Safety assessment of pasteurised milk products fermented with *Bacteroides xylanisolvens* DSM 23964

Name of Applicant: **Avitop GmbH**
Robert Rössle Str. 10
D-13125 Berlin
Germany

Contact person(s): Dr. Albert Bär, Bioresco

Novel Food Classification: 2.2

Introduction

An application for the authorisation of pasteurised milk products fermented with *Bacteroides xylanisolvens* (DSM 23964) was submitted to the Food Safety Authority of Ireland (FSAI) by Avitop GmbH, Berlin in accordance with *Article 4* of the Novel Food Regulation (EC) No. 258/97. The application was accepted by the FSAI on the 18th of December, 2012.

The intensive production of many fermented foods involves well defined bacterial starter cultures, though there are a few on the EU market for which the precise identity of the functional microorganisms involved are unknown. It is estimated that 250 bacterial species belonging to 60 genera have a history of safe use in fermented foods, or as adventitious components in food. However, the use of *Bacteriodes* in food production in the EU before May 1997 has not been reported, and *B. xylanisolvens* has not been subject to the qualified presumption of safety (QPS) procedure by EFSA. Therefore, foods produced *via* fermentation with *B. xylanisolvens* fall within the scope of the novel food Regulation (EC) No 258/97.

The German Federal Institute for Occupational Safety and Health (BAuA) has assigned *B. xylanisolvens* Risk Group 1 status (microorganisms which in their viable form can be handled without posing a health risk to humans). The applicant proposes that except for a minor variance in acidity and texture characteristics, milk products fermented by *B. xylanisolvens* closely resemble those fermented with more traditional starter cultures such as *Lactobacilli, Bifidobacteria* and *Streptococci* subspecies. The

applicant intends to market this novel ingredient in liquid and semi-liquid form, to be used as an alternative to existing low-fat or non-fat yoghurt, acidophilus milk, or cultured buttermilk. A spray-dried form will also be available for use in fillings and coatings.

The applicant considered the precise classification of this novel food to be difficult as in their view it could fit into a few categories. However, they conclude that the novel food is best placed in the category of "foods and food ingredients consisting of or isolated from micro-organisms, fungi or algae" in line with *Article 1(2)(d)* of the Novel Food Regulation (EC) No 258/97. For the purposes of the safety assessment, the applicant places the novel food in Class 2.2 in accordance with Commission Recommendation 97/618/EC: (complex novel food from non-GMO sources – the source of the novel food has no history of food use in the Community).

I. Specification of the novel food

The novel food that is the subject of this application is a pasteurised milk product fermented with *B. xylanisolvens* DSM 23964 in which the bacterium has been heat-inactivated. Fermented milk products are not standardised compositionally in the EU, though a Codex standard (Standard 243-2003) does exist for fermented milks including heat treated fermented milks, concentrated fermented milks and composite milk products based on these products for direct consumption or further processing. This standard specifies particular starter cultures for fermented milk products but also allows for the use of "Other suitable and harmless microorganisms". Rather than a product specification, the applicant has chosen to compare the novel food to traditional fermented milk products and demonstrates the compositional and nutritional similarities in the application dossier.

II. Effect of the production process applied to the novel food

The fermentation process begins with the addition of *B. xylanisolvens* to pasteurised low-fat or skim milk. Sugars such as glucose and other fermentable sugars like xylan-containing plant materials may also be added to aid bacterial growth. The milk product is homogenised at the conclusion of the fermentation process and pasteurised once more to inactivate the starter culture. The fermented milk product may then be either processed and packaged like a traditional liquid fermented milk product, or spray-dried for use as a fermented milk powder.

Microbial and molecular analysis is conducted throughout the process to ensure the absence of contaminating microorganisms and to demonstrate the absence of viable *B. xylanisolvens* from the final product.

III. History of the organism used as the source of the novel food

Bacteroides xylanisolvens is a natural component of the normal human intestinal microflora and is believed to account for a significant proportion of all *Bacteriodes* sub-species in the human gastrointestinal tract. In addition, it is estimated that *Bacteriodes* sub-species account for approximately a quarter of all anaerobic microorganisms residing in the human colon. *Bacteriodes* are largely non-pathogenic commensals, though certain strains of *Bacteriodes fragilis* have been reported to be both pathogenic and toxigenic.

B. xylanisolvens is a propionic acid-producing Gram-negative, rod-shaped bacterium and a strict anaerobe. It is non-motile and unlike other *Bacteriodes* sub-species is unable to degrade starch. *B. xylanisolvens* grows optimally at 38^{o}C and a pH of 6.8, and can utilise a variety of sugars, including lactose (for carbon and energy) and xylan (for acetate, propionate and succinate). The specific *B. xylanisolvens* starter culture strain used in the fermentation process was originally isolated from the faeces of a healthy human. It has been extensively characterised by the applicant and has been deposited with the German Resource Centre for Biological Material (DMZ) which assigned the specific identification number DSM 23964.

