COMMITTEE PAPER FOR DISCUSSION **ACNFP/105/5**

ADVISORY COMMITTEE FOR NOVEL FOODS AND PROCESSES

CLOSTRIDIUM BUTYRICUM PROBIOTIC

ISSUE

An application has been submitted to the UK Competent Authority for authorisation of *Clostridium butyricum* as a probiotic food supplement under the novel foods regulation (EC) No 258/97. The Committee is asked to review this new application and advise whether the available data provide an adequate basis for a safety assessment of this novel ingredient, and if it recommends authorisation of the product.

Background

1. An application has been submitted to the UK by the Japanese company Miyarisan Pharmaceutical Co. Ltd for authorisation of *Clostridium butyricum* as a novel ingredient for use in food supplements in the EU. The application was received by the UK Competent Authority in January 2012. In accordance with Article 6(3) of Regulation (EC) No 258/97, the UK has 3 months from formal acceptance of the application to prepare an initial assessment report. The initial assessment will then be circulated for review by the Competent Authorities in the other Member States.

2. Clostridium is a large bacterial genus with more than 150 species. Although the genus contains pathogenic species, notably *Clostridium botulinum, Clostridium difficile, Clostridium perfringens* and *Clostridium tetani,* the applicant points out that fewer than 10% of this genus produce toxins. The applicant draws attention to the fact that most Clostridial species, especially gut-associated clostridial species (including the *Clostridium butyricum* strain to be marketed by the applicant) are non-pathogenic gut commensals which form an important part of the lower gut flora of humans and animals.

3. The *Clostridium butyricum* strain (CBM 588) intended to be marketed by the applicant is a Gram positive, spore forming, obligate anaerobic, non-pathogenic, non-genetically modified bacterium.

   To note: this paper refers to:

   a) *Clostridium butyricum / C.butyricum* (the broad genus and species);

b) <u>CBM 588</u> (the specific strain of C. butyricum that is being proposed as a novel ingredient).

4. The applicant's intention is to market *C. butyricum* (strain CBM 588) as viable spores in tablet form intended for use as a probiotic food supplement to support, maintain or restore healthy gut flora physiology and/or function. The applicant intends to make a parallel application under the EU Nutrition and Health Claims Regulation.

5. The applicant has marketed preparations of this *C. butyricum* strain (CBM 588) for use as a probiotic in Japan and other Asian countries for several decades. This strain of *Clostridium butyricum* has also received EU approval as a microbial feed additive for chickens for fattening and weaned piglets and minor avian and porcine species in 2009 and 2011, respectively **Annex 4 and 6.**

6. The present application for authorisation of CBM 588 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. CBM 588 has been classified as a complex novel food from a non-GM source, the source of the novel food has no history of food use in the Community (class 2.2). The requirements for a submission for this class are as follows:

I	**Specification of the NF**	**X**
II	**Effect of the production process applied to the NF**	**X**
IV	*Effect of the genetic modification on the properties of the host organism*	**-**
V	*Genetic stability of the GMO*	**-**
VI	*Specificity of expression of novel genetic material*	**-**
VII	*Transfer of genetic material from GM microorganisms*	**-**

VIII	*Ability to survive in and colonise the human gut*	**-**
IX	**Anticipated intake/extent of use of the NF**	**X**
XI	**Nutritional information on the NF**	**X**
XII	**Microbiological information on the NF**	**X**
XIII	**Toxicological information on the NF**	**X**

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

7. This is the first time the Committee has been required to assess a live microorganism under the Novel Foods Regulation. Commission Recommendation 97/618/EC does not address the specific information that should be supplied by applicants for this type of ingredient. However, the

European Food Safety Authority (EFSA) has established a framework known as the Qualified Presumption of Safety (QPS) concept which provides a generic assessment system 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 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.

8. *C. butyricum* was considered by EFSA's BIOHAZ Panel in its 2011 update to the QPS list (Annex 5). EFSA concluded that "the safety of *Clostridium butyricum* is a strain-related property, therefore *Clostridium butyricum* should not be recommended for the QPS list." This conclusion was based on the observation that a minority of strains contain a gene coding for botulinum neurotoxin type E (see paragraph 14 below) and there is only limited knowledge of human and animal exposure to this species. As QPS does not apply, the micro-organism should undergo a full novel food assessment.

