COMMITTEE PAPER FOR DISCUSSION

ADVISORY COMMITTEE FOR NOVEL FOODS AND PROCESSES

PASTEURISED FERMENTED MILK PRODUCTS WITH HEAT-INACTIVATED BACTEROIDES XYLANISOLVENS

ISSUE

The Irish Competent Authority will shortly issue its initial opinion on pasteurised fermented milk products with heat-inactivated Bacteroides xylanisolvens under the novel foods regulation (EC) No 258/97. This paper describes the application dossier and provides an opportunity for Members to raise questions and make any comments in advance of opinion being circulated. Members will be consulted by post once the opinion is available and their advice will form the basis for the UK’s formal response to the Commission.

Introduction

1. The European Commission will shortly forward to Member States the Irish Competent Authority’s (CA) initial opinion on an application made by Avitop GmbH under Article 4 (1) of Regulation (EC) 258/97, for the authorisation of pasteurised fermented milk products with Bacteroides xylanisolvens strain DSM 23964.

2. The applicant intends to employ B. xylanisolvens DSM 23964 as a starter culture to produce fermented milk products (pasteurised fermented low fat milk and skimmed milk products). The novel micro-organism will be delivered to the consumer in a heat-inactivated form as a result of pasteurisation of the milk products. To note: Due to the novel nature of the micro-organism, the safety assessment will be based on both the micro-organism and the fermented milk products produced from it.

3. The applicant highlights that use of B. xylanisolvens results in the formation of fermented milk products with softer acidity and smoother texture than conventional low-fat yoghurt. Otherwise these novel fermented milk products closely resemble the corresponding existing products which are typically produced with the aid of Lactobacilli, Bifidobacteria, Streptococcus thermophilus and some other traditionally used microorganisms.

4. The Commission will shortly be seeking the views of other Member States on this application dossier and on the Irish CA’s initial assessment of this novel ingredient. Member States must provide any comments or reasoned objections to the Irish assessment within 60 days.

5. The application dossier is attached as Annex A (this Annex has two appendices). The favourable Irish opinion will be circulated to the Committee on receipt.
Background

6. The applicant states that *Bacteroides* account for about a quarter of all anaerobic bacteria residing in the human colon and *B. xylanisolvens* is a regular commensal of the human intestinal microbiota, accounting for a significant proportion of all *Bacteroides* occurring in human faeces.

7. The applicant highlights that while certain strains of *Bacteroides fragilis* may be pathogenic and toxigenic, *Bacteroides* in general (including the strain DSM 23964) are largely non-pathogenic commensals and some species may have favourable effects on the immune system.

8. The European Food Safety Authority (EFSA) has established a framework known as the Qualified Presumption of Safety (QPS) that can be applied to all requests received for the safety assessments of microorganisms deliberately introduced into the food chain. Microorganisms granted QPS by EFSA have been placed on a list\(^1\) thus avoiding the extensive investigation of organisms known not to cause concern. Microorganisms not considered suitable for QPS remain subject to a full safety assessment.

9. The applicant states that as yet, no *Bacteroides* species is a known food fermentation micro-organism with a history of safe use and therefore the safety of *B. xylanisolvens* for its intended use cannot be based on QPS but must be based on a conventional safety assessment of this micro-organism and the milk fermented with it.

10. The present application for authorisation of pasteurised fermented milk products with heat-inactivated *Bacteroides xylanisolvens* as a novel food was prepared pursuant to Commission Recommendation (97/618/EC) of 29 July 1997 concerning the scientific aspects and presentation of information necessary to support applications for the placing on the market of novel foods and novel food ingredients. The novel food is classified as a complex food from a non-GM source, where the source of the novel food does not have a history of food use in the Community (Class 2.2). The requirements for a submission for this class are as follows:

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The information presented in the dossier is structured accordingly and is considered below under these schemes.

I. Specification of the novel food

11. The applicant states that the *B. xylanisolvens* strain which may be used for the production of pasteurised fermented milk products has been deposited by the applicant with the German Resource Centre for Biological Material (DSMZ) and has been attributed the strain number DSM 23964. *B. xylanisolvens* DSM 23964 is specified by its identity with the deposited strain.

12. *B. xylanisolvens* are strictly anaerobic, Gram negative, non-spore forming, non-motile rods, which unlike other *Bacteroides* spp. are unable to degrade starch. The applicant states that the strain is able to utilise a variety of sugars, including lactose as a carbon and energy source. The strain is also able to metabolise xylan (from plant material) to propionate, acetate and succinate.

