Miya-Pro

(*Clostridium butyricum* MIYAIRI 588)

PUBLIC VERSION - *CLOSTRIDIUM BUTYRICUM* MIYAIRI 588 NOVEL FOOD APPLICATION

PROBIOTIC FOOD SUPPLEMENT

MIYARISAN PHARMACEUTICAL CO. LTD.

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Table 1. Abbreviations	
ACNFP	Advisory Committee on Novel Foods and Processes
CBM 588	*Clostridium butyricum* MIYAIRI 588 [FERM BP-2789]
CRL	Community Reference Laboratory
EC	European Community
EFSA	European Food Safety Authority
EU	European Union
FAO	Food & Agriculture Organisation
GLP	Good Laboratory Practice
GM	Genetically Modified
GMM	Genetically Modified Microorganism
GMO	Genetically Modified Organism
GMP	Good Manufacturing Practice
GOT	Glutamic Oxaloacetic Transaminase
GPT	Glutamate Pyruvate Transminase
GVP	Good Pharmacovigilance Practice
HACCP	Hazard Analysis & Critical Control Points
IBS	Irritable Bowel Syndrome
ISR	Intergenic Spacer Region
JP	Japanese Pharmacopoeia
JGMP	Japanese Pharmaceutical Good Manufacturing Practice
LDH	Lactate Dehydrogenase
NF	Novel Foods
NOAEL	No Observed Adverse Effect Level
NOEL	No Observed Effect Level
OTC	Over The Counter
PCR	Polymerase Chain Reaction
PFGE	Pulsed Field Gel Electrophoresis
QC	Quality Control
QPS	Qualified Presumption of Safety
RAPD	Random Amplification of Polymorphic DNA
RASFF	Rapid Alert System for Food
TAMC	Total aerobic microbial counts
TYMC	Total yeast & mould counts
WHO	World Health Organisation

Table 2. List of annexes and references

Annex or Reference	Authors	Year	Full Title	Study Code/Site	Further details	Nº Pages
Annex 1	Yumoto	2008	Strain deposit certificate & viability statement of *Clostridium butyricum* MIYAIRI 588 (CBM 588)	International Patent Organism Depositary National Institute of Advance Industrial Science & technology	Depositary Nº FERM BP-2789	1-10
Annex 2 CONFIDENTIAL	Miyarisan Pharmaceutical Co. Ltd.	2011	Geographical markets, sales data, pharmacovigilance statistics & GVP monitoring documents	Miyarisan Pharmaceutical Co. Ltd.	Post-marketing monitoring	1-4
Annex 3	Miyarisan Pharmaceutical Co. Ltd	2011	Comparison of legal classifications of drugs, foods & food supplements (EU & Japan)	Miyarisan Pharmaceutical Co. Ltd.	Human consumption	1
Annex 4 CONFIDENTIAL	Noguchi & Hayano	1963	Effects of CBM on constipation in pregnant women	Department of Obstetrics & Gynaecology, Nigata University School of Medicine, Japan	Case reports of *in vivo* use of CBM 588 in pregnant women	1-4
Annex 5	Miyarisan Pharmaceutical Co. Ltd	2011	Outcome of structured schemes followed for CBM 588.	Miyarisan Pharmaceutical Co. Ltd.	Structured schemes	1-8
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Annex 10 CONFIDENTIAL	Japanese Ministry of Health, Labour & Welfare	2006	Pharmaceutical manufacturing licence of Miyarisan Pharmaceutical Co. Ltd.	CBM 588 production plant, Miyarisan Pharmaceutical Co. Ltd., Nagano, Japan	Miya-Pro production site	1-1
Annex 11 CONFIDENTIAL	Japanese Ministry of Health, Labour & Welfare	2007	GMP Certification of Miyarisan Pharmaceutical Co. Ltd.	CBM 588 production plant, Miyarisan Pharmaceutical Co. Ltd., Nagano, Japan	Miya-Pro production site	1-1
Annex 12 CONFIDENTIAL	Kamiya *et al*	2008	Search for *Clostridium perfringens* toxin genes & in *Clostridium butyricum* MIYAIRI 588 strain	Division of Medical Microbiology, Department of Infectious Diseases, Kyorin University School of Medicine, Japan	Demonstration of absence of genes coding for toxins in CBM 588	1-8
Annex 13 CONFIDENTIAL	Shimizu & Nakanishi	2008	Assessment of the presence of botulinum neurotoxin genes & nontoxic nonhaemagglutinin gene in the probiotic bacterium *Clostridium butyricum* MIYAIRI 588 strain (CBM 588)	Department of Bacteriology, Graduate School of Medical Science, Kanazawa University, Japan	Demonstration of absence of genes coding for toxins in CBM 588	1-8
Annex 14 CONFIDENTIAL	Miyarisan Pharmaceutical Co. Ltd.	Updated 2008	Sensitivity of CBM 588 to antibiotics	Miyarisan Pharmaceutical Co. Ltd., Japan	Sensitivity of CBM 588 to antibiotics	1-3
BS 5763-13 1998	British Standards Institution (BSI)	1998	BS 5763-13. Methods for microbiological examination of food & animal feeding stuffs. Enumeration of *Escherichia coli*. Colony-count technique at 44ºC using membranes.	http://www.bsigroup.com/	Official EU analytical method/s for food products	1-1

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BS EN 15763 2008	British Standards Institution (BSI)	2008	BS EN 15763. Foodstuffs. Determination of trace elements. Determination of arsenic, cadmium, mercury & lead in foodstuffs by inductively coupled plasma mass spectrometry (ICP-MS) after pressure digestion.	http://www.bsigroup.com/	Official EU analytical method/s for food products	1-1
BS EN ISO 6579:2002+A1:2007	British Standards Institution (BSI)	2008	BS EN ISO 6579:2002+A1:2007. Microbiology of food & animal feeding stuffs. Horizontal method for the detection of *Salmonella* spp.	http://www.bsigroup.com/	Official EU analytical method/s for food products	1-1
BS EN ISO 6888-3:2003	British Standards Institution (BSI)	2003	BS EN ISO 6888-3:2003. Microbiology of food & animal feeding stuffs. Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* & other species). Detections & MPN technique for low numbers.	http://www.bsigroup.com/	Official EU analytical method/s for food products	1-2
Cianferoni & Spergel 2009	Cianferoni & Spergel	2009	Food allergy: review, classification & diagnosis	Allergology International, 2009 **58**(4):457-466	Review article	1-10
Com. Rec. 97/618	EU Commission	1997	Commission Recommendation 97/618/EC of 29 July 1997 concerning the scientific aspects & the presentation of information necessary to support applications for the placing on the market of novel foods & novel food ingredients & the preparation of initial assessment reports under Regulation (EC) No. 258/97 of the European Parliament & of the Council.	http://eur-lex.europa.eu	Novel foods	1-36
Com. Reg. 903/2009	EU Commission	2009	Commission Regulation (EC) No. 903/2009 concerning the authorisation of the preparation of *Clostridium butyricum* MIYAIRI 588 (FERM-P 1467) as a feed additive for chickens for fattening (holder of authorisation Miyarisan Pharmaceutical Co. Ltd, represented by Mitsui & Co. Deutschland GmbH).	http://eur-lex.europa.eu	First EU authorisation of CBM 588 strain as a feed additive in broilers	1-2
Com. Reg. 1881/2006	EU Commission	2006	Commission Regulation (EC) Nº 1881/2003 setting maximum levels for certain contaminants in foodstuffs	http://eur-lex.europa.eu	Food law	1-26
Com. Reg 373/2011	EU Commission	2011	Commission Implementing Regulation (EU) No 373/2011 0f 15 April 2011 concerning the authorisation of the preparation of *Clostridium butyricum* MIYAIRI 588 (FERM-BP 2789) as a feed additive for minor avian species except laying birds, weaned piglets and minor porcine species (weaned) and amending Regulation (EC) No 903/2009 (holder of authorisation Miyarisan Pharmaceutical Co. Ltd, represented by Miyarisan Pharmaceutical Europe SLU)	http://eur-lex.europa.eu	Confirmation of strain identity. EU authorisation of CBM 588 as a feed additive in weaned piglets & minor avian & porcine species	1-3

