# Scientific Opinion on the safety of 'heat-treated milk products fermented with Bacteroides xylanisolvens DSM 23964' as a novel food

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## SCIENTIFIC OPINION

# Scientific Opinion on the safety of 'heat-treated milk products fermented with *Bacteroides xylanisolvens* DSM 23964' as a novel food[1]

**EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA)**[2,3]

European Food Safety Authority (EFSA), Parma, Italy

## ABSTRACT

Following a request from the European Commission, the EFSA NDA Panel was asked to carry out the additional assessment for 'pasteurised milk products fermented with *Bacteroides xylanisolvens* DSM 23964' as a novel food (NF) in the context of Regulation (EC) No 258/97. Pasteurised or ultra-high-temperature-treated milk is used for the fermentation process with *B. xylanisolvens* DSM 23964. After fermentation the product is heat treated for one hour at 75 °C to ensure the absence of viable *B. xylanisolvens* DSM 23964. The Panel considers the information provided on the identity and characterisation of *B. xylanisolvens* DSM 23964 to be sufficient. The production process encompasses standard techniques used by the dairy industry, is sufficiently described by the applicant and does not give rise to safety concerns. The Panel considers that the information provided on the production process and on the content of vitamins $B_2$ and $B_{12}$ and furosine in heat-treated fermented milk products does not give rise to concerns regarding disadvantageous nutritional effects. The Panel considers that the microbiological data provided do not give rise to safety concerns. The Panel also notes that a pilot study and a RCT over six weeks with 140 volunteers receiving daily doses of a spray-dried heat-treated fermented milk product containing intakes of up to $1 \times 10^{12}$ inactivated bacterial cells of *B. xylanisolvens* DSM 23964 were provided. No clinical effects related to the treatment were observed in the two studies. Although no information has been provided to conclude on the risk of allergic reactions caused by the NF, the Panel considers that it is unlikely that its allergenic potential is dissimilar to that of other fermented dairy products. The Panel concludes that the NF 'heat-treated milk products fermented with *B. xylanisolvens* DSM 23964' is safe for the proposed uses and at the proposed use levels.



## KEY WORDS

*Bacteroides xylanisolvens*, fermentation, dairy, novel food

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[1] On request from the European Commission, Question No EFSA-Q-2014-00295, adopted on 10 December 2014.

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## SUMMARY

Following a request from the European Commission, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA Panel) was asked to carry out the additional assessment for 'pasteurised milk products fermented with *Bacteroides xylanisolvens* DSM 23964' as a novel food (NF) in the context of Regulation (EC) No 258/97, taking into account the comments and objections of a scientific nature raised by Member States.

The NF products pertaining to this application are low-fat and skimmed milk products that have been manufactured using *B. xylanisolvens* DSM 23964 as a starter culture, have been heat treated after fermentation and do not contain viable *B. xylanisolvens* bacteria. For the specification of the NF, the applicant proposes, the 'absence of viable *B. xylanisolvens*' by using a modified method for the detection and enumeration of *Enterobacteriaceae* (DIN EN ISO 21528-2) in addition to general microbiological analyses applicable for fermented milk products.

Neither *B. xylanisolvens* DSM 23964 nor any other *Bacteroides* strain has a history of use in food production and consumption.

*Bacteroides* spp. are commensal bacteria of the human intestinal microbiota, and the species *B. xylanisolvens* has been reported to be one of most abundant *Bacteroides* spp. in the human intestine. *B. xylanisolvens* DSM 23964 has been isolated from the faeces of a healthy human adult. In 2010, it was recognised as an independent strain. The sequence analysis of a 480-bp fragment of the 16S ribosomal RNA gene and DNA–DNA hybridisation analyses, as well as random amplified polymorphic DNA analysis, were used to identify this bacterium at species and strain level, respectively. The biochemical identification and characterisation of *B. xylanisolvens* DSM 23964 included 12 enzymes and 6 metabolites. The Panel considers the information provided on the identity and the genotypic and phenotypic characteristics of *B. xylanisolvens* DSM 23964 to be sufficient.

Pasteurised or ultra-high-temperature-treated (UHT) milk is used for fermentation with *B. xylanisolvens* DSM 23964. After fermentation, the product is heat treated for one hour at 75 °C to ensure the absence of viable *B. xylanisolvens* DSM 23964. The Panel notes that the production process encompasses standard techniques used by the dairy industry and considers that it is sufficiently described by the applicant and does not give rise to safety concerns. According to analyses provided by the applicant, heat treatment has no significant adverse effect on the content of vitamins $B_2$ and $B_{12}$ and lysine. Based on the information provided on the production process and composition, the Panel considers that consumption of the NF is not nutritionally disadvantageous.

The applicant provided studies evaluating the presence of antibiotic resistance and plasmids, potential virulence genes, extracellular enzymes and pathogenic factors, and the adhesion of *B. xylanisolvens* DSM 23964 to Caco-2 cells. The *cepA* gene conferring resistance to β-lactam antibiotics was identified in the genomic DNA of this strain and plasmids were not detected. The Panel notes that resistance to β-lactam antibiotics is widespread among *Bacteroides* spp. and that the *cepA* gene has not been reported to be associated with mobile elements (e.g. conjugative transposon) that mediate gene transfer. The Panel considers that the chromosomal location of *cepA*, the absence of detectable plasmids and the heat inactivation make it unlikely that horizontal gene transfer will occur from this bacterium. An abscess formation test with *B. xylanisolvens* DSM 23964 was negative. The Panel considers that the data provided do not give rise to safety concerns with regard to microbiological risks of the NF.

According to the intake estimate performed by the applicant, the estimated number of inactivated (heat-treated) *B. xylanisolvens* cells ingested with fermented milk products was similar for the different age groups. On a kilogram body weight (bw) per day basis, intake was estimated to be highest in two- to five-year-old children, with mean and 90$^{th}$ percentile values of $2 \times 10^{10}$ cells/kg bw per day and $3.8 \times 10^{10}$ cells/kg bw per day, respectively. These estimates are based on the conservative assumption that *B. xylanisolvens* DSM 23964 is used for all fermented milk products consumed.