IV. – VIII. GM Aspects

B. xylanisolvens DSM23964 is not genetically modified and therefore these sections are not applicable.

IX. Anticipated intake/extent of use of the novel food

The pasteurised fermented milk product produced with *B. xylanisolvens* DSM 23964 is to be marketed in liquid and semi-liquid form in fermented milks, buttermilks, yoghurts and yoghurt drinks and as spray-dried powder for use like yogurt powders, e.g. in fillings and coatings of cereals, cereal bars, fruits and nuts. The final pasteurised fermented milk products may also be supplemented with other ingredients such as sugars, flavours, fruit preparations and fibre.

The intakes of milk fats were estimated using US data since equivalent European data is not available. Intakes were estimated using a "worst case scenario" where the novel food was assumed to replace all the yoghurts, buttermilks and acidophilus milks consumed in the USA. Based on the US NHANES food intake data and the associated Food Commodity Intake Database of raw agricultural values, mean daily intake (MDI) of non-fat milk-derived solids for all study participants were estimated as 1.6g/d rising to 6.3g/d for the 90th percentile. Absolute intakes were approximately 15-16 times higher for yoghurts (MDI 1.5 g/d), than for buttermilk and other fermented milks (MDI 0.1 g/d) in the population, although there was a slightly higher percentage intake of buttermilk/fermented milks (20%) compared to yoghurts (13%).

Absolute intakes of all fermented milk products combined were highest for children aged 2-5 years (MDI 6.4 g/d), but the 90th percentile intakes were highest for males aged 20 years or older (17.4 g/d). On a bodyweight per day basis, the highest intakes of all fermented milk products combined were reported for children aged 2-5 years (MDI 0.38 g/kg bw/day; 90th percentile 0.96 g/kg bw/day), and were significantly higher than the comparable figures for users from all age groups combined (MDI 0.10 g/d, 90th percentile; 0.29 g/d). Intake from yoghurts was highest for male consumers aged 11-19 years (MDI 12.7g/d). However, only 8% of this age group reported consuming yoghurts at all. Intakes of the fermented milks were highest for males aged 20 years or older (MDI 1.1 g/d), with a 17% user rate.

The applicant also estimated the level of intake of the heat inactivated starter culture (*B. xylanisolvens*) *via* the consumption of yoghurts which represent the highest potential intake of the novel food. The estimated number of *B. xylanisolvens* cells ingested in fermented milk products was similar for the different age groups, but on a bodyweight per day basis was highest for users in the 2-5 year old age group (2 x 10^{10} cells/kg bw/d). The cell number estimate was based on the assumption that 100 ml of pasteurised fermented low or non-fat milk products contains 14 g non-fat milk solids and 0.5 x 10^{12} (150 – 300 mg) *B. xylanisolvens* cells.

The highest estimated intake of the novel food in terms of bodyweight was in yoghurt consumed by 2-5 year olds which represents a worst case scenario. However, this is over one hundred-fold less than the proposed NOAEL of 3.2 x 10^{12} *B. xylanisolvens* cells/kg bw/day which was based on a 90-day oral toxicity study in mice that included both heat inactivated and viable bacteria. The applicant estimates the intake of 4 x

10^{11} *B. xylanisolvens* cells/day is similar in number to that of other microorganisms traditionally used as starter cultures in other fermented milk products.

XI. Nutritional information on the novel food

Nutritional data is presented for spray-dried skim milk fermented by *B. xylanisolvens* DSM 23964 in comparison with six fermented milk products already on the market. The data indicates that levels of carbohydrates (total, glucose, fructose, saccharose, maltose), protein, fat (saturates, monounsaturates, polyunsaturates and trans-fats), ash, total acids, purine nitrogen and water are all within the ranges of the existing products. Micronutrient analysis was not completed, though a reference is cited which concludes that yoghurt and other fermented milk products are not known to be significant sources of micronutrients. The "softer acidity" attributed to the novel food by the applicant is most likely due to proportion of specific short chain fatty acids in the novel food. The presence of *B. xylanisolvens*, viable or inactivated, would not be expected to contribute significantly to the nutritional value of the food. Though it is not clear how relevant US dietary intake data is for the EU population, it is not expected that the novel food would have any negative nutritional effect on the diet of EU consumers.