13. The microorganism that is the subject of this dossier was classified as *B. xylanisolvens* based on an examination of its phenotypical and biochemical properties, by 16S rRNA gene sequencing and DNA-DNA hybridization with *B. xylanisolvens* DSM 18836 and closely related *Bacteroides* species. A detailed description of all these investigations and of the classification of *B. xylanisolvens* DSM 23964 has been published (Ulsemmer et al., 2012a).

14. The applicant states that although pasteurised fermented milk products with *B. xylanisolvens* have a smoother texture and softer acidity, in terms of nutritional composition, they are essentially identical to other traditionally fermented milk products. Table 1 in Appendix 1 to Annex A provides further details on nutritional comparison (see also Section XI below).

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

15. Pasteurised milk is fermented with a starter culture of *B. xylanisolvens* using optimal conditions to sustain the fermentation. Following further pasteurisation after fermentation, the fermented product (containing heat inactivated *B. xylanisolvens*) is cooled and can either be processed and packaged like existing liquid fermented milk products, or spray dried for use as a fermented milk powder like yoghurt powder.

16. The applicant has provided details of quality control procedures employed throughout the entire production process (microscopic examination, molecular biological tests such as species specific PCRs and genotyping, and quality control of the fermented milk product) to ensure the absence of microbial contamination.

17. Further details can be found in Annex A (Protect:Commercial).
III. History of the organism used as a source of the novel food
Annex A, p24-25

18. The applicant has highlighted the natural presence of *B. xylanisolvens* in the human gut as part of the intestinal microflora and the significant presence of this micro-organism in human faeces. The applicant states that this strain has originally been isolated from the faeces of a healthy human adult.

19. The applicant states that although intentional use of this strain in food is not known, the strain may have reached foods inadvertently by contamination as with other faecal micro-organisms. The applicant has performed an extensive literature search to investigate the pathogenic potential of this strain and concluded that there have been no reports of food poisoning or human disease associated with this species.

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

20. *B. xylanisolvens* is intended to be used as a starter culture for the production of pasteurised fermented low fat or skimmed milk products which are intended to be consumed in either liquid, semi liquid or spray-dried form. The dossier is somewhat vague about the exact types of fermented milk products intended to be marketed but implies that these are likely to be an alternative to traditional fermented milk products. Further details can be found in Appendix 2 to Annex A.

21. To note: The applicant highlights that in the absence of an EU standard for fermented milks, that the Codex Standard (243-2003) specifies the species of microorganism that may be used as starter cultures for the production of yoghurt, alternate culture yoghurt, acidophilus milk, kefir and Kumys. For the production of all other types of fermented milk, any appropriate harmless microorganisms may be used as starter cultures. Pasteurised fermented milk products with *B. xylanisolvens* are therefore heat-treated, fermented milk products, according to the Codex Standard.

22. Estimates of the potential intake of non-fat milk solids, which would result from the consumption of fermented milk products produced with *B. xylanisolvens* DSM 23964 as starter culture, were calculated using US food consumption data and details can be found in Appendix 2 to Annex A.

23. Applying a "worst case" approach, it was assumed that low-fat or non-fat milk cultured with *B. xylanisolvens* DSM 23964 would substitute for all yoghurt, buttermilk and acidophilus milk that is currently consumed in the US, be it in semi-liquid or frozen form, or as a spray-dried powder, or as an ingredient of certain processed foods.

24. The results of these intake calculations reveal a current mean and 90th percentile intake of non-fat, milk-derived solids of 5.4 and 15.3 g/d, respectively, for users of all fermented milk products combined. On a bodyweight basis and across all groups, the mean and 90th percentile user of such products consumes 0.104 and 0.288 g/kg bodyweight/d, respectively, of non-fat milk derived solids. The highest intake occurs in 2 – 5 year old children with a milk solids intake from all fermented milk products combined of 0.383 and 0.964 g/kg bw/d for the mean and 90th percentile user, respectively.
25. The applicant states that in comparison to yoghurt, the daily intake of non-fat milk solids with buttermilk and acidophilus milk is more than fifteen times lower for both the mean and 90th percentile consumer and each age group, the yoghurt consumer is, therefore, the consumer with the potentially highest intake of *B. xylanisolvens* DSM 23964.

26. The estimated intake of *B. xylanisolvens* DSM 23964 cells from its intended use in low-fat and non-fat yoghurt is shown for the mean consumer, per age group, in Table 5, Appendix 2, to Annex A. While the intake of *B. xylanisolvens* DSM 23964 is about the same in the different age groups (4 x 10^{11} cells/d), intake per kg bodyweight is highest in 2 – 5 year old children (2 x 10^{10} cells/kg bw/d).