The Panel also notes the results of a pilot study and a randomised controlled trial (RCT) in which 140 adult volunteers received daily doses of a spray-dried heat-treated fermented milk product providing up to $1 \times 10^{12}$ inactivated cells of *B. xylanisolvens* DSM 23964, which is above the 90$^{th}$ percentile intake of a conservative intake scenario. No haematological, immunological, gastrointestinal or other clinical adverse effects were observed related to the treatment in these two human studies.

Although no information has been provided to allow conclusions to be drawn on the risk of allergic reactions caused by the NF, the Panel considers that it is unlikely that the allergenic potential of the NF is dissimilar to that of other fermented dairy products.

The Panel concludes that the NF, heat-treated milk products fermented with *B. xylanisolvens* DSM 23964, is safe for the proposed uses and at the proposed use levels.

## TABLE OF CONTENTS

## BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

On 18 December 2012, the company Avitop GmbH submitted a request in accordance with Article 4 of the Novel Food Regulation (EC) No 258/97 to place on the market pasteurised milk products fermented with *Bacteroides (B.) xylanisolvens* as a novel food.

On 21 June 2013, the competent authority of Ireland (FSAI) forwarded to the Commission their initial assessment report, which came to the conclusion that pasteurised milk products fermented with *B. xylanisolvens* meet the criteria for acceptance of a novel food defined in Article (3)1 of Regulation (EC) No 258/97.

On 4 September 2013, the Commission forwarded the initial assessment report to the other Member States. Several Member States submitted comments or raised objections.

In consequence, a decision is now required by the Commission under Article 7(1) of Regulation (EC) No 258/97.

The concerns of a scientific nature raised by the Member States can be summarised as follows:

- A clear product specification is lacking, and the production organism is not fully analysed genetically. *Bacteroides xylanisolvens* is presented as a commensal in the human intestine, but information regarding this organism in scientific literature seems to be rather scarce as yet.
- There are no publications on the origin of the strain *B. xylanisolvens* DSM 23964 in contrast to the strain DSM 18836 (Chassard el al., 2008). The information on strain DSM 23964 is not as detailed as for the other one. The strain *B. xylanisolvens* DSM 23964 was only recently discovered and therefore little information is known and is closely related to *Bacteroides ovatus* which is potentially pathogenic in intra-abdominal infections.
- It would be desirable to receive meaningful data on the comparability of the products in question with traditional products.
- If pasteurisation of the milk products is unlikely to inactivate hydrolytic enzymes, enhanced colonic fermentation may increase the energy value of the consumed diet, which may not be beneficial to certain consumer groups.
- Information regarding remaining hydrolytic enzyme activities in the final pasteurised food products containing *B. xylanisolvens* is lacking. These activities may enhance hydrolysis of other food components particularly those containing dietary fibre in consumers' diet. The colonic fermentation may be enhanced to a level which may cause flatulence and intestinal discomfort in sensitive subjects.
- Because of their enzyme make-up, *Bacteroides* species are suspected of being capable of converting precarcinogens into carcinogens.
- Data for the EU on the anticipated intake of the NF including for children should be provided.
- No information is presented regarding specific vulnerable groups within the population.
- Possible allergenicity of heat-inactivated *B. xylanisolvens* has not been addressed.

## TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

In accordance with Article 29(1)(a) of Regulation (EC) No 178/2002, the European Commission asks the European Food Safety Authority to provide a scientific opinion by carrying out the additional assessment for pasteurised milk products fermented with *B. xylanisolvens* as a novel food in the context of Regulation (EC) No. 258/97.

## ASSESSMENT

In accordance with Commission Recommendation 97/618/EC, pasteurised milk products fermented with *B. xylanisolvens* was allocated to class 2.2 (complex novel food from non-GM source which has no history of food use in the Community) by the competent authority of Ireland in its initial assessment report. The assessment of the safety of this novel food (NF) is based on data supplied in the original application, the initial assessment by the competent authority of Ireland (Food Safety Authority of Ireland, FSAI), the concerns and objections of the other Member States, and the responses of the applicant. The data are required to comply with the information required for novel foods of class 2.2, i.e. structured schemes I, II, III, IX, XI, XII and XIII of Commission Recommendation 97/618/EC. In the text, these structured schemes are covered in nine sections. This assessment concerns only the risk that might be associated with consumption, and it is not an assessment of the efficacy of pasteurised milk products fermented with *Bacteroides xylanisolvens* with regard to any claimed benefit. Council Directive 92/46/EEC of 16 June 1992 states in Annex C that 'Pasteurized milk must have been obtained by means of a treatment involving a high temperature for a short time (at least 71.7 °C for 15 seconds or any equivalent combination) or a pasteurization process using different time and temperature combinations to obtain an equivalent effect.' In this opinion, the Panel therefore uses 'heat treatment' to describe the process of treatment at 75 °C for one hour, rather than 'pasteurisation', as proposed by the applicant.

### 1. Specification of the novel food

The novel food products pertaining to this application are low-fat and skimmed milk products that have been manufactured using *B. xylanisolvens* DSM 23964 as a starter culture, have been heat treated after fermentation and do not contain viable *B. xylanisolvens* bacteria.

Both the applicant and the FSAI noted that 'fermented milk products' are not standardised compositionally in the EU, but noted the Codex standard for fermented milks including heat-treated fermented milks, concentrated fermented milks and composite fermented milk products for direct consumption or further processing (Codex Standard 243-2003; FAO, 2011). This standard specifies particular starter cultures for fermented milk products but also allows for the use of 'other suitable and harmless microorganisms'.

For the specification of the NF, the applicant proposes, the 'absence of viable *B. xylanisolvens*' by using a modified method for the detection and enumeration of *Enterobacteriaceae* (DIN EN ISO 21528-2) in addition to general microbiological analyses applicable for fermented milk products. Regarding the modified DIN EN ISO 21528-2 analysis, the applicant stated that agar plates using Wilkins–Chalgren anaerobe agar CM0619 (Oxoid) were used with incubation for 48 hours at 37 °C, appropriate dilutions of *B. xylanisolvens* in Wilkins–Chalgren anaerobe broth CM0643 (Oxoid) were included as positive controls, and that all steps were conducted under anaerobic conditions.

