bacterium.

Acquired: most strains of a bacterial species are usually susceptible to a given antibiotic. However, some strains may be resistant, having adapted to survive antibiotic exposure. Possible explanations for this include:

- A mutation in the gene coding for the antibiotic's target can make the antibiotic less efficient. This type of antibiotic resistance is usually not transferable.
- A resistance gene may have been acquired from a bacterium.

Of the acquired resistances, the latter is of most concern, as it may also be passed

on to other (potentially pathogenic) bacteria.

Much concern has arisen in recent years regarding vancomycin resistance, as vancomycin-resistant enterococci are a leading cause of hospital-acquired infections and are refractory to treatment. The transmissible nature of genetic elements that encode vancomycin resistance in these enterococci is an important mechanism of pathogenicity.

Resistance to vancomycin in certain lactobacilli, pediococci and leuconostoc is due to intrinsic factors related to the composition of their cell wall. It is not due to any transmissible elements (21).

As yet, no case of antibiotic resistance transfer has ever been identified and

reported for the lactic acid bacteria used in foods and feed.

L. acidophilus La-14 is vancomycin sensitive.

The antibiotic susceptibility patterns for *L. acidophilus* La-14 are summarised in table 1.

APPLICATIONS AND STABILITY

L. acidophilus La-14 is available as a high-count freeze-dried material with very good stability in powder formulations, taking advantage of Danisco's patented stabilisation technology, FloraFit® RT (22). *L. acidophilus* La-14 is suitable for food and dietary supplement applications.

BENEFIT SUMMARY

Extensive *in vitro* and *in vivo* studies support the health-enhancing probiotic properties of *L. acidophilus* La-14. Following is a summary of these attributes:

- Long history of safe use
- Well-suited for intestinal survival
 - High tolerance to gastrointestinal conditions (acid, bile, pepsin and pancreatin)
 - Strong adhesion to intestinal cell lines
- Ability to inhibit common pathogens
- Beneficial modulation of immune functions
 - May improve specific immune response, as demonstrated in a human clinical study (manuscript submitted)
 - May influence immune regulation, as demonstrated by the induction of IL-12 and moderate induction of tumour necrosis factors *in vitro*

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Lactobacillus acidophilus La-14 antibiogram

Amoxicillin	S
Ampicillin	S
Ceftazidime	I
Chloramphenicol	I
Ciprofloxacin	R
Clindamycin	I
Cloxacillin	S
Dicloxacillin	S
Erythromycin	S
Gentamicin	R
Imipenem	R
Kanamycin	R
Neomycin	R
Nitrofurantoin	R
Penicillin G	S
Polymixin B	R
Rifampicin	S
Streptomycin	R
Sulfamethoxazole	R
Tetracycline	R
Trimethoprim	R
Vancomycin	S

S = Susceptible (minimum inhibitory concentration ≤ 4µg/ml)

I = Intermediate (minimum inhibitory concentration = 8 to 32µg/ml)

R = Resistant (minimum inhibitory concentration ≥ 64µg/ml)

Table 1

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