XII. Microbiological information on the novel food

B. xylanisolvens DSM 23964 is a Gram-negative obligate anaerobe which is non-spore-forming and non-motile. It belongs to the genus *Bacteroides* which forms a major part of the human intestinal flora and has only relatively recently been identified as an individual species.

Its virulence potential was investigated, specifically its ability to produce the *bft* enterotoxin, a capsular polysaccharide (PS), extracellular hydrolytic enzymes such as collagenase and hyaluronidase, and the tendency to bind to host cells such as those of the intestinal tract. *B. xylanisolvens* DSM 23964 was shown to lack such virulence factors, in contrast to *Bacteroides fragilis* which is recognised as an opportunistic pathogen. This strain produces beta-lactamase and hence is resistant to penicillin, ampicillin and other beta-lactam antibiotics. It is however, sensitive to metronidazole and clindamycin and does not carry plasmids which could transfer antibiotic resistance genes.

On the basis of this information, the German Federal Institute for Occupational Safety and Health (BAuA) allocated *B. xylanisolvens* to Risk Group 1, namely those microorganisms which can be handled in a viable form without risk to human health.

Monitoring by microbiological and molecular analysis takes place during and after the production process in order to detect any microbial contamination. The final fermented milk product is tested for microbial pathogens (*E. coli, Salmonellae, Staphylococci* and *Enterobacteria*) and yeast, while the absence of viable *B. xylanisolvens* is also confirmed.

XIII. Toxicological information on the novel food

It is anticipated that *B. xylanisolvens* will be degraded in the gastrointestinal tract of humans, or experimental animals in the same way as other bacterial cells ingested with traditional fermented milk products. As the starter culture is non-viable in the final product, it is not expected to have any influence on the normal gut microflora. The safety of the *B. xylanisolvens* fermented milk product was addressed in two human studies. In a three week pilot study, two groups of male and female volunteers consumed daily portions of 100 ml pasteurised low-fat milk cultured with *B. xylanisolvens* (5×10^{11} and 8×10^{11}inactivated cells/portion) and flavoured with cherry pulp. The product was well-tolerated and no significant effects were observed in the parameters monitored. An increase in TNF-α in both groups at the end of the treatment period was interpreted by the study authors as a beneficial effect, but was not observed in the subsequent main study.

In the main study, 140 healthy male and female volunteers were randomly assigned to four treatment groups: (1) control group (milk powder as placebo); (2) low dose; (3) mid dose and (4) high dose groups ingesting a nominal daily intake of zero, 1×10^{10}, 2.5×10^{11} and 1×10^{12} *B. xylanisolvens* cells per day, respectively for 6 weeks. Gastrointestinal side-effects due to the treatment were not reported and treatment-related effects were not observed for the various immunological parameters, plasma CRP and liver enzymes monitored.

The applicant also provided results of an abscess formation test in mice which indicated that viable *B. xylanisolvens* was non-pathogenic and was eradicated by the immune system. Viable and heat-inactivated *B. xylanisolvens* were non-mutagenic in a bacterial mutagenicity study (Ames test) with and without metabolic activation. In

addition, *B. xylanisolvens* was not found to be clastogenic or cytotoxic in human peripheral lymphocytes (with and without metabolic activation).

The toxicity of *B. xylanisolvens* was examined in a 90-day oral toxicity study in mice using daily doses of heat-inactivated and viable cells. This was a guideline study, with full examination on all standard parameters including functional tests. Treatment-related effects were not reported, and the highest dose tested (1×10^{11}) was considered the NOAEL by the applicant, which is equivalent to 3.2×10^{12} viable or heat-inactivated cells/kg bw/day. The NOAEL of 3.2×10^{12} cells/kg bw/day can be used to derive a margin of safety for humans. On a body weight basis, the highest intake of 2.0×10^{10} cells/kg bw/day *B. xylanisolvens* DSM 23964 was estimated for 2–5 year old children (2.0×10^{10} cells/kg bw/day), providing a margin of safety of >100. For the mean adolescent or adult consumer (age ≥11 years), the margin of safety was proportionately higher, but for the 90th percentile of consumers, the margins of safety is a factor of approximately two lower.

Conclusions

Though there is a considerable history of consumption of various fermented milk products in the EU and globally, most are relatively poorly characterised. The data provided in this application indicate that compositionally and nutritionally, milk products fermented by *B. xylanisolvens* DSM 23964 are broadly similar to equivalent fermented milk products already on the EU market. The toxicological and microbiological data presented in the dossier and cited texts have not identified specific safety concerns associated with the consumption of the novel food in the target food categories and at the estimated intake levels. Therefore the FSAI considers that this novel food meets the criteria set out in *Article 3.1.* of the novel food Regulation (EC) No 258/97.