#### Identity and characterisation of B. xylanisolvens DSM 23964

The *B. xylanisolvens* strain concerned was deposited by the applicant at the German Resource Centre for Biological Material (DSMZ) and has been assigned the reference number DSM 23964 (DSMZ, 2010). The applicant notes that, before allocation of a reference number by the DSMZ, *B. xylanisolvens* DSM 23964 was referred to as *B. xylanisolvens* CTC1. This older designation was used in early study reports. The applicant provided a signed copy from the DSMZ that confirmed the acceptance of and viability of *B. xylanisolvens* CTC1 (DSM 23964) (DSMZ, 2010).

According to the applicant, *B. xylanisolvens* DSM 23964 has been isolated from the faeces of a healthy adult human subject. *Bacteroides* spp. account for about one-quarter of all anaerobic microorganisms inhabiting the human colon (Salyers, 1984). *Bacteroides xylanisolvens* is a strictly anaerobic, Gram-negative species. The organisms are non-spore-forming, non-motile rods, which, unlike members of other *Bacteroides* spp., are unable to degrade starch (Chassard et al., 2008). *Bacteroides xylanisolvens* grows optimally at 38 °C and a pH value of about 6.8 under strictly

anaerobic conditions. It can utilise a variety of sugars, including lactose, and metabolises xylan to acetate, propionate and succinate. *Bacteroides xylanisolvens* DSM23964 was classified on the basis of its phenotypical and biochemical properties and by sequencing the 16S ribosomal RNA (rRNA) gene and by DNA–DNA hybridisation analyses (Ulsemer et al., 2012a). These authors constructed a phylogenetic tree covering the *Bacteroides* strain that is the subject of this application (DSM 23964), *B. xylanisolvens* DSM 18836 and 20 other characterised *Bacteroides* strains from other culture collections such as the American Type Culture Collection (ATCC), DSMZ or the Japan Collection of Microorganisms (JCM). In this analysis, a 480-bp sequence from the 16S rRNA of *B. xylanisolvens* DSM 23964 and *B. xylanisolvens* DSM 18836 showed 100 % similarity. The similarity to other related species was significantly lower: 97.5 %, 94.2 % and 92.2 % similarity to *B. ovatus* ATCC 8483, *B thetaiotaomicron* ATCC 29148 and *B. finegoldii* DSM 17565, respectively. DNA–DNA hybridisation performed by the DSMZ Identification Service revealed 98.65 % similarity to *B. xylanisolvens* DSM 18836 and similarities of about 25–30 % to other investigated *Bacteroides* species (including *B. ovatus* ATCC8483).

A random amplified polymorphic DNA (RAPD) analysis of *B. xylanisolvens* DSM 23964 and *B. xylanisolvens* DSM 18836 revealed different profiles, indicating that *B. xylanisolvens* DSM 23964 is a strain distinct from *B. xylanisolvens* DSM 18836 (Culebras et al., 2012).

The biochemical identification of *B. xylanisolvens* DSM 23964 was performed with rapid ID 32A and API 20A biochemical test kits (bioMerieux, Marcy l'Etoile, France) covering the activity of 12 enzymes (*N*-acetyl-β-glucosaminidase, glutamic acid decarboxylase, α-fucosidase, indole production, arginine arylamidase, phenylalanine arylamidase, leucine arylamidase, tyrosine arylamidase, glycine arylamidase, histidine arylamidase, glutamyl glutamic acid arylamidase, serine arylamidase) and six metabolites (D-mannitol, salicin, glycerol, D-melezitose, D-sorbitol, D-trehalose). *Bacteroides xylanisolvens* DSM 23964 and *B. xylanisolvens* DSM 18836 showed an identical profile, and both were different from other *Bacteroides* spp.

The applicant provided a comparison of some compositional parameters of skimmed milk fermented with *B. xylanisolvens* and of commercial milk products fermented with *Lactobacillus reuteri*, *L. rhamnosus* or yoghurt cultures, respectively (see Table 2, below). The Panel notes that information on the lactose content was not provided.

The Panel considers the information provided on the identity and the genotypic and phenotypic characteristics of *B. xylanisolvens* DSM 23964 to be sufficient.

## 2. Production process applied to the novel food

The applicant provided details about the production of the *B. xylanisolvens* DSM 23964 starter culture. Sugars such as glucose and other fermentable sugars, such as xylan-containing plant materials, may be added to aid bacterial growth during fermentation. At the end of the fermentation process, the biomass culture is recovered, washed and concentrated about 50 times by ultracentrifugation. The *B. xylanisolvens* DSM 23964 pellets obtained may be used directly as a starter culture for milk fermentation or they may be frozen and lyophilised and stored for later use. The identity of the starter culture was confirmed by molecular analyses (species-specific polymerase chain reaction (PCR) and RAPD-PCR). Routine quality control procedures applied by the dairy industry are followed in the different steps of this process.

In the production of milk products fermented with the *B. xylanisolvens* DSM 23964 starter culture, low-fat milk (< 1.8 % fat) or skimmed milk (< 0.3 % fat) is pasteurised or ultra-heat treated immediately before starting the fermentation process. After inoculation with the starter culture, the fermentation is run at 38.5 °C under constant gassing with carbon dioxide and without stirring for 14 to 16 hours. The milk product is homogenised at the end of the fermentation process and then heat treated at 75 °C for one hour to inactivate the starter culture. For quality control purposes, samples are taken before and after the second heat treatment. The fermented milk product may then be either

packaged like a traditional liquid fermented milk product or spray dried for use as a fermented milk powder. Quality control tests, including microbiological tests in accordance with the European Pharmacopoeia, are conducted throughout the process to ensure the absence of contaminating microorganisms and to check the absence of viable *B. xylanisolvens* in the final product.

The applicant also provided a study report on the effect of the heat treatment after fermentation of the milk with *B. xylanisolvens* DSM 23964 at either 75 °C for various times (15, 30, 60, 120, 180 seconds) or 65 °C for 30 minutes (Toutounian, 2008). Heat-treated milk before inoculation of *B. xylanisolvens* DSM 23964 was used as negative control. Heat-treated and fermented milk before the second heat treatment but after fermentation was used as positive control. No microbial colony could be observed on any of the samples heat treated after fermentation under cultivation conditions (Wilkins–Chalgren agar, anaerobic conditions).