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Consol. Dir. 90/496	EU Commission	1990	Council Directive of 24 September 1990 on nutritional labelling for foodstuffs	http://eur-lex.europa.eu	Food law	1-28
Consol. Dir. 2000/13	EU Commission	2000	Directive 2000/13/EC of the European Parliament & of the Council on the approximation of the laws of the Member States relating to the labelling, presentation & advertising of foodstuffs	http://eur-lex.europa.eu	Food law	1-36
Consol. Dir. 2002/46	EU Commission	2002	Directive 2002/46/EC of the European Parliament & of the Council on the approximation of the laws of the Member States relating to food supplements	http://eur-lex.europa.eu	Food law	1-15
Consol. Reg. 258/97	EU Commission	1997	Regulation (EC) No. 258/97 of the European Parliament & of the Council of 27th January 1997 concerning novel foods & novel food ingredients.	http://eur-lex.europa.eu	Food law	1-9
Consol. Reg. 852/2004	EU Commission	2004	Regulation (EC) No. 852/2004 of the European Parliament & of the Council of 29th April 2004 on the hygiene of foodstuffs.	http://eur-lex.europa.eu	Food law	1-23
Consol. Reg. 1924/2006	EU Commission	2006	Regulation (EC) No. 1924/2006 of the European Parliament & of the Council of 20th December 2006 on nutrition & health claims made of foods.	http://eur-lex.europa.eu	Food law	1-26
Consol. Reg. 2073/2005	EU Commission	2005	Commission Regulation (EC) N° 2073/2005 of 15th November 2005 on microbiological criteria for foodstuffs.	http://eur-lex.europa.eu	Food law	1-29
CRL 2008	Community Reference Laboratory	2008	CRL evaluation report on the analytical methods submitted in connection with section II, 2.5 (control methods) of the application for authorisation as a feed additive according to Regulation (EC) N° 1831/2003 (*Clostridium butyricum* MIYAIRI 588)	http://irmm.jrc.ec.europa.eu	CRL evaluation report of analytical & identification methods used for CBM 588	1-6
CRL 2010	Community Reference Laboratory	2010	CRL evaluation report on the analytical methods submitted in connection with the application for authorisation as a feed additive according to Regulation (EC) N° 1831/2003 (*Clostridium butyricum* MIYAIRI 588)	http://irmm.jrc.ec.europa.eu	CRL evaluation report of analytical & identification methods used for CBM 588	1-7
Dir. 2003/89	EU Commission	2003	Directive 2003/89/EC of the European Parliament & of the Council as regards indication of the ingredients present in foodstuffs	http://eur-lex.europa.eu	Food law	1-4
Durre 2009	Durre	2009	Chapter 25: The Genus Clostridium	In: Practical Handbook of Microbiology, 2009, 2nd Edition E. Goldman & L.H. Green	CRC Press, ISBN 978-0-8493-9365-5	1-15
EFSA 2008	European Food Safety Authority	2008	EFSA technical guidance on the update of the criteria used in the assessment of bacterial resistance to antibiotics of human or veterinary importance	The EFSA Journal 2008 **732**:1-15 http://www.efsa.europa.eu	EFSA guidance on acceptable criteria for live micro-organisms used in the food chain, with reference to antimicrobial resistance	1-15
EFSA 2009	European Food Safety Authority	2009	Safety & efficacy of Miya-Gold® S (*Clostridium butyricum* MIYAIRI 588) as a feed additive for chickens for fattening.	The EFSA Journal 2009 **1039**:1-16 http://www.efsa.europa.eu	First EFSA opinion on safety quality & efficacy of CBM 588 strain (as an EU feed additive in chickens)	1-16

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EFSA 2010b	European Food Safety Authority	2010	Scientific/technical report submitted to EFSA. Bibliographic review on the potential of microorganisms, microbial products & enzymes to induce respiratory sensitisation	http://www.efsa.europa.eu	Review paper	1-95
EFSA 2011a	European Food Safety Authority	2011	Safety & efficacy of Miya-Gold® S (*Clostridium butyricum* MIYAIRI 588) as a feed additive for weaned piglets & minor avian species	EFSA Journal 2011; **9**(1): 1951-1965 http://www.efsa.europa.eu	Second EFSA opinion on safety quality & efficacy of CBM 588 strain (as an EU feed additive in weaned piglets & minor avian species)	1-15
EFSA 2011b	European Food Safety Authority	2011	Technical guidance on the assessment of the toxigenic potential of *Bacillus* species used in animal nutrition	EFSA Journal 2011; **9**(11): 2445-2456 http://www.efsa.europa.eu	EFSA guidance on *in vitro* methods to asses toxins & virulence factors	1-13
EFSA 2011c	European Food Safety Authority	2011	Scientific opinion on the maintenance of the list of QPS (qualified presumption of safety) biological agents intentionally added to food and feed (2011 update)	EFSA Journal 2011; **9**(12):2497 http://www.efsa.europa.eu	EFSA confirm strain-specific safety evaluation for EU food chain micro-organisms	1-82
Finegold *et al* 1983	Finegold *et al*	1983	Chapter 1 – Normal Indigenous Intestinal Flora	In: Human Intestinal Microflora in Health & Disease, 1983	Academic Press Inc ISBN 0-12-341280-3	1-17
Fujita *et al* 1986	Fujita *et al*	1986	Studies on the anti-diarrheal activity of *Clostridium butyricum* Miyairi II 588 - effects of *Clostridium butyricum* Miyairi II 588 on the production of enterotoxin from enterotoxigenic *Escherichia coli.*	Japanese Pharmacology & Therapeutics, 1986 **14**, Nº 10, p 33(6073)-40(6080) October 1986	*In vivo* studies on CBM 588 in mice	1-8
Fujita & Takashi 1986	Fujita &Takashi	1986	Studies on the anti-diarrheal activity of *Clostridium butyricum* Miyairi II 588 - effects of *Clostridium butyricum* Miyairi II 588 on the fluid accumulation induced by enterotoxigenic *Escherichia coli* in the rabbit intestinal loops.	Japanese Pharmacology & Therapeutics, 1986 **14**, Nº 7, p 137(4651)-141(4655) July 1986	*Ex vivo* studies on CBM 588 in rabbit intestinal loops	1-5
Fujita & Takashi 1987	Fujita & Takashi	1987	Studies on the anti-diarrheal activity of *Clostridium butyricum* Miyairi II 588 - effects of *Clostridium butyricum* Miyairi II 588 on the fluid accumulation induced by enterotoxigenic in the mouse intestinal loop.	Japanese Pharmacology & Therapeutics, 1987 **15**, Nº 3, p 239(1219)-243(1223)	*Ex vivo* studies on CBM 588 in mouse intestinal loops	1-5