9. The application dossier is attached as **Annex 1** (Protect Commercial). The dossier contains fourteen appendices some of which can be regarded as commercially sensitive and are attached as **Annex 2** (Protect Commercial)**. Annexes 3-6** contain relevant EFSA opinions. A non-confidential version of the application dossier is being placed on the FSA website for a period of 21 days to allow the public to contribute to the assessment. Any comments received will be tabled at the meeting or presented to a future meeting.

I. Specification of the novel food

Annex 1, p 14-22

10. The applicant intends to market *Clostridium butyricum* tablets in two forms, standard and strong, containing a minimum of $3x10^5$ and $4.5x10^5$ viable cells per tablet, respectively. The tablets in different strengths are intended to suit the needs of the consumer; the applicant has clarified to the Secretariat that the need for this probiotic may vary amongst individuals. Data on individual batches (page 21 of Annex 1) indicate that the actual content of the tablets is substantially higher than the quoted minimum (standard: 5 to 7.1 x 10^6; strong: 1.1 to 1.7 x 10^7). According to a certificate of analysis (Appendix 6 to Annex 2) the content of "strong" tablets should not exceed 4.5 x 10^7 CFU. The specification for tablets containing CBM 588 has been established by the applicant and can be found in the table below.

Specification	Detail
Appearance	Round tablet, 9mm diameter, white or pale grey, with characteristic odour and sweet taste.
Total aerobic count	< 10^3 CFU/g
E. coli in 1 g sample	not detected
Staphylococcus aureus in 1 g sample	not detected
Pseudomonas aeroginosa in 1 g sample	not detected
Yeasts & moulds	< 10^2 CFU/g

11. The applicant states that the product complies with the limits for food supplements that are set out in Commission Regulation (EC) 1881/2006 on maximum levels for certain contaminants in foodstuffs. The applicant also states that the product specifications comply with the Japanese Pharmacopoeia. A certificate of analysis is provided in Appendix 6 of Annex 2**.**

12. The applicant states that the original wild strain of *C. butyricum* MIYAIRI (CBM 588) was isolated in 1963 from a soil sample sourced in Nagano, Japan. This strain is deposited at the Fermentation Research Institute, Agency of Industrial Science and Technology, Japan under the strain name *Clostridium butyricum* MIYAIRI 588 strain, deposit number FERM BP-2789. The applicant has preserved their collection of *Clostridium butyricum* MIYAIRI strains by freeze-drying and freezing methods since 1986. Subculture of CBM 588 master cell banks and working cell banks is performed at appropriate intervals. The applicant has provided details of quality control procedures employed for each lot of the novel ingredient which include methods to confirm strain identity. (Appendix 8 part of Annex 2; Protect Commercial).

13. Genetic and biochemical stability of CBM 588 has been accepted by EFSA in the context of its use in animal feed (Annexes 4 and 6) and full details of the relevant tests are provided in Appendix 8 of Annex 2 (Protect Commercial).

14. The applicant states that the strain of *C. butyricum* intended to be marketed does not carry any genes encoding any toxins and virulence factors associated with clostridium or other enteropathogens. Absence of neurotoxin production was demonstrated by PCR and Southern blot hybridisation for type E botulinum toxin gene. The absence of genes encoding botulinum neurotoxin A,B,F and genes encoding non-toxic haemagglutinin (NTNH) and genes encoding *Clostridium perfringens* toxins (alpha, beta, epsilon and iota) was demonstrated by PCR assay. The applicant acknowledges that the presence of a single cryptic plasmid of 6.5 kb has been noted in this strain of *C. butyricum* but the nucleotide sequence of this plasmid was analysed and data

showed that none of the nine putative open reading frames encoded any known virulence factor of Clostridium spp. (EFSA 2009, 2011, Annexes 4 and 6). The applicant has provided further details of the methods and results that demonstrate the absence of the above in Appendix 12 and 13 (Annex 2).