27. In comparison, a daily intake of 3.2 x 10^{12} heat-inactivated and non heat-inactivated cells/kg bw/d was found to be a NOAEL in a 90-day oral toxicity study in mice (see Section XIII below). This provides a margin of safety of >100 for the mean, 2-5 year old consumer. For the mean adolescent or adult consumers (age ≥11 yrs), there is an even higher safety margin. For the 90th percentile consumers of yoghurt, the margins of safety will be approximately 2-fold lower.

**X. Information from previous human exposure to the novel food or its source**  
*Annex A, p29*

22. The applicant states that while there is ample evidence of the safety of pasteurised fermented milk products there is no history of use of pasteurised fermented milk products with *B. xylanisolvens* DSM 23964.

**XI. Nutritional information on the novel food**  
*Annex A, p 30-32*

23. The applicant states that pasteurised *B. xylanisolvens* fermented milk products are substantially equivalent to the corresponding traditional products in terms of composition and nutritional value and presents data in Annex A, Appendix 1, Table 1 to support this. The applicant mentions elsewhere in the dossier that *B. xylanisolvens* fermented milk products have a smoother texture and softer acidity compared to traditional counterparts; the latter is attributed to the slightly different proportions of fermentatively formed acids (mainly acetic, propionic, lactic and succinic acids). No explanation is offered for the differences in texture.

**XII. Microbiological information on the novel food**  
*Annex A, p33*

24. The microbiological purity of cultured *B. xylanisolvens* 23964 and the fermented milk product is controlled during the production process and is analytically verified in the final products to ensure compliance with specifications.

25. The applicant has presented microbiological specifications for the fermented milk product in Annex A, Appendix 1, Table 3. The microbiological specifications acknowledge *E. coli*, coagulase positive *Staphylococcus* spp., *Bacillus cereus*, *Clostridium perfringens*, *Salmonella* spp and *Listeria monocytogenes*. Absence of viable *Bacteroides xylanisolvens* is confirmed in each batch of milk products.
XIII. Toxicological information on the novel food

26. The applicant highlights that, although *B. xylanisolvens* DSM 23964 will be ingested only in a heat inactivated form, it has nonetheless examined all safety related characteristics of the viable form.

27. The absence of pathogenicity of *B. xylanisolvens* DSM 23964 was demonstrated by an abscess formation test in mice. Further details of this test are not supplied in the dossier but the applicant has cited two references where further details will be available (Annex A, p 35).

28. The applicant states that examination of *B. xylanisolvens* DSM 23964 revealed that this strain does not carry virulence genes or plasmids that could transfer antibiotic resistance genes. Although details of the methodology used are not described in the dossier, the applicant cites a reference where further details can be found.

29. The mutagenicity of both heat inactivated and non-heat inactivated forms of this strain was examined in standard Ames tests with and without metabolic activation using *S. typhimurium* TA 98, TA 100, TA 102, TA 1535 and TA 1537. The applicant states that the results show that no mutagenic effect was observed under the test conditions.

30. The clastogenic activity of heat-inactivated and non-heat inactivated *B. xylanisolvens* DSM 23964 was examined in human peripheral lymphocytes with and without metabolic activation. No signs of cytotoxicity or clastogenic activity were observed under the conditions of this test.

31. The toxigenicity of both heat-inactivated and non-heat inactivated *B. xylanisolvens* DSM 23964 was examined in a 90-day oral toxicity study in mice. Mice received daily by gavage either $1 \times 10^9$, $1 \times 10^{10}$ or $1 \times 10^{11}$ non-heat inactivated cells of *B. xylanisolvens* DSM 23964 or $1 \times 10^{11}$ heat inactivated cells of the same strain. The NOAEL was established to be the highest dose tested.

32. The applicant reports the findings of a human tolerance study with 140 subjects. The study investigated the effects of daily ingestion of spray dried fermented milk containing up to $1 \times 10^{12}$ *B. xylanisolvens* DSM 23964. The applicant reports that there were no adverse effects relating to the clinical parameters assessed and no treatment related side effects.

XIII. Allergenicity

33. The applicant does not appear to have specifically addressed allergenicity in the dossier. It is possible however, that this information could have been requested from the applicant by the Irish Authorities during initial assessment and will become apparent once the Irish opinion is issued.

COMMITTEE ACTION REQUIRED
34. The Committee is asked whether it wishes to make any comments on this application or raise any questions, in advance of receipt of the initial opinion of the Irish authorities.

35. An addendum to this paper will be circulated to Members by post once the Irish assessment is available.

Secretariat
June 2013

Annexes attached

Annex A Application for the approval of pasteurised fermented milk products with heat-inactivated *Bacteroides xylanisolvens* (PROTECT – REGULATORY). This annex has two appendices.