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Ghoddusi & Sherburn 2010	Ghoddusi & Sherburn	2010	Preliminary study on the isolation of *Clostridium butyricum* strains from natural sources in the UK & screening the isolates for presence of the type E botulinal toxin gene.	International Journal of Food Microbiology, 2010 **142**, Issues 1-2: 202-206	Field study	1-5
Hippe *et al* 1992	Hippe *et al*	1992	Chapter 81: The Genus *Clostridium* - Nonmedical	The prokaryotes: a handbook on the biology of bacteria. 1992, 2nd edition: ecophysiology, isolation, identification & applications. ISBN 0387972587	Textbook chapter	1-68
Ikeda *et al* 1988	Ikeda *et al*	1988	Phenotypic characteristics in distinguishing *Clostridium butyricum* from *Clostridium beijerinckii.*	Bifidobacteria Microflora, 1988 **7**(1): 57-60	Includes CBM 588	1-4
Imase *et al* 2008	Imase *et al*	2008	Efficacy of *Clostridium butyricum* preparation concomitantly with *Helicobacter pylori* eradication therapy in relation to changes in the intestinal microbiota	Microbiology & Immunology, 2008 **52**:156-161	*In vivo* studies with CBM 588 in humans	1-6
ISO 15213 2003	International Organization for Standardization	2003	Microbiology of food & animal feeding stuffs – horizontal method for the enumeration of sulfite-reducing bacteria growing under anaerobic conditions.	http://www.iso.org/iso/home.htm	Use of iron sulphite agar for culture of anaerobic sulphite-reducers	1-12
Ito *et al* 1997	Ito *et al*	1997	Effects of administration of *Clostridium butyricum* to patients receiving long-term tube feeding	Japanese Journal of Geriatrics, 1997 **34**:298-304	Use of CBM 588 in elderly humans	1
Kamiya *et al* 1997	Kamiya *et al*	1997	Bacterioprophylaxis using *Clostridium butyricum* for lethal caecitis by *Clostridium difficile* in gnotobiotic mice.	Reviews in Medical Microbiology, 1997 **8** (S1):S57-S59	*In vivo* studies with CBM 588 in mice	1-3
Kitajo *et al* 1990	Kitajo *et al*	1990	Effect of feeding viable *Clostridium butyricum* MIYAIRI 588 to lactating cows on ruminal *Lactobacilli* & coliforms.	Japanese Journal Zootechnical Science 1990 **61**(4):344-348	*In vivo* studies with CBM 588 in dairy cows	1-5
Kitajo *et al* 1991	Kitajo *et al*	1991	Influence of oral administration of *Clostridium butyricum* MIYAIRI 588 preparation for growth & survival ratio in suckling piglets.	Research of Animal Husbandry, 1991 **45**(6): 724-728	*In vivo* studies with CBM 588 in sows & piglets	1-5
Kobashi *et al* 1983	Kobashi *et al*	1983	Cholesterol-lowering effect of *Clostridium butyricum* in cholesterol-fed rats	Digestion, 1983 **26**:173-178	*In vivo* studies with CBM 588 in rats	1-6
Kobayashi *et al* 1976	Kobayashi *et al*	1976	Change of intestinal flora following oral administration of *Clostridium butyricum* MIYAIRI strain..	Chiba Medical Journal, 1976 **52**:121-127	*In vivo* studies with CBM 588 in humans	1-7
Kuga 1985	Kuga	1985	Pharmacological approach to bacteria preparations: biochemical & pharmacological aspects of *Clostridium butyricum* MIYAIRI.	Journal of Medicine, 1985 **21**(12):1-7	Review of CBM 588 studies	1-7

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Kurata *et al* 1988	Kurata *et al*	1988	Prophylaxis of diarrhoea due to antibiotic administration by a *Clostridium butyricum* preparation (Miya BM)	Japanese Journal of Paediatrics 1988 **41:**1-6	*In vivo* studies with CBM 588 in infants & children	1-6
Kuroiwa *et al* 1990a	Kuroiwa *et al*	1990a	Inhibition of enteropathogens by *Clostridium butyricum* MIYAIRI 588.	The Journal of the Japanese Association for Infectious Diseases, 1990 **64**(3):257-263	*In vitro* studies with CBM 588	1-18
Kuroiwa *et al* 1990b	Kuroiwa *et al*	1990b	Preventive effect of *Clostridium butyricum* M588 against the proliferation of *Clostridium difficile* during antimicrobial therapy.	The Journal of the Japanese Association for Infectious Diseases, 1990 **64**(3):1425-1432	*In vivo* studies with CBM 588 in humans	1-8
Kuroiwa *et al* 1995	Kuroiwa *et al*	1995	The effect of antimicrobial feed additives on probiotics during co-administration.	Animal Husbandry, 1995 **49**(10):1-5	*In vivo* studies with CBM 588 in rats	1-5
Machii & Kuga 1989	Machii & Kuga	1989	Inhibition of 5-hydroxytryptamine-induced contraction of the isolated ileum by culture filtrate of *Clostridium butyricum* MIYAIRI	Pharmacometrics 1989 **37**(1) pages 9-16 (In Japanese with English abstract)	*Ex vivo* studies with CBM 588	1-8
Maebashi *et al* 1998	Maebashi *et al*	1998	Implication of "harmful" intestinal microflora in the pathogenesis of diseases with immune dysfunction	Biosciences Microflora, 1998 **17**(1):55-60	Use of CBM 588 in humans with immune deficiency	1-6
Maeda *et al* 1986	Maeda *et al*	1986	KM1, a bacteriophage of *Clostridium butyricum*	Journal of General Microbiology **132**:2271-2275	KM1 is strain-specific to CBM 588	1-6
Mountzouris *et al* 2002	Mountzouris *et al*	2002	Intestinal microflora of human infants & current trends for its nutritional modulation.	British Journal of Nutrition, 2002 **87**:405-420	Review article	1-16
Nakanishi *et al* 2005	Nakanishi *et al*	2005	Rapid species identification & partial strain differentiation of *Clostridium butyricum* by PCR using 16S-23S rDNA intergenic spacer regions.	Microbiology & Immunology, 2005 **49 (**7):613-621	*In vitro* identification of CBM 588 & comparison with other strains of *C. butyricum*	1-9
Nakatsuji *et al* 1995	Nakatsuji *et al*	1995	Efficacy of oral administration of *Clostridium butyricum* MIYAIRI 588 preparation for prevention of diarrhea in suckling calves.	Research Bulletin of the University Farm, Hokkaido University, 1995 **29**:55-61	*In vivo* studies with CBM 588 in milk-fed calves	1-6
Okabayashi & Kobari 1994	Okabayashi & Kobari	1994	The effects of Miya-BM® on bowel movements & abdominal symptoms in the elderly	Journal of New Remedies & Clinics, 1994 **43**(2), pages 116(300)-136(320)	*In vivo* studies of CBM 588 in the elderly	1-28
Okamoto *et al* 2000	Okamoto *et al*	2000	Preventive efficacy of butyrate enemas & oral administration of *Clostridium butyricum* M588 in dextran sodium sulfate-induced colitis in rats.	Journal of Gastroenterology, 2000 **35**: 341-346	*In vivo* studies with CBM 588 in rats	1-6

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Quiberoni *et al* 2008	Quiberoni *et al*	2008	New & classical spoilage bacteria causing widespread blowing in Argentinean soft & semi-hard cheeses	International Journal of Dairy Technology, 2008 **61**(4):358-363	Wild *C. butyricum* isolated from cheese	1-6
RASFF 2010	EU Commission	2010	The Rapid Alert System for Food & Feed	RASFF Annual Report 2010	Summary of food safety hazards notified in the EU	1-76
Reg 1169/2011	EU Commission	2011	Regulation (EU) Nº 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) Nº 1924/2006 and (EC) Nº 1925/2006 of the European Parliament and of the Council and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council , Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) Nº 608/2004	http://eur-lex.europa.eu	Food Law	1-46
Sato & Tanaka 1996	Sato & Tanaka	1996	Multiplication of orally-administered *Clostridium butyricum* in rats	Microbial Ecology in Health & Disease, 1996 **9**:115-122	*In vivo* studies on CBM 588 in the gut of rats	1-8
Sato & Tanaka 1997	Sato & Tanaka	1997	Intestinal distribution & intraluminal localization of orally administered *Clostridium butyricum* in rats.	Microbiology & Immunology, 1997 **41**, (9):665-671	*In vivo* tracking of CBM 588 in the intestinal tract of rats – spores & vegetative cells, live & dead cells	1-7
Seki *et al* 2003	Seki *et al*	2003	Prevention of antibiotic associated diarrhea in children by *Clostridium butyricum* MIYAIRI 588	Paediatrics International, 2003 **45**: 86-90	*In vivo* studies with CBM 588 in children	1-5
Shimbo *et al* 2005	Shimbo *et al*	2005	Effect of *Clostridium butyricum* on fecal flora in *Helicobacter pylori* eradication therapy	World Journal of Gastroenterology, 2005 **11**(47): 7520-7524	*In vivo* studies with CBM 588 in adults	1-5
Smith 1992	Smith	1992	Chapter 82: The Genus *Clostridium* - Medical	The prokaryotes: a handbook on the biology of bacteria. 1992, 2nd edition: ecophysiology, isolation, identification & applications. ISBN 0387972587	Textbook chapter	1-12
Taguchi *et al* 1988	Taguchi *et al*	1988	Prevention of experimental antibiotic-associated diarrhea by *Clostridium butyricum*	Japanese Journal of Bacteriology 1988 **43**, Nº 4, pages 829-835	*In vivo* studies with CBM 588 in hamsters	1-24
Takahashi *et al* 2000	Takahashi *et al*	2000	Studies of the effect of *Clostridium butyricum* on *Helicobacter pylori* in several test models including gnotobiotic mice	Journal of Medical Microbiology, 2000 **49**:635-642	*In vitro* & *in vivo* studies with CBM 588	1-8
Takahashi *et al* 2004	Takahashi *et al*	2004	The effect of probiotic treatment with *Clostridium butyricum* on enterohaemorrhagic *Escherichia coli* O157:H7 infection in mice	FEMS Immunology & Medical Microbiology, 2004 **41**:219-226	*In vitro* & *in vivo* studies with CBM 588	1-8
Takashi *et al* 1989	Takashi *et al*	1989	Effects of short chain fatty acids on the production of heat-labile enterotoxin from enterotoxigenic *Escherichia coli*	Japanese Journal of Pharmacology, 1989 **50**:495-498	*In vitro* studies to elucidate the mode of action of CBM 588 in the gut	1-4
Takeda *et al* 1976	Takeda *et al*	1976	Experience with the use of *Clostridium butyricum* MIYAIRI preparation (MIYA BM)	Journal of New Remedies & Clinics, 1976 **25**:1505-1510	*In vivo* studies with CBM 588 in infants & children	1-6
Takeda *et al* 1983	Takeda *et al*	1983	Effect of *Clostridium butyricum* on the formation & dissolution of gallstones in experimental cholesterol cholelithiasis	Life Sciences, 1983 **32:**541-564	*In vivo* studies with CBM 588 in mice	1-4
Tuohy *et al* 2003	Tuohy *et al*	2003	Using probiotics & prebiotics to improve gut health	Drug Discovery Today, 2003 **8**, Nº 15: 692-700	Review article	1-9