15. The susceptibility of this strain of *C. butyricum* to key antibiotics as recommended by EFSA was tested. The applicant reports that the minimum inhibitory concentrations of these key antibiotics were lower than the EFSA breakpoints confirming that CBM 588 is not resistant to antibiotics of human or veterinary importance (EFSA 2008, 2009, 2011; Annexes 3,4,6).

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

Annex 1, p 23-24

16. The applicant's *Clostridium butyricum* supplement is produced by submerged anaerobic fermentation followed by centrifugation, drying, blending and packaging to produce either strong or standard tablets. The process complies with Japanese Good Pharmaceutical Manufacturing Practice and details can be found in Appendix 8 and Appendix 9 (Confidential).

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

Annex 1, p 25

17. The applicant has considered historical and current consumption patterns of CBM 588 in non-EU countries in order to derive appropriate daily intakes of this food supplement in the EU. The applicant states that daily intake of CBM 588 as a food supplement in the EU as intended for market is expected to be within the range of 3×10^5 to 1.35×10^8 CFU/day (one standard tablet to three strong tablets per day). The supplement is intended for healthy adults.

18. The applicant states that an effective and optimum daily dose may vary between adults but the appropriate daily dose is anticipated to provide gut health benefits such as improved gut transit time, improved faecal bulk and consistency and more comfortable bowel movements.

19. The applicant states that CBM 588 does not establish permanently in the gut, in common with other orally administered probiotic bacteria.

XI. Nutritional information on the novel food

Annex 1, p 26

20. The applicant states that CBM 588 is not intended to replace any other foods or nutrients in the diet, and does not supply significant dietary macro or micro nutrients.

XII. Microbiological information on the novel food

Annex 1, p 26-27

21. Microbiological specifications are presented above in Section I. The applicant has also acknowledged the possibility that CBM 588 may have effects on the intrinsic gut flora of animals and humans and has highlighted a number of published studies illustrating that CBM 588 has no adverse effects on beneficial gut flora of humans or animals.

XIII. Toxicological information on the novel food

Annex 1, p 27-29

22. The applicant highlights that the safety of CBM 588 has been reviewed by EFSA in 2009 and 2011 and that EFSA concluded that its use in animal nutrition is safe for animals, consumers, industrial workers/users and the environment. The applicant reiterates that CBM 588 does not pose a risk to humans as the strain does not carry genes encoding relevant toxins and virulence factors, nor does it harbour acquired or transferable antibiotic resistance.

23. The applicant has described a series of toxicological studies using CBM powder (the dried fermentation concentrate of CBM 588). An acute oral toxicity study in rats showed that the acute oral toxicity of CBM powder is in excess of 5000 mg/kg body weight. A subacute (5 week) oral toxicity study investigating the effects of CBM powder in beagle dogs showed a NOEL of 2000 mg/kg body weight/day (the highest dose tested). Chronic oral toxicity of CBM powder was investigated in SPF Fischer 344 rats over a twelve month period. Some effects (increased blood glucose and increased urine volume and kidney weights in males) were observed at the highest dose tested (50 g/kg diet) but macroscopic and microscopic pathological examinations revealed no differences between treated and untreated rats. The NOEL (No Observed Effect Level) was therefore determined to be 5 g/kg diet (equivalent to 241 mg/kg body weight/day in male rats and 288 mg/kg body weight/day in female rats).

24. The applicant highlights that the optimum CBM 588 intake in the EU may vary between individuals but emphasises that the maximum intake envisaged in healthy adults in the EU is 100 fold less than the NOEL calculated from toxicological studies in laboratory animals. The lowest NOEL of CBM powder was determined as 241 mg/kg bodyweight per day in male rats. From these data, the NOEL can be extrapolated to a 60 kg human as 14.46 g/day (0.241 g x 60 = 14.46 g). CBM powder contains $\geq 1 \times 10^9$ CFU CBM 588 per g, so the NOEL is equivalent to a dose of 1.45×10^{10} CFU/60 kg adult/day. The highest anticipated dose of CBM 588 (3 Strong Tablets per 60 kg adult per day, each

containing up to 4.5 x 10^7 CFU) is 1.35 x 10^8 CFU/adult/day. This is 107 times less than the human equivalent of the NOEL.