In response to a question raised by a Member State, the applicant answered that the standards (Good Manufacturing Practice, GMP) and quality control measures (Hazard Analysis and Critical Control Point, HACCP) that are applied in the production of the NF are up to date and that the compliance of the production of the NF with the applicable standards for food production will fall within the scope of the same rules and within the competence of the same authorities as the production of the corresponding traditional foods.

The Panel notes that the production process encompasses standard techniques used by the dairy industry and considers that it is sufficiently described by the applicant and does not raise safety concerns.

## 3. History of the organism used as a source

*Bacteroides* spp. account for about one-quarter of all anaerobic microorganisms inhabiting the human colon (Salyers, 1984). *Bacteroides* spp. are largely non-pathogenic commensals, although certain strains of the species *B. fragilis* have been reported to be potentially pathogenic and toxigenic (Brook, 1989; Sánchez et al., 2012).

*Bacteroides xylanisolvens* is a regular commensal of the human intestinal microbiota (Marschal et al., 2011; Ponnusamy et al., 2011; Zitomersky et al., 2011). Kulagina et al. (2012) reported that *B. xylanisolvens*, together with *B. vulgatus* and *B. uniformis*, were the most prevalent and abundant *Bacteroides* species in the faeces of 36 healthy subjects aged 1 – 33 years, in whom bacterial counts of these species were in the order of 8.3 – 9.9 lg colony-forming units (CFU)/g faeces.

Neither *B. xylanisolvens* DSM 23964 nor any other *Bacteroides* strain has a history of use in food production and consumption.

## 4. Anticipated intake/extent of the use of the novel food

The applicant intends to market the NF in liquid and semi-liquid forms in fermented low-fat and skimmed milk products, i.e. fermented milks, buttermilks, yoghurts and yoghurt drinks, and as spray-dried powder to be used like yoghurt powders, e.g. in fillings and coatings of cereals, cereal bars, fruits and nuts. The final heat-treated fermented milk products may also be supplemented with other ingredients such as sugars, flavours, fruit preparations and fibre.

The intakes of non-fat milk solids were estimated using US consumption data. The applicant stated that equivalent European data were not available to him. The applicant considered a conservative scenario in which the NF was assumed to replace all the yoghurts, buttermilks and acidophilus milks. Based on the US National Health and Nutrition Examination Survey (NHANES) food intake data and the associated Food Commodity Intake Database of raw agricultural consumption data, mean daily intakes of non-fat solids from yoghurts, buttermilks and acidophilus milks for all survey participants (> 2 years of age) were estimated as 1.6 g per day rising to 6.3 g per day for the 90$^{th}$ percentile

(Appendix A). Considering consumers only, the corresponding figures were 5.5 g per day and 15.3 g per day, respectively.

The highest mean and 90th percentile values for daily intakes were estimated for consuming children aged 2–5 years (6.4 g per day and 14.9 g per day, respectively) and for males aged 20 years or older (5.4 g per day and 17.4 g per day, respectively). On a per kilogram body weight per day basis, the highest daily intakes of all fermented non-fat milk solids combined were reported for children aged 2–5 years (mean 0.38 g/kg bw per day; 90th percentile 0.96 g/kg bw per day). When considering only yoghurt consumers, the highest mean and 90th percentile intake on a per kilogram body weight per day basis was estimated for children aged 2–5 years (0.57 and 1.1 g/kg bw per day).

In a second step, the applicant estimated the level of intake of the heat-inactivated starter culture (*B. xylanisolvens* DSM 23964) via the consumption of yoghurts, which represents the highest potential intake of the NF. The estimated ingestion of non-viable cells of *B. xylanisolvens* in fermented milk products expressed as CFU was similar for the different age groups. On a per kilogram body weight per day basis it was highest for two- to five-year-old old children (mean $2 \times 10^{10}$ CFU/kg bw per day; 90th percentile $3.8 \times 10^{10}$ CFU/kg bw per day), which could be considered as a conservative scenario (Table 1). The 90th percentile intake of 964 mg of non-fat milk solids from all fermented milk products, combined per kilogram body weight by children aged 2–5 years, would correspond to an intake of $3.4 \times 10^{10}$ CFU of non-viable *B. xylanisolvens* per kilogram body weight per day for this population group.

**Table 1:** Estimated intake of non-fat milk solids and heat-inactivated *B. xylanisolvens* DSM 23964 for the mean and the 90th percentile consumption of yoghurt by age group

| Age group, gender | Non-fat milk solids | | | | *B. xylanisolvens* DSM 23964 | | | |
|---|---|---|---|---|---|---|---|---|
| | g per day | | mg/kg bw per day | | Cells per day [(a)] | | Cells/kg bw per day | |
| | Mean | 90th | Mean | 90th | Mean | 90th | Mean | 90th |
| 2–5 years | 9.5 | 16.5 | 570 | 1084 | $3.4 \times 10^{11}$ | $5.9 \times 10^{11}$ | $2.0 \times 10^{10}$ | $3.8 \times 10^{10}$ |
| 6–10 years | 9.6 | 20.5 | 357 | 793 | $3.4 \times 10^{11}$ | $7.3 \times 10^{11}$ | $1.3 \times 10^{10}$ | $2.9 \times 10^{10}$ |
| 11–19 years, m | 12.7 | 25.0 [(a)] | 225 | 475 [(a)] | $4.5 \times 10^{11}$ | $8.9 \times 10^{11}$ | $0.80 \times 10^{10}$ | $1.7 \times 10^{10}$ |
| 11–19 years, f | 11.0 | 23.1 | 190 | 403 | $3.9 \times 10^{11}$ | $8.1 \times 10^{11}$ | $0.68 \times 10^{10}$ | $1.4 \times 10^{10}$ |
| > 20 years, m | 12.1 | 23.1 | 147 | 282 | $4.3 \times 10^{11}$ | $8.2 \times 10^{11}$ | $0.52 \times 10^{10}$ | $1.0 \times 10^{10}$ |
| > 20 years, f | 11.7 | 22.5 | 171 | 339 | $4.2 \times 10^{11}$ | $8.1 \times 10^{11}$ | $0.61 \times 10^{10}$ | $1.2 \times 10^{10}$ |
| All | 11.4 | 22.5 | 225 | 464 | $4.1 \times 10^{11}$ | $7.9 \times 10^{11}$ | $0.80 \times 10^{10}$ | $1.6 \times 10^{10}$ |

[(a)] Calculated on basis of the assumption that 100 ml heat-treated fermented low-fat or non-fat milk product contains 14 g non-fat milk solids and $0.5 \times 10^{12}$ CFU of non-viable *B. xylanisolvens* DSM 23964.