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Wang *et al* 2000	Wang *et al*	2000	Comparative analysis of nontoxigenic & neurotoxigenic *Clostridium butyricum* by molecular typing methods	Japanese Pharmacological Therapy, 2000 **28**(12): 999-1004	*In vitro* differentiation of CBM 588 from neurotoxigenic strains by RAPD & PFGE	1-6
Yamazaki *et al* 1996	Yamazaki *et al*	1996	Basic study of effect on using antibiotics with MIYA BM® fine granule (*Clostridium butyricum* MIYAIRI 588)	Journal of New Remedies & Clinics, 1996 **45**:155-160	*In vivo* studies on CBM 588 & antibiotics in mice	1-4
Yuzawa *et al* 1987a	Yuzawa *et al*	1987	Twelve month chronic toxicity study of *Clostridium butyricum* Miyairi powder in rats	Pharmacometrics, 1987 **33**(4):683-694	Chronic oral toxicity of CBM 588 in rats	1-13
Yuzawa *et al* 1987b	Yuzawa *et al*	1987	Tests for the acute oral toxicity in rats & mutagenicity of *Clostridium butyricum* Miyairi powder	Pharmacometrics, 1987 **34**(2): 215-221	Acute oral toxicity of CBM 588 in rats & *in vitro* mutagenicity tests	1-7
Yuzawa *et al* 1987c	Yuzawa *et al*	1987	A five week sub-acute oral toxicity & recovery test of *Clostridium butyricum* Miyairi powder in beagle dogs	Pharmacometrics, 1987 **34**(2): 223-237	Subacute oral toxicity of CBM 588 in dogs	1-16
Zhang *et al* 1998	Zhang *et al*	1998	Intestinal microflora changes in patients with irritable bowel syndrome after ingestion of *Clostridium butyricum* preparation	Chinese Journal of Gastroenterology, 1998 **3**(Nº 2):1-5	*In vivo* studies with CBM 588 in humans	1-5

1. Administrative data

(a) Name and address of the applicant & manufacturer

Miyarisan Pharmaceutical Co. Ltd. (Miyarisan),
1-10-3, Kaminakazato, Kita-Ku,
Tokyo 114-0016, Japan
Tel Nº +81-3-3917-1260, Fax Nº +81-3-3940-1142
Represented in the EU by: Miyarisan Pharmaceutical Europe, S.L.U.
Contact person: Dr Motomichi Takahashi, motomichi.takahashi@miyarisan.com

(b) Name and address of company preparing dossier

Pen & Tec Consulting S.L.
Plaza Ausias March 1, 4º, D01, Mirasol, 08195 Sant Cugat del Vallés, Catalunya, ES-08195 Spain.
Tel +34 936 758 015, Fax +34 936 758 016, Cell +34-699-053-898.
Dossier contact person: Dr Elinor McCartney, elinor@pentec-consulting.eu

Date of submission: 31 January 2012

1.1. Note on confidentiality:

According to guidance of the UK ACNFP Secretariat this dossier and relevant parts of key safety studies and safety-related data are made public. Information and texts for which the applicant claims confidentiality because it pertains to the manufacturing process, business data, marketing know-how, or for which third parties have given limited access permission (i.e. permission to be used for regulatory purposes, but not to be placed in the public domain) are marked [redacted] in the public version of the dossier. Related Annexes are listed as "CONFIDENTIAL" in the index and tables of contents, and are confidential in their entirety, namely:

Confidential Annex 2 – Geographical markets & CBM 588 products sold, sales data, prescribing & pharmacovigilance statistics, examples of GVP monitoring documents.

Confidential Annex 4 – Case studies - effects of CBM 588 on constipation in pregnant women.

Confidential Annex 8 – Detailed manufacturing & quality control processes for Strong Miyarisan Tablets.

Confidential Annex 10 - Pharmaceutical manufacturing licence of Miyarisan Pharmaceutical Co. Ltd.

Confidential Annex 11 – GMP certificate of Miyarisan Pharmaceutical Co. Ltd.

Confidential Annexes 12, 13, 14 – Unpublished data generated to meet EFSA guidance on microbial strain safety.

2. General introduction & description of Clostridium butyricum MIYAIRI 588 (CBM 588)

This application provides evidence for the safety of CBM 588 as a novel probiotic food supplement, intended for use in the general healthy adult population. The application has been prepared in accordance with Regulation (EC) Nº 258/97 and Commission Recommendation 97/618/EC. A novel food/ingredient is one that does not have a significant history of consumption within the European Union (EU) before 15 May 1997.

The food ingredient referred to in this dossier is a live microorganism, a non-pathogenic, non-genetically-modified bacterium, destined for use as a food supplement in the EU. The microorganism is *Clostridium butyricum* MIYAIRI 588 (CBM 588), presented as viable CBM 588 spores in tablets, under the proposed EU trade name of Miya-Pro. CBM 588 fulfils the criteria of a novel food, namely *´foods and food ingredients consisting of or isolated from microorganisms, fungi or algae´,* under the Class 2.2 category: *´complex novel food from non-GM source, the source of the novel food has no history of food use in the Community´*.

Although the purpose of this application is to obtain a novel food authorisation for Miya-Pro (viable spores of *Clostridium butyricum* MIYAIRI 588) as a food supplement for human consumption in the EU, the future intention of the applicant is to obtain EU approval of claims for CBM 588 under the nutrition and health claims regulation, Regulation (EC) Nº 1924/2006. The intended claims are in relation to support of gut function and physiology in the healthy adult population.

Clostridium butyricum MIYAIRI 588 (CBM 588) is a strain of *Clostridium butyricum*. The first *Clostridium butyricum* MIYAIRI strain was isolated from the faeces of a healthy human by Dr. Chikaji Miyairi in Japan in 1933, and CBM 588 is the 588th MIYAIRI strain, isolated from a soil sample in Nagano, Japan, in 1963.

Recent EFSA opinions confirm the official strain nomenclature as *Clostridium butyricum* FERM-BP 2789, and support strain identity and safety (Annex 1, EFSA 2009, 2011a). For convenience, in this novel food application, the strain name is abbreviated to CBM 588.

Preparations based on CBM 588 have a long history of safe use in human populations in Asia, especially Japan (Confidential Annex 2), where such products are variously classed as pharmaceutical drugs, “quasi drugs”, and OTC (Over The Counter) probiotics (Annex 3). The safe history of CBM 588 in human Asian populations is supported by various peer-reviewed publications and case studies dating back to 1963 (e.g. Confidential Annex 4), including reports of CBM 588 use in severely-ill, immune-compromised and hospitalized patients, whose ages range from infants to elderly people, and include pregnant women.