25. The applicant also details a study looking into mutagenicity of CBM powder by way of reverse mutation assays and chromosome aberration assays; the study highlights that CBM 588 does not exhibit any mutagenicity or clastogenicity.

26. Although CBM 588 does not have a history of consumption as a food ingredient in the EU, the applicant draws attention to other examples of previous human exposure to *Clostridium butyricum*. The applicant refers to studies which show that *C. butyricum* strains are commensals in the gut of humans and may colonise the gut of infants after birth.

27. The applicant has sold preparations of CBM 588 in Japan and other Asian countries for both human and animal use since the 1960s and there have been no reports of adverse events or allergenicty.

Consumer Access and Choice

28. The Secretariat has considered the issues of access and choice in relation to CBM 588. If authorised, this probiotic food supplement would be available for use across the UK and subsequently in other EU Member States. In practical terms, access to products containing CBM 588 could be limited by a high price or by limited geographic distribution, which are both driven by commercial considerations that cannot be predicted at this stage.

29. It is envisaged that the introduction of products containing CBM 588 will increase existing consumer choice. The consumer would be aware of the presence of CBM 588 through the ingredient list and, most likely through special marketing that highlights its contribution to the food supplements that contain it.

Health and Nutrition Claims

30. The Committee's assessment focuses on safety and labelling and does not address any nutrition or health benefits that may be claimed for the novel ingredient or for foods that containing it. Nutrition or health claims may only be made if they are specifically authorised under EU Regulation (EC) No 1924/2006.

COMMITTEE ACTION REQUIRED

31. The Committee is asked whether the available data provide a satisfactory basis for evaluating the safety of this novel food ingredient, when used in food

supplements. Members are asked to consider whether the data provided are sufficient to demonstrate that the novel ingredient is a) safe for consumers, b) not nutritionally disadvantageous to the consumer and c) does not mislead the consumer.

32. If so the Committee is asked whether it is content to recommend approval of Miyarisan's *Clostridium butyricum* (CBM 588) as a food supplement.

33. If not, the Committee is asked to indicate what additional data would be required.

Secretariat
February 2012

Annexes attached:

Annex 1 Application for the approval of Clostridium butyricum (Protect Commercial)

A non-confidential version of the application dossier is available via the ACNFP website http://acnfp.food.gov.uk

Annex 2 Appendices to the dossier (Protect Commercial).

Annex 3 EFSA opinion (2008): Update of the criteria used in the assessment of bacterial resistance to antibiotics of human or veterinary importance. http://www.efsa.europa.eu/en/efsajournal/doc/732.pdf

Annex 4 EFSA opinion (2009): Safety and efficacy of Miya-Gold®S (*Clostridium butyricum*) as feed additive for chickens for fattening. http://www.efsa.europa.eu/en/efsajournal/doc/1039.pdf

Annex 5 EFSA opinion (2011) on the maintenance of the list of QPS biological agents intentionally added to food and feed (2011 update) http://www.efsa.europa.eu/de/efsajournal/doc/2497.pdf

Annex 6 EFSA opinion (2011) on Miya-Gold® (*Clostridium butyricum*) as a feed additive for weaned piglets, minor weaned porcine species and minor avian species. http://www.efsa.europa.eu/en/efsajournal/doc/1951.pdf

Annex 1 to ACNFP/105/5

ADVISORY COMMITTEE ON NOVEL FOODS AND PROCESSES

PROTECT COMMERCIAL

Application for approval of C. butyricum strain CBM 588

Secretariat
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Annex 2 to ACNFP/105/4

ADVISORY COMMITTEE ON NOVEL FOODS AND PROCESSES

PROTECT COMMERCIAL

Appendices to dossier

Secretariat
February 2012

Annex 3-6 to ACNFP/105/5

ADVISORY COMMITTEE ON NOVEL FOODS AND PROCESSES

Relevant EFSA opinions

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