## 5. Nutritional information on the novel food

The compositional data provided by the applicant includes information on macronutrients (Table 2).

**Table 2:** Composition of spray-dried skimmed milk cultured with *B. xylanisolvens* DSM 23964 and commercial fermented milk products

| Composition (g/100 g) | Skimmed milk with *B. xylanisolvens* | Fermented milk *L. reuteri* [(a)] | Fermented milk *L. rhamnosus* [(a)] | Low-fat yoghurt [(b)] |
|---|---|---|---|---|
| Carbohydrates | 48.8 | 37.3 | 41.0 | 35.7 |
| Glucose | 3.6 | 0.4 | < 0.10 | n.a. |
| Fructose | < 0.10 | < 0.10 | < 0.10 | n.a. |
| Saccharose | 1.5 | 0.15 | 0.15 | n.a. |
| Maltose | < 0.10 | < 0.10 | < 0.10 | n.a. |
| Protein [(c)] | 30.9 | 32.5 | 32.1 | 42.6 |

| Composition (g/100 g) | Skimmed milk with *B. xylanisolvens* | Fermented milk *L. reuteri* [(a)] | Fermented milk *L. rhamnosus* [(a)] | Low-fat yoghurt [(b)] |
|---|---|---|---|---|
| Fat [(d)] | 0.4 | 13.9 | 14.0 | 0.98 |
| Saturated | 0.21 | 9.78 | 9.86 | n.a. |
| MUFAs | 0.12 | 3.27 | 3.28 | n.a. |
| PUFAs | 0.014 | 0.36 | 0.36 | n.a. |
| *trans*-fats | 0.012 | 0.36 | 0.37 | n.a. |
| Ash | 8.17 | 6.78 | 6.73 | 8.8 |
| Total acid [(e)] | 4.84 | 5.62 | 2.51 | 11.9 |
| Purine nitrogen | 0.025 | 0.012 | 0.013 | n.a. |
| Water | 6.86 | 3.91 | 3.67 | – |

(a): Product prepared by Avitop (similar process as that for skimmed milk fermented with *B. xylanisolvens*, except for the applied starter culture and the addition of glucose).
(b): Souci et al., 1994—values calculated on dry matter basis.
(c): Kjeldahl analysis (N × 6.25); Codex Standard 243-2003: > 2.7 %.
(d): Weibull–Stoldt analysis; Codex Standard 243-2003: < 10 %.
(e): Titration-calculated as citric acid; Codex Standard 243-2003: > 0.3 %.
n.a., not available; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.

In order to address effects of the long heat treatment process (75 °C for 60 minutes) on the vitamins for which fermented milk products could be a relevant source and on the extent of Maillard reactions which could effect the lysine content, EFSA asked the applicant to provide analyses of the content, in at least three batches, of vitamins $B_2$ and $B_{12}$ and lysine, as well as furosine, as a marker for Amadori products formed from early Maillard reactions before and after this heat treatment. The results of these analyses are provided in Table 3. According to these results, there is no effect of this heat treatment step on the vitamin $B_2$ and vitamin $B_{12}$ content. The measurement of 'free lysine' is considered not an appropriate parameter to address potential lysine loss by Maillard reaction, but, considering the results for furosine, the Panel considers that the one-hour heat treatment does not have a significant effect on the lysine content.

**Table 3:** Effect of the heat treatment (75 °C for 60 minutes) on the content of certain heat-sensitive nutrients and furosine in UHT low-fat milk before and after fermentation with *B. xylanisolvens* DSM 23964

| | UHT control | Batch No 1 | Batch No 2 | Batch No 3 | UHT control | Batch No 1 | Batch No 2 | Batch No 3 | UHT control | Batch No 1 | Batch No 2 | Batch No 3 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 min | | | | 5 min | | | | 60 min | | | |
| Vitamin $B_2$ (mg/l) | 1.8 | 1.6 | 1.7 | 1.7 | 1.9 | 2.1 | 2.1 | 1.9 | 1.7 | 1.8 | 1.7 | 1.7 |
| Vitamin $B_{12}$ (µg/l) | 1.4 | 1.5 | 1.4 | 1.3 | 1.3 | 1.4 | 1.3 | 1.3 | 1.3 | 1.3 | 1.1 | 1.2 |
| Free lysine (mg/l) | 3.5 | 25.7 | 25.5 | 24.8 | 2.7 | 23.1 | 24.2 | 23.5 | 3.1 | 21.6 | 18.9 | 18.8 |
| Furosine (mg/kg) | 26.6 | 26.6 | 26.1 | 24.8 | 27.1 | 25.6 | 25.9 | 26.0 | 29.0 | 26.9 | 27.0 | 27.5 |

In response to the question raised by EFSA regarding the lactose content of the NF, the applicant stated that lactose content has not been analysed, but that it could be estimated by the difference between the figures given for total carbohydrates and the specifically determined sugars (glucose, fructose, saccharose and maltose) provided in Table 2. The applicant estimated that 43.7 % of the total solids of fermented milk would be lactose, which would be within the range of low-fat dried dairy products available on the market.

Based on the information provided on the production process and composition, the Panel considers that consumption of the NF is not nutritionally disadvantageous.

## 6. Microbiological information on the novel food

The applicant and the FSAI noted that the use of *Bacteroides* in food production was not reported in the EU before May 1997 and that *B. xylanisolvens* was not assessed under the Qualified Presumed Safety (QPS) scheme at the time when this novel food was assessed by Member States. However, a QPS assessment is triggered when EFSA receives an application for a novel bacterial strain or a product which intentionally contains novel bacteria. Thus, in 2014, the EFSA Panel on Biological Hazards has assessed *B. xylanisolvens* under the QPS scheme (EFSA BIOHAZ Panel, 2014). The QPS assessment by the Panel on Biological Hazards is independent of the assessment of application dossiers, which remain the responsibility of the EFSA Panel to which the risk assessment is mandated. The QPS assessment therefore does not consider dossier-specific data such as the production process (e.g. pasteurisation or inactivation steps) or unpublished data contained in application dossiers. Further, the EFSA Panel on Biological Hazards noted that, although no safety concerns have been observed (as only a β-lactamase resistance gene (*cepA*) has been identified in the genomic DNA, which is unlikely to be transferable), the studies published on *B. xylanisolvens* are not sufficient for the inclusion of this species in the QPS list.