The applicant, Miyarisan Pharmaceutical Co. Ltd. (Miyarisan), has followed the relevant structured schemes established for novel foods (Annex 5). Miyarisan proposes 2 concentrations of CBM 588 (Miya-Pro) tablets as novel food supplements in the EU, manufactured to appropriate quality standards, labelled in accordance with EU legislation and compliant with relevant EU food legislation (Annexes 6-10).

In this application, the safety of the CBM 588 strain for healthy adults in the EU is based on 3 premises:

* Microbial techniques that establish strain identity, characteristics and safety (Annexes 1, 8-14),
* A long, safe history of use in human populations in Asian countries, since the 1960s,
* Classic toxicology testing in laboratory animals permitting extrapolation of safety to humans.

3. Identification of essential information requirements

For the safety evaluation of a Class 2.2 category novel food product, the following information is required in accordance with Commission Recommendation 97/618/EC. Please refer to Annex 5 for further details of the outcome of the structured schemes that were followed for CBM 588.

I. Specification of the novel food,
II. Effect of the production process applied to the novel food,
III. History of the organism used as the source of the novel food,
IX. Anticipated intake/extent of use of the novel food,
XI. Nutritional information on the novel food,
XII. Microbiological information on the novel food,
XIII. Toxicological information on the novel food.

4. Consultation of structured schemes (decision trees)

I. Structured Scheme I: Specification of the novel food

The novel food is defined as *Clostridium butyricum* MIYAIRI 588 (CBM 588), presented as viable spores in tablet form, for intended use as a probiotic food supplement in the EU (Consol. Dir. 2002/46). The brand name proposed for EU use is Miya-Pro, although the basic formulation is identical to Asian brands of CBM 588 tablets (i.e. Strong Miyarisan Tablet = Strong Miya-Pro Tablet; Miyarisan Tablet = Standard Miya-Pro Tablet). The only difference is the concentration of CBM 588 per tablet (Table 3).

Specified reference material for EU regulatory authorities is available on request to Miyarisan Pharmaceutical Co. Ltd. Such material may include samples of Miya-Pro Tablets with corresponding Certificates of Analysis, or permission to access the strain deposit of *Clostridium butyricum* MIYAIRI 588 (Annex 1).

Clostridium butyricum MIYAIRI 588 is a strain of *Clostridium butyricum*, isolated from a soil sample in Nagano, Japan, in 1963.

Miya-Pro Tablets are produced from CBM powder, a concentrate of viable spores of CBM 588. Appropriate excipient/s are used to produce the appropriate potency of each tablet. Miya-Pro Tablets are formulated in 2 strengths, with different quantities of CBM 588. Miya-Pro Tablets meet the required purity and quality standards for food supplements in the EU (Table 3). The composition of each tablet, apart from viable spores of CBM 588, includes the following excipients, in descending order by weight: corn starch, lactose, hydrated magnesium silicate, microcrystalline cellulose, magnesium stearate and sucrose.

Table 3. Specifications of Miya-Pro Tablets

Minimum CBM 588 content per tablet	
Standard Miya-Pro Tablets = Miyarisan Tablets	$\geq 3x10^5$
Strong Miya-Pro Tablets = Strong Miyarisan Tablets	$\geq 4.5x10^5$
Other quality specifications	
Appearance	Round tablet, 9 mm diameter, white or pale grey, with characteristic odour & sweet taste
Total aerobic count	$< 10^3$ CFU/g
Enterobacteriaceae in 1 g sample	not detected
Staphylococccus aureus in 1 g sample	not detected
Pseudomonas aeroginosa in 1 g sample	not detected
Yeasts & moulds	$< 10^2$ CFU/g
Miya-Pro tablet specifications comply with Commission Regulation (EC) Nº 1881/2006 on maximum levels for certain contaminants in foodstuffs (food supplements as sold).	
Notes: Miya-Pro tablet specifications comply with the Japanese Pharmacopoeia. An example certificate of analysis is supplied in Annex 6 & proposed label texts in Annex 7. Details of the manufacturing process & quality control procedures are provided in Confidential Annex 8 & summarized in Annex 9. Miyarisan is licensed by the Japanese government as a pharmaceutical manufacturer & the manufacturing process complies with Japanese Good Pharmaceutical Manufacturing Practice. Please refer to Confidential Annexes 10 & 11.	

I.1 Clostridium butyricum MIYAIRI 588 (CBM 588) strain origin, identity and physical properties

CBM 588 is a gram-positive, spore-forming, obligate anaerobic, non-pathogenic, non-genetically modified bacterium. The original wild strain of 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 (Annex 1). Miyarisan Pharmaceutical Co. Ltd. (Miyarisan) 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.

Viable spores of CBM 588 are naturally resistant to environmental stressors such as heat, acid, and alkalis. *In vivo* compatibility of CBM 588 has been demonstrated with a wide rage of antimicrobials (Kuroiwa *et al* 1990b, Kuroiwa *et al* 1995). After oral ingestion, viable CBM 588 spores pass through the stomach without inactivation by gastric juices. Once in the anaerobic conditions of the intestine viable CBM 588 spores germinate and undergo vegetative growth. CBM 588 is excreted in faeces mainly as vegetative cells, which die immediately on exposure to air, since CBM 588 is a strict anaerobe. When intestinal or faecal samples are exposed to air, only a small percentage of CBM 588 survive, as viable spores (Sato & Tanaka 1997). Culture systems such as CO_2 displacement and catalysed anaerobiosis are markedly inferior to continuous flow anaerobiosis, the system applied routinely by Miyarisan. Therefore, for optimum detection and enumeration of CBM 588, especially for detection and enumeration in intestinal contents or faeces, samples are taken, handled, transported and cultured under strict anaerobic conditions.

I.2 Clostridium butyricum MIYAIRI 588 (CBM 588) strain identification & characterisation

EFSA (2009) characterized the CBM 588 strain thus:

The Clostridium butyricum MIYAIRI 588 strain is deposited at the Fermentation Research Institute, Agency of Industrial Science and Technology, Japan, but not in a European culture collection. Biochemical and physiological characteristics of the strain Clostridium butyricum MIYAIRI 588 have been described and the strain has been characterized by means of biochemical and genetic techniques, such as the partial sequence of the rrn operon. Phage typing and RAPD PCR analysis were used to assess the identity of the production strain by analysing isolates from production cultures. The absence of neurotoxin production was assessed by PCR assay and Southern blot hybridisation for type E botulinum toxin gene, the most frequently encountered neurotoxin in C. butyricum. Moreover, a second study demonstrated the absence of genes coding for botulinum neurotoxin A, B, F and of genes encoding for the non-toxic non-haemagglutinin (NTNH) in CBM 588. A third PCR study demonstrated the absence in CBM 588 of genes encoding for C. perfringens toxins (α, β, ε and ι). CBM 588 contains a single plasmid of 6.5 kb. The nucleotide sequence of this plasmid has been analysed and none of the nine putative open reading frames codes for a known virulence factor of clostridia. The production strain has not been genetically modified.

EFSA (2011a) considered that the most appropriate strain nomenclature for CBM 588 in EU authorisation/s should be as *Clostridium butyricum* FERM-BP 2789 corresponding to the current strain deposition number at the National Institute of Advanced Industrial Science and Technology, International Organism Depository (AIST) in Japan. Please also refer to Annex 1.

Further details of the CBM 588 strain and its characterisation are provided below.

CBM 588 has been identified by biochemical and molecular parameters described in detail in 3 peer-reviewed published papers (Ikeda *et al* 1988, Nakanishi *et al* 2005, Wang *et al* 2000).

Ikeda *et al* (1988) used 90 strains of *Clostridium butyricum* or *Clostridium beijerincki* to establish phenotypic characteristics distinguishing *Clostridium butyricum* from *Clostridium beijerinckii* as morphological properties of both bacteria are similar, and both produce butyric acid. *Clostridium butyricum* strains were differentiated from *Clostridium beijerinckii* strains by their ability to grow in 20% bile, to produce lactic acid from peptone-yeast-extract-glucose (PYG) broth, to ferment ribose, and the failure to produce butanol from PYG broth or to ferment inositol, melezitose and sorbitol. These biochemical tests classified CBM 588 as *Clostridium butyricum*. CBM 588 exhibited 91% DNA homology with the type strain *Clostridium butyricum* ATCC 19398^T. CBM 588 was also classified as Biotype II, the same biotype as *Clostridium butyricum* ATCC 19398^T, and exhibited GC mol% of 25.9 (*Clostridium butyricum* ATCC 19398^T exhibited 24.7 GC mol%).