In addition to the information given in section 1 on the identification and phenotypic and genotypic characterisation of *B. xylanisolvens* DSM 23964, the information provided by the applicant also contained an examination of the presence of antibiotic resistance and plasmids, potential virulence genes, extracellular enzymes and pathogenic factors, and determination of the adhesion of *B. xylanisolvens* DSM 23964 to Caco-2 cells (Ulsemer et al., 2012a).

According to the results of the investigations by Ulsemer et al. (2012a), *B. xylanisolvens* DSM 23964 is resistant to β-lactam antibiotics owing to the presence of β-lactamase activity encoded by the *cepA* gene. This gene (encoding a class 2e cephalosporinase) has not been previously associated with mobile elements (e.g. conjugative transposon) that facilitate gene transfer and was identified in the genomic DNA of this strain. This resistance to β-lactam antibiotics is widespread among species of the genus *Bacteroides* (Wexler, 2007; Eitel, 2013). A test determining plasmids with a detection limit of about 150 kb and using *Escherichia coli* strains containing low-copy-number plasmids as positive controls was negative, suggesting the chromosomal location of the β-lactamase gene. *Bacteroides xylanisolvens* DSM 23964 was sensitive to the antibiotics metronidazole, meropenem agents and clindamycin.

The Panel considers that, owing to the chromosomal location of the *cepA* gene, the absence of detectable plasmids (with a detection limit of 150 kb) and the heat inactivation, gene transfer is not expected to occur.

Analyses of *B. xylanisolvens* DSM 23964 for eight potential virulence genes, as identified for *B. fragilis*, *B. caccae* and *B. ovatus*, provided negative results (Ulsemer et al., 2012a). The established strains and clinical isolates of these three species were used as positive controls for potential virulence factors and production of extracellular enzymes.

The most relevant exoenzyme activities were analysed by means of PCR (for the neuraminidase gene) or with enzymatic assays. *Bacteroides xylanisolvens* DSM 23964 showed no DNase, chondroitinase, hyaluronidase or neuraminidase activity and only very weak β-haemolytic and collagenase activities (Ulsemer et al., 2012a).

*Bacteroides xylanisolvens* DSM 23964 differs from three *B. ovatus* strains, DSM 1896 and two clinical isolates (MN23 and MN7) in several of the investigated characteristics: *B. ovatus* strains were positive for the *cefoxitin (cfxA*) gene, which was reported to be horizontally transmitted (in addition to *cepA*) and were used as a positive controls for neuraminidase, haemolysin and collagenase activity.

*Bacteroides xylanisolvens* DSM 23964 was negative in an adhesion test with Caco-2 cells compared with *L. acidophilus* DSM 9126 and *B. fragilis* DSM 1396, which were used as positive controls.

Based on available information as well as data on *B. xylanisolvens* DSM 23964 provided by the applicant, the German Federal Institute for Occupational Safety and Health (BAuA) allocated *B. xylanisolvens* to risk group 1 (RG1), which contains those microorganisms which in their viable form can be handled without a health risk to humans (and other vertebrates), according to current knowledge (BAuA, 2011). Thus, *B. xylanisolvens* DSM 23964 is considered to belong to those biological agents that are unlikely to cause human disease, as defined in Directive 2000/54/EC on the protection of workers from risks related to exposure to biological agents at work (Deutsche Gesetzliche Unfallversicherung, 2010).

The Panel considers that the data provided are sufficient and do not raise safety concerns with regard to the microbiological risks of the NF.

## 7. Toxicological information on the novel food

The toxicological evaluation covered the genotoxicity and subchronic toxicity of heat-inactivated and non-heat-inactivated *B. xylanisolvens* DSM 23964 examined by studies that followed the appropriate OECD guidelines and GLP. In addition, an abscess formation test was conducted in mice to investigate the potential pathogenicity of the *B. xylanisolvens* DSM 23964.

### 7.1. Genotoxicity

The applicant provided the study reports on an *in vitro* test for gene mutations (Ames test) and on an *in vitro* chromosome aberration test using lyophilisate containing purified live and heat-inactivated *B. xylanisolvens* DSM 23964 in accordance with OECD Guidelines 471 and 473 with negative outcomes (Leuschner 2010a, b; Ulsemer et al., 2012b). However, the Panel considers that there is no rationale for genotoxicity testing of live and heat-inactivated microorganisms such as *B. xylanisolvens* DSM 23964.

### 7.2. Subchronic toxicity

The toxicity of *B. xylanisolvens* DSM 23964 was examined in a 90-day oral toxicity study following OECD Guideline 408 (Leuschner, 2010c; Ulsemer et al., 2012b). Five dose groups (10 male and 10 female animals per group) were studied: each mouse received by gavage daily doses of either $1 \times 10^9$ viable, $1 \times 10^{10}$ viable, $1 \times 10^{11}$ viable or $1 \times 10^{11}$ heat-inactivated cells of *B. xylanisolvens* DSM 23964 or the vehicle (control). No mortality was noted during the course of the study. No differences between groups were found in behaviour, external clinical appearance, faeces and functional observations between the animals treated with viable or inactivated *B. xylanisolvens* DSM 23964. Nor were any differences in body weight or food or water consumption observed. No treatment-related effects were found in the haematological and clinical biochemical, ophthalmological, macroscopic post-mortem examinations, organ weights or histology. As for genotoxicity testing, the Panel considers that there is no rationale for toxicity testing of live and heat-inactivated microorganisms such as *B. xylanisolvens* DSM 23964.

### 7.3. Abscess formation test

*Bacteroides xylanisolvens* DSM 23964 was also studied in an intraperitoneal abscess formation model in mice (Toutounian, 2010, unpublished; Ulsemer et al., 2012a). Twenty-six male Webster mice were used for this experiment. The mice were divided into seven groups, of which three groups received an intraperitoneal injection of 200 µl of viable *B. xylanisolvens* DSM 23964 at three different doses ranging from $5 \times 10^6$ to $1 \times 10^9$ CFU per mouse. Three groups received the same amounts of *B. fragilis* RMA 6791 as a positive control and one group received sterilised supernatant of phosphate-buffered saline-suspended rat faeces (negative control). *B. fragilis* RMA 6791 induced abscess formation in all three dose groups, confirmed by species-specific PCR, while no mouse receiving *B. xylanisolvens* DSM 23964 developed an abscess.

### 7.4. Human data

The safety of the *B. xylanisolvens* fermented milk product was addressed in one pilot study and a randomised controlled trial (RCT).

In a three-week non-controlled pilot study, two groups, each of 10 male and 10 female volunteers, consumed daily portions of 100 ml heat-treated low-fat milk cultured with *B. xylanisolvens* ($5 \times 10^{11}$ or $8 \times 10^{11}$ inactivated cells/portion) and flavoured with cherry pulp. The product was well tolerated and no significant effects were observed in the parameters (haematological analyses, immunoglobulins, cytokines, phagocytosis, NK cells) monitored (Ulsemer et al., 2012c).

In a subsequent RCT over six weeks, 140 adult volunteers were divided into four groups, which received, once per day, (1) milk powder as a placebo or spray-dried heat-treated milk fermented by *B. xylanisolvens* DSM 23964; (2) $1 \times 10^{10}$ CFU *B. xylanisolvens* DSM 23964; (3) $2.5 \times 10^{11}$ CFU *B. xylanisolvens* DSM 23964; or (4) $1 \times 10^{12}$ CFU *B. xylanisolvens* DSM 23964 that had been inactivated. No gastrointestinal problems occurred. No effects on the clinical, haematological and immunological parameters analysed were observed (Ulsemer et al., 2012c). No information on the source of the milk used for fermentation was provided in the article, but the applicant informed EFSA that this was cow's milk.

In addition to the human studies, the applicant also conducted an extensive literature search and investigations of the Center for Disease Control and Prevention and the Food and Drug Administration's databases, including Medwatch, which did not reveal any report of food poisoning or human disease associated with *B. xylanisolvens*.

## 8. Allergenicity

No data regarding allergenicity of the novel food were provided by the applicant.

The Panel notes that the effects of heat treatment of milk on its allergenicity have been considered by EFSA (EFSA NDA Panel, 2014).

Although no information has been provided to allow conclusions to be drawn on the risk of allergic reactions caused by the NF, the Panel considers that it is unlikely that the allergenic potential of the NF is dissimilar to that of other fermented dairy products.

## DISCUSSION

The NF products pertaining to this application are heat-treated low-fat and skimmed milk products, including spray-dried products, fermented with *B. xylanisolvens* DSM 23964. The applicant noted that the NF should comply with the Codex Standard for fermented milk and fermented milk products and with microbiological criteria in EU regulations set for fermented milk products. In addition, the absence of viable *B. xylanisolvens* DSM 23964 was proposed by the applicant to serve as the specification of this NF.

*Bacteroides xylanisolvens* is a commensal bacterium of the human intestinal microbiota and has been reported to be one of most abundant *Bacteroides* species in the human intestine. *Bacteroides xylanisolvens* DSM 23964 has been isolated from the faeces of a healthy human adult. It was characterised at strain level and differentiated from the type strain *B. xylanisolvens* DSM 18836. The sequence analysis of a 480-bp fragment of the 16S rRNA gene and DNA–DNA hybridisation, as well as RAPD analysis, were used to identify this bacterium at species and strain level, respectively. The biochemical identification and characterisation of *B. xylanisolvens* DSM 23964 included the detection of 12 enzyme activities and 6 metabolites. The Panel considers the information provided on the identity of *B. xylanisolvens* DSM 23964 to be sufficient.

Pasteurised or UHT skimmed or low-fat milk is used for the fermentation process with *B. xylanisolvens* DSM 23964. After fermentation the product is heat treated for one hour at 75 °C to ensure the absence of viable *B. xylanisolvens* DSM 23964. The Panel notes that the production process encompasses standard techniques used by the dairy industry and considers that it is sufficiently described by the applicant and does not give rise to safety concerns. The Panel also considers that the information provided on the production process and on its effect on the content of vitamins $B_2$ and $B_{12}$ and furosine of the heat-treated fermented milk products do not give rise to concerns regarding disadvantageous nutritional effects.

The applicant provided studies evaluating the antibiotic resistances and the presence of related genes and plasmids, potential virulence genes, extracellular enzymes and pathogenic factors and the adhesion of *B. xylanisolvens* DSM 23964 to Caco-2 cells. The *cepA* gene conferring resistance to β-lactam antibiotics was identified in the genomic DNA of this strain. The Panel notes that this resistance to β-lactam antibiotics is widespread among species of the genus *Bacteroides* and that the *cepA* gene has not been previously associated with mobile elements (e.g. conjugative transposon) that facilitate gene transfer. The Panel considers that the chromosomal location of *cepA*, the absence of detectable plasmids (detection limit 150 kb) and the heat inactivation make it unlikely that horizontal gene transfer occurs from the starter culture. *Bacteroides xylanisolvens* DSM 23964 did not show potential pathogenic features and adverse enzymatic activities such as DNase, chondroitinase, hyaluronidase or neuraminidase activity and only very weak β-haemolytic and collagenase activity. An abscess formation test with viable *B. xylanisolvens* DSM 23964 cells was negative. The Panel considers that the microbiological information does not give rise to safety concerns.

According to the intake estimate performed by the applicant, the ingestion of inactivated (non-viable) cells of *B. xylanisolvens*, expressed as CFUs, ingested with fermented milk products was similar for the different age groups. On a kilogram body weight per day basis, intake was estimated to be highest in two- to five-year-old children, with mean and 90$^{th}$ percentile values of $2 \times 10^{10}$ CFU/kg bw per day and $3.8 \times 10^{10}$ CFU/kg bw per day, respectively. These estimates are based on the conservative assumption that *B. xylanisolvens* DSM 23964 is used for all consumed fermented milk products.