Wang *et al* (2000) analysed CBM 588 by RAPD and PFGE, showing unique, strain-specific patterns in comparison with 14 other strains of *Clostridium butyricum*.

A study, carried out by Nakanishi *et al* (2005), aimed to establish whether there are sufficient genetic variations in the 16S-23S intergenic spacer regions (ISRs) to discriminate *Clostridium butyricum* at the biovar level. ISRs from 5 reference strains of *Clostridium butyricum*, CBM 588, and 22 other *Clostridium butyricum* isolates were amplified.

The strains were classified into 4 groups on the basis of amplification patterns (types A, B, C, D). However, amplification of ISRs was not sufficient for discriminating strains. Subsequently, genetic structures of the ISRs were compared.

Sequence analysis revealed that the size variations of ISRs were generated by the insertion of tRNA genes and unique sequences into the internal portion, while the external portions were highly conserved. On the basis of the highly conserved nucleotide sequences within the ISRs, a PCR primer set specific to *Clostridium butyricum* was developed. In addition, the PCR primer designed from the unique inserted sequence in type B strain was useful to differentiate probiotic strains at the biovar level, and CBM 588 was identified as a type B strain. Thus in conclusion, PCR using both *Clostridium butyricum* specific primers and an ISR type-B specific primer could directly detect the *Clostridium butyricum* strain in intestinal contents.

The EU Community Reference Laboratory (CRL 2008, CRL 2010, EFSA 2009, EFSA 2011a) recommends the use of PFGE (Wang *et al* 2000) for unambiguous identification of the CBM 588 strain and the ISO Standard 15213 (ISO 15213 2003) to isolate and enumerate CBM 588 for official control purposes in the EU. The ISO 15213 2003 standard uses iron sulphite agar as the medium. CBM 588 colonies may be identified on iron sulphite agar after 24 to 48 hours anaerobic incubation at 37ºC, as round, opaque, black colonies, up to 10 mm in diameter. Iron sulphite agar is a suitable medium for isolation of CBM 588 from simple matrices such as Miya-Pro Tablets, or highly concentrated preparations of CBM 588. A CBM 588 selective blood agar is required to isolate CBM 588 from complex organic matrices such as food or feed samples, gut contents or faeces, matrices in which there may high numbers of contaminating flora and where CBM 588 is present in lower concentrations (CRL 2008, CRL 2010, Sato & Tanaka 1997).

I.3 Identification of CBM 588 vegetative bacteria

A sample colony is derived from a presumptive CBM 588 colony isolated from test matrices such as Miya-Pro Tablets, intestinal contents or faeces. The sample colony is first sub-cultured on plate count agar and incubated for 24 hours at 37ºC under anaerobic conditions, to obtain a pure subculture. The sample colony selected from the pure subculture must be an isolated, individual colony. An inoculation loop of water is placed on a slide, and a small portion of the colony obtained from the pure subculture is added using an inoculation needle. The mixture is stirred and suspended, expanded to a thin film, and dried at room temperature or over gentle heat. The slide is then passed through a flame a few times to fix the bacteria on the slide. Gram staining shows CBM 588 vegetative bacteria as large, straight or slightly curved, gram-positive rods.

I.4 Identification of CBM 588 spores

A sample colony is derived from a presumptive CBM 588 colony isolated from test matrices such as Miya-Pro Tablets, intestinal contents or faeces. The sample colony is first sub-cultured on plate count agar and incubated for 24 hours at 37ºC under anaerobic conditions to obtain a pure subculture. The sample colony selected from the pure subculture must be a well-isolated, individual colony. An inoculation loop of water is placed on a slide, and a small portion of the colony obtained from the pure subculture is added using an inoculation needle. The mixture is stirred and suspended, expanded to a thin film, and dried at room temperature or over gentle heat. The slide is then passed through a flame a few times to fix the bacteria on the slide. Spore staining will show oval sub-terminal endospores stained light to dark green.

I.5 No growth in aerobic conditions

CBM 588 is a strict anaerobe. There is no bacterial growth when a CBM 588 colony is sub cultured on plate count agar and incubated aerobically at 36 to 38°C for 1-2 days.

I.6 Production of butyric acid

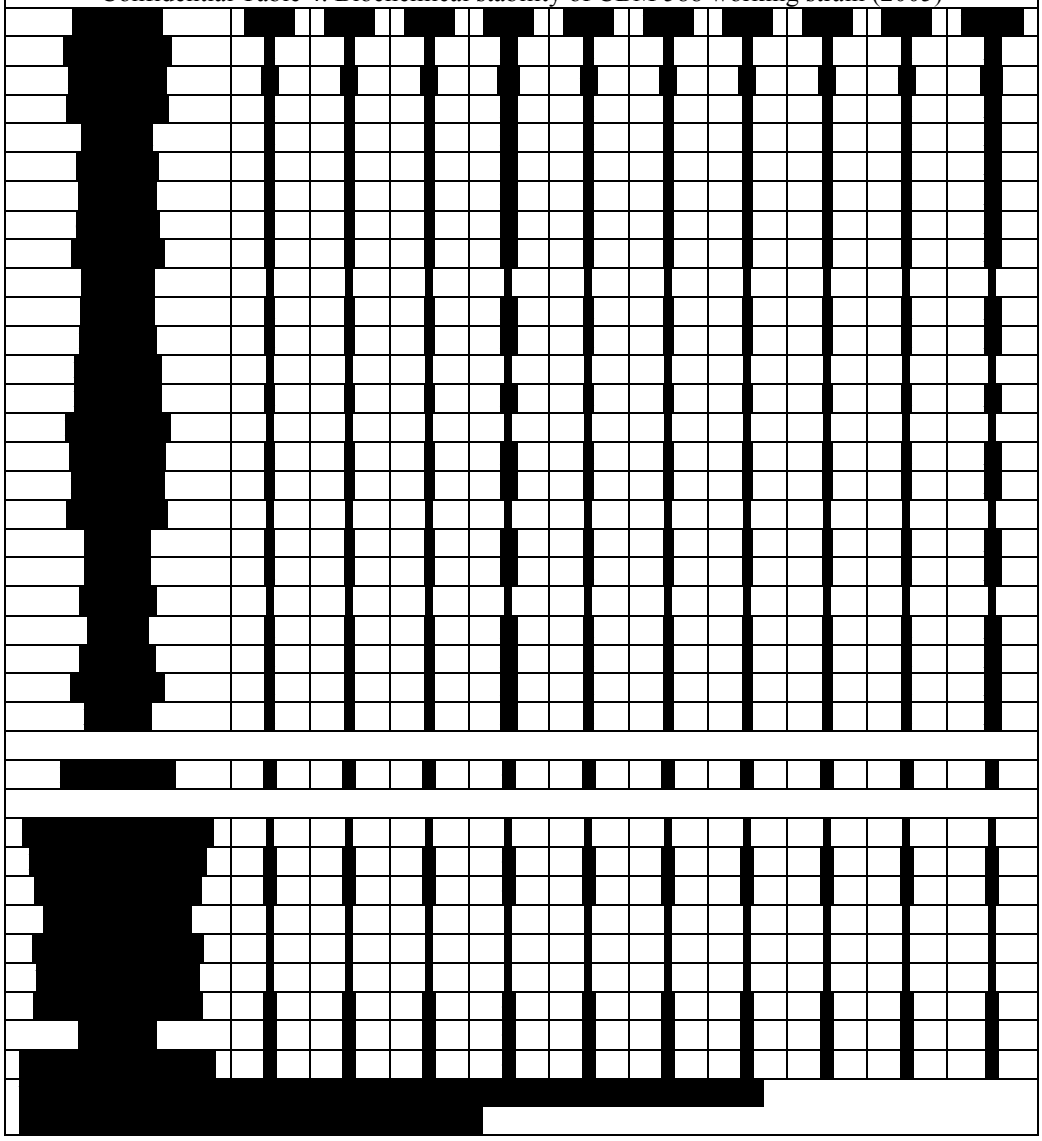
To determine the ability of CBM 588 to produce butyric acid a sample colony for this test is a presumptive CBM 588 colony isolated from test matrices such as Miya-Pro Tablets. The presumptive CBM 588 colony is first sub cultured on plate count agar and incubated (24-48 hours, 37ºC, anaerobic conditions) to obtain a pure subculture. The sample colony selected from the pure subculture must be a well-isolated, individual colony. The sample colony is then inoculated into butyric acid assay medium and cultured at 37°C for 7 days under anaerobic conditions.