The Panel also notes a pilot study and a RCT in which 140 adult volunteers received daily doses of a spray-dried heat-treated fermented milk product providing up to $1 \times 10^{12}$ inactivated CFU of *B. xylanisolvens* DSM 23964, which is above the 90$^{th}$ percentile intake of a conservative intake scenario. No haematological, immunological, gastrointestinal or other clinical adverse effects were observed related to the treatment in these two human studies.

Although no information has been provided to allow conclusions to be drawn on the risk of allergic reactions caused by the NF, the Panel considers that it is unlikely that the allergenic potential of the NF is dissimilar to that of other fermented dairy products.

## CONCLUSIONS

The Panel concludes that the novel food 'heat treated milk products fermented with *Bacteroides xylanisolvens* DSM 23964' is safe for the proposed uses and at the proposed use levels.

## DOCUMENTATION PROVIDED TO EFSA

1. Dossier on 'pasteurised milk products fermented with *Bacteroides xylanisolvens* DSM 23964' by Bioresco on behalf of Avitop GmbH, received on 10 April 2014. Additional information was provided on 10 October and 28 November 2014.

2. Letter from the European Commission to the European Food Safety Authority with the request for an opinion on the safety of 'pasteurised milk products fermented with *Bacteroides xylanisolvens* as a novel food'. Ref. Ares (2014)1132458-10/04/2014.

3. Initial assessment report carried out by the competent authority of Ireland 'Safety assessment of pasteurised milk products fermented with *Bacteroides xylanisolvens* DSM 23964'.

4. Member States' comments and objections.

5. Response by the applicant to the initial assessment report and the Member States' comments and objections.

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## Appendix A. Estimated daily intake of milk non-fat solids from fermented milk products by the US population, NHANES 2003–2008

| Fermented milk category and subpopulation | Unweighted sample size | Unweighted number of users | % Users | Estimated intake of non-fat milk solids (g per day) | | | | Estimated intake of non-fat milk solids (mg/kg bw per day) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Per capita | | Per user | | Per capita | | Per user | |
| | | | | Mean | 90th | Mean | 90th | Mean | 90th | Mean | 90th |
| **Yogurt** | | | | | | | | | | | |
| US 2+ years | 22 602 | 2 727 | 13 | 1.5 | 5.7 | 11.4 | 22.5 | 30 | 87 | 225 | 464 |
| Children 2–5 years | 2 149 | 467 | 25 | 2.4 | 9.6 | 9.5 | 16.5 | 144 | 529 | 570 | 1084 |
| Children 6–10 years | 2 202 | 311 | 15 | 1.4 | 4.7 | 9.6 | 20.5 | 52 | 159 | 357 | 793 |
| Males 11–19 years | 2 667 | 155 | 8 | 1.0 | 0 | 12.7 | 25.0 [(a)] | 17 | 0 | 225 | 475 [(a)] |
| Females 11–19 years | 2 719 | 257 | 10 | 1.1 | 0.7 | 11.0 | 23.1 | 19 | 13 | 190 | 403 |
| Males 20+ years | 6 113 | 521 | 9 | 1.1 | 0 | 12.1 | 23.1 | 14 | 0 | 147 | 282 |
| Females 20+ years | 6 752 | 1 016 | 16 | 1.9 | 8.1 | 11.7 | 22.5 | 27 | 116 | 171 | 339 |
| **Buttermilk and acidophilus milk** | | | | | | | | | | | |
| US 2+ years | 22 602 | 4 144 | 20 | 0.1 | 0.3 | 0.7 | 1.0 | 1.9 | 3.9 | 9.8 | 15.4 |
| Children 2–5 years | 2 149 | 308 | 17 | 0.1 | 0.1 | 0.3 | 0.7 | 2.9 | 6.2 | 17.0 | 35.7 |
| Children 6–10 years | 2 202 | 425 | 20 | 0.1 | 0.2 | 0.3 | 0.7 | 2.2 | 6.6 | 11.4 | 27.8 |
| Males 11–19 years | 2 667 | 427 | 16 | 0.1 | 0.2 | 0.5 | 1.1 | 1.2 | 3.1 | 7.2 | 17.0 |
| Females 11–19 years | 2 719 | 552 | 22 | 0.1 | 0.3 | 0.4 | 0.8 | 1.4 | 4.5 | 6.2 | 14.6 |
| Males 20+ years | 6 113 | 1 007 | 17 | 0.2 | 0.3 | 1.1 | 1.4 | 2.3 | 3.0 | 13.7 | 14.8 |
| Females 20+ years | 6 752 | 1 425 | 23 | 0.1 | 0.3 | 0.5 | 0.9 | 1.6 | 4.1 | 7.1 | 12.7 |
| **All fermented milk products combined** | | | | | | | | | | | |
| US 2+ years | 22 602 | 6 359 | 30 | 1.6 | 6.3 | 5.4 | 15.3 | 31 | 94 | 104 | 288 |
| Children 2–5 years | 2 149 | 699 | 38 | 2.4 | 9.7 | 6.4 | 14.9 | 146 | 531 | 383 | 964 |
| Children 6–10 years | 2 202 | 675 | 32 | 1.5 | 4.7 | 4.6 | 13.8 | 54 | 161 | 171 | 500 |
| Males 11–19 years | 2 667 | 549 | 22 | 1.1 | 0.7 | 4.8 | 15.0 | 19 | 11 | 84 | 247 |
| Females 11–19 years | 2 719 | 758 | 30 | 1.2 | 1.4 | 4.0 | 13.8 | 21 | 26 | 69 | 233 |
| Males 20+ years | 6 113 | 1 454 | 25 | 1.3 | 1.4 | 5.4 | 17.4 | 16 | 16 | 65 | 207 |
| Females 20+ years | 6 752 | 2 224 | 35 | 2.0 | 8.7 | 5.7 | 15.5 | 29 | 119 | 82 | 243 |

(a): Estimates may be less statistically reliable based on the sample size.

## ABBREVIATIONS

| | |
|---|---|
| ATCC | American Type Culture Collection |
| bw | body weight |
| DSMZ | German Resource Centre for Biological Material |
| FAO | Food and Agriculture Organisation of the United Nations |
| GMP | Good Manufacturing Practice |
| HACCP | Hazard Analysis and Critical Control Point |
| JCM | Japan Collection of Microorganisms |
| kb | kilobase |
| NF | novel food |
| PCR | polymerase chain reaction |
| RAPD | random amplified polymorphic DNA |