The supernatant becomes the pre-sample solution. 1.0 % of reagent grade butyric acid diluted with reagent grade diethyl ether is used as pre-control solution. 0.1 mL of sulphuric acid (50% solution, diluted with the same volume of distilled water) and 0.5 mL of diethyl ether are added to 0.5 mL of pre-sample and pre-control solution, then mixed by gently swirling around 20 times, then allowed to stand for 10 minutes. Each solution is then centrifuged at 3,000 rpm and the supernatants become the sample and control solutions, respectively. Aliquots of 1 microL aliquots of sample and control solutions are then examined by gas chromatography to establish that retention times of both sample and control solutions are the same. If no gas chromatography is available, pure CBM 588 colonies grown on commercially available plate count agar will give off a characteristic smell of butyric acid.

I.7 Genetic and biochemical stability of CBM 588

Genetic and biochemical stability of the CBM 588 strain have been established (CRL 2008, CRL 2010, EFSA 2009, EFSA 2011a), and are confirmed by suitable quality control procedures on every production lot of CBM powder, the concentrate used to formulate Miya-Pro Tablets. [redacted] Additionally, in 2005, Miyarisan reconfirmed the genetic stability and identity of a working strain of CBM 588 and a CBM 588 strain from CBM powder, Lot Nº 53G1, in comparison with the strain of *Clostridium butyricum* FERM BP-2789, originally deposited in 1972, and obtained from the Japanese National Institute of Advanced Industrial Science and Technology, International Organism Depository, and the type strain (*Clostridium butyricum* ATCC 19398^{T}). [redacted] The deposited strain (*Clostridium butyricum* FERM BP-2789), the stock strain of CBM 588 and CBM 588 isolated from CBM G powder Lot Nº 53G1 showed identical amplification patterns. However, the type strain, *Clostridium butyricum* ATCC 19398^{T}, showed a different pattern (Figure 1 of Confidential Annex 8). Full details of testing for biochemical and genetic stability of the CBM 588 strain are provided in Confidential Annex 8.

Confidential Table 4. Biochemical stability of CBM 588 working strain (2005)



I.8 Assessment of CBM 588 strain identity, characterisation & microbial safety by microbiological techniques

EFSA scientific panels evaluating microorganisms intended for use in the EU food chain take a common approach to strain identity, characterisation and strain safety (EFSA 2011c). In recent opinions, EFSA (EFSA 2009, EFSA 2011a) considered that the biochemical and physiological characteristics of the CBM 588 production strain, including genetic stability of the strain, were adequately established and described by both biochemical and genetic techniques, such as the partial sequence of the *rrn* operon, phage typing and RAPD PCR analysis. Additionally, the strain does not carry toxins and virulence factors associated with clostridium or other enteropathogens (Wang *et al* 2000, EFSA 2009, EFSA 2011a). Absence of neurotoxin production was demonstrated by PCR assay and Southern blot hybridisation for type E botulinum toxin gene. The absence of genes coding for botulinum neurotoxin A, B, F and of genes encoding for the non-toxic non-haemagglutinin (NTNH), and genes encoding for *C. perfringens* toxins (α, β, ε and ι) was demonstrated by PCR assay. EFSA (2009) noted the presence of a single cryptic plasmid of 6.5 kb in CBM 588. The nucleotide sequence of this plasmid was analysed and the data showed that none of the nine putative open reading frames coded for a known virulence factor of *Clostridium* spp. (EFSA 2009, EFSA 2011a). The susceptibility of the CBM 588 production strain to the antibiotics recommended by EFSA (2008) was tested by an appropriate dilution method. The minimum inhibitory concentrations (MICs) of key antibiotics to CBM 588 were lower than the EFSA (2008) breakpoints, thereby confirming that CBM 588 is not resistant to antibiotics of human or veterinary importance (Confidential Annex 14, EFSA 2009, EFSA 2011a).

Ikeda *et al* (1988) used biochemical tests to classify CBM 588 as *Clostridium butyricum*. CBM 588 exhibited 91% DNA homology with the type strain *Clostridium butyricum* ATCC 19398^T. CBM 588 was also classified as Biotype II, the same biotype as *Clostridium butyricum* ATCC 19398^T, and exhibited GC mol% of 25.9 (*Clostridium butyricum* ATCC 19398^T exhibited 24.7 GC mol%).

Wang *et al* (2000) analysed CBM 588 by RAPD and PFGE, showing unique, strain-specific patterns in comparison with 14 other strains of *Clostridium butyricum*.

Using modern molecular techniques such as PCR, Nakanishi *et al* (2005) classified CBM 588 as a type B strain of *Clostridium butyricum*.

With respect to strain safety, several studies have examined CBM 588 to demonstrate absence of toxins and virulence factors and absence of transferrable antibiotic resistance.

Wang *et al* (2000), noting that neurotoxigenic *Clostridium butyricum* occur as wild strains investigated 8 non-toxigenic strains, including CBM 588, and 7 neurotoxigenic strains of *C. butyricum*. Strains were compared by PCR assay and southern blot hybridisation for the type E botulinum toxin gene (*bont*/E), RAPD (random amplified polymoprphic DNA) assay and PFGE (pulsed-field gel electrophoresis). The *bont*/E gene, previously established as occurring in the chromosome and not in plasmids, was detected by PCR and Southern blot hybridisation in all 7 neurotoxigenic strains of *C. butyricum* but not in non-toxigenic strains such as CBM 588, indicating the absence of partial *bont*/E gene fragments.

Kamiya *et al* (Confidential Annex 12) searched for *Clostridium perfringens* toxin genes in CBM 588, using a PCR assay and appropriate gene primers for alpha, beta, iota and epsilon genes. The bacterial strains examined included CBM 588 and 5 strains of *Clostridium perfringens*. All 5 *C. perfringens* strains produced alpha toxins and 1 strain (JCM 3817) also produced beta and epsilon toxins. No iota-producing strains of *C. perfringens* were located at the culture deposits consulted.

[redacted]

All 5 *C. perfringens* strains tested exhibited a gene coding for alpha toxin, and strain JCM 3817 also exhibited genes coding for beta and epsilon toxins. However, genes coding for alpha, beta or epsilon toxins were not detected in CBM 588.

Shimizu & Nakanishi (Confidential Annex 13) assessed CBM 588 for the presence of botulinum neurotoxin (BoNT) genes and the non-toxic non-haemagglutinin gene (NTNH). The study used extracted DNA, amplified with universal primers and multiplex real time PCR. As positive controls, the study included 3 *Clostridium botulinum* strains, and a *Clostridium butyricum* strain, all producing BoNT and NTNH. The results demonstrated that CBM 588 does not harbour genes coding for BoNT or NTHT whereas these genes were detected in al 4 positive control strains (Figures 1 and 2 of Confidential Annex 13).

Reports by Nakanishi and Tanaka (Confidential Annex 14) tested the sensitivity of CBM 588 to various antibiotics, demonstrating that CBM 588 is sensitive to antibiotics considered by EFSA (2008) to be of human or veterinary importance, and indicating a lack of mobile genetic elements capable of transferring resistance in the food chain (Confidential Table 5).

Confidential Table 5. Minimum inhibitory concentrations (mg/L) of antibiotics to CBM 588

	Ampicillin	Vancomycin	Gentamicin	Kanamycin	Sterptomycin	Erythromycin	Clindamicin	Quinupristin + Dalfopristin	Tetracylcine	Chloramphenical
EFSA 2008 breakpoints (Gram +)	1	2	4	16	8	0.5	0.25	0.5	2	2
CBM 588	[redacted]									

The data summarised above indicate that CBM, as assessed by microbiological techniques, is a well-characterised, strain, adequately and correctly identified, biochemically and genetically stable, that does not possess genes coding for toxins or virulence factors, nor does it possess mobile genetic elements capable of transferring resistance to antibiotics of human or veterinary importance.

I.9 Specification & composition of Miya-Pro Tablets

Miya-Pro Tablets are produced from CBM powder. The production method of CBM powder involves submerged anaerobic fermentation of CBM 588 in a liquid medium (Confidential Annex 8, Annex 9). Manufacturing standards of both CBM powder and Miya-Pro Tablets are certified JGMP (Japanese Good Pharmaceutical Manufacturing Practice), quality standards that meet World Health Organization recommendations and the Japanese Pharmaceutical Affair Law (Confidential Annex 10, Confidential Annex 11).

CBM powder is a dried concentrate of viable spores of CBM 588. Appropriate excipient/s are used to produce the appropriate potency of each CBM 588 product. Miya-Pro Tablets are formulated in 2 strengths, to supply different quantities of CBM 588 (Tables 3 & 6). Miya-Pro Tablets meet the required purity and quality standards for food supplements in the EU.

Table 6. Batch variation data - Miya-Pro Tablets

Minimum CBM 588 content per tablet*		Lot 1	Lot 2	Lot 3	Lot 4	Lot 5
Standard Miya-Pro Tablets	$\geq 3x10^5$	$5x10^6$	$6.5x10^6$	$5.5x10^6$	$8.2x10^6$	$7.1x10^6$
Strong Miya-Pro Tablets	$\geq 4.5x10^5$	$1.4x10^7$	$1.4x10^7$	$1.5x10^7$	$1.7x10^7$	$1.1x10^7$
*An example certificate of analysis is supplied as Annex 6						

Miya-Pro Tablets will be labelled in accordance with relevant provisions in Directive 2002/46 for food supplements, Directive 2000/13 and Council Directive 90/496 on foodstuffs labelling, Regulation (EU) Nº 1169/2011 on the provision of food information to consumers, and any future amendments to these. Proposed label texts are provided in Figures 1 & 2, (Annex 7).

Figure 1. Label text for Strong Miya-Pro Tablets - Food Supplement

Strong Miya-Pro Tablets contain *Clostridium butyricum* MIYAIRI 588 (CBM 588, guaranteed minimum $4.5x10^5$ viable spores per tablet).

Recommended daily consumption: 1 - 3 tablets daily. Quantity of tablets per glass container: 330.

Warnings: Do not exceed the recommended daily consumption. Keep out of the reach of children.

Food supplements should not be used as a substitute for a varied diet. Contains lactose.

Other ingredients: Corn starch, lactose, hydrated magnesium silicate, microcrystalline cellulose, magnesium stearate, sucrose.

Distributed in the EU by: Name, Address, Telephone, E-mail, Web Page.

EU contact address: Miyarisan Pharmaceutical Europe S.L.U., Barcelona, Spain.

Store in cool dry place in original closed container. Lot Nº: xxxxx, Best before date: dd/mm/yyyy.

Figure 2. Label text for Standard Miya-Pro Tablets - Food Supplement

Standard Miya-Pro Tablets contain *Clostridium butyricum* MIYAIRI 588 (CBM 588, guaranteed minimum $3x10^5$ viable spores per tablet).

Recommended daily consumption: 1 - 3 tablets daily. Quantity of tablets in container (330).

Warnings: Do not exceed the recommended daily consumption. Keep out of the reach of children.

Food supplements should not be used as a substitute for a varied diet. Contains lactose.

Other ingredients: Corn starch, lactose, hydrated magnesium silicate, microcrystalline cellulose, magnesium stearate, sucrose.

Distributed in the EU by: Name, Address, Telephone, E-mail, Web Page.

EU contact address: Miyarisan Pharmaceutical Europe S.L.U., Barcelona, Spain.

Store in cool dry place in original closed container. Lot Nº: xxxx, Best before date: dd/mm/yyyy.

II. Structured Scheme II: Effect of the production process applied to the novel food

The basic production process and quality control procedures are detailed in Figure 3, Annex 8 (Confidential) and Annex 9. The basic production process is submerged anaerobic fermentation, not uncommon in EU food production (e.g. pickles, sauerkraut & vinegar). Final Miya-Pro Tablets comply with all relevant EU food hygiene and safety regulations. The CBM 588 strain used in Miya-Pro Tablets is a strain of *Clostridium butyricum* that has not been modified genetically, and is genetically stable. There is no evidence of adverse consumer safety or quality effects due to the production process.

II.1. Production process

Please refer to Figure 3, Annex 8 (Confidential) and Annex 9 for information on the manufacturing process and quality control procedures applied to every batch of Miya-Pro Tablets.

Figure 3. Production flow chart & quality control (QC) of CBM powder & Strong Miyarisan® Tablets

	Production Flow Chart	QC - every lot is tested as below
	CBM 588 cell bank	Species & strain characterization by classic culture, biochemical & molecular microbiological techniques. Sensitivity of CBM 588 to strain-specific KM1 phage.
	Fermentation process	↓
	Centrifugation process	
	Drying process (with carriers)	
	Blending process (with excipients)	
↓	CBM Powder	Appearance of CBM 588 powder CBM 588 strain identity confirmed by classic culture, morphology, biochemical & molecular techniques CBM 588 quantification Microbial purity Safety test Heavy metals & arsenic Loss on drying
	Formulation process (with excipients)	↓
	Packaging process	
	Strong Miyarisan Tablets (= Strong Miya-Pro Tablets)	Appearance of Strong Miyarisan Tablets Tablet disintegration test Tablet weight variation test CBM 588 identity confirmed by classic culture, morphology & biochemical tests CBM 588 quantification Microbial purity Loss on drying
Complies with Japanese Good Pharmaceutical Manufacturing Practice (JGMP)		

Confidential: With respect to the manufacturing process (Figure 3), appropriate quality control procedures are applied to every batch (Table 4 & Confidential Annex 8):

III. Structured Scheme III: History of the organism used as the source of the novel food

Over 500 different species of micro-organisms inhabit the human gut, some of which are harmless or beneficial to the host and others potentially harmful (Tuohy *et al* 2003). *Clostridium* is a large bacterial genus with more than 150 species (Durre 2009; Hippe *et al* 2002), all gram positive, spore-forming bacteria, obligate anaerobes. Most scientists are aware of the pathogenic *Clostridium* species, notably *Clostridium botulinum*, *Clostridium difficile*, *Clostridium perfringens* and *Clostridium tetani* (Smith 1992). However, only a few members of this genus, less than 10%, form dangerous toxins (Durre 2009). Most clostridial species, especially gut-associated clostridial species such as *Clostridium butyricum*, are non-pathogenic gut commensals, which dominate and form an important part of the lower gut flora of both man and animals (Finegold *et al* 1983). The importance of clostridial species as a major bacterial component in the normal gut flora of all mammals and birds had been underestimated until the advent of modern molecular microbiological methods, possibly due to historical difficulties of cultivation *in vitro*, associated with the requirements of the genus *Clostridium* for strict anaerobic conditions.

Nowadays it is appreciated that after birth, the infant intestine is progressively colonised by facultative and strictly anaerobic bacteria, including bacteria from the *Clostridium* genus (Mountzouris *et al* 2002). *Clostridium butyricum* was first isolated and characterised by Prazmowski in 1880 (cited by Durre 2009), and may be found in the intestinal content of healthy humans, colonising the colon of infants soon after birth and forming part of the dominant, non-pathogenic, commensal clostridial group. Surveys have identified *Clostridium butyricum* in 20% of human faecal samples by microbial culture. Higher detection rates are noted when intestinal contents are sampled and strict anaerobiosis is maintained from sampling to culture (Finegold *et al* 1983; Sato & Tanaka 1997). A recent UK study (Ghoddusi & Sherburn 2010) yielded presumptive *Clostridium butyricum* strains from 302 of 978 environmental samples tested (31%). The highest percentage of positive isolations came from soil, potato skins, Swede skin, yoghurt and cream. Early blowing by *Clostridium butyricum* of Grana cheese has been reported (Bottazzi 2001, cited by Quiberoni *et al* 2008). In these respects *Clostridium butyricum* is not novel to the human gut, since it may be consumed with common food products and it forms a natural part of the gut flora of a significant proportion of human infants, children and adults, as well as being a common environmental commensal.

Clostridium butyricum MIYAIRI 588 (CBM 588) is a wild strain of *Clostridium butyricum*, originally isolated from a soil sample in Nagano, Japan, in 1963 and deposited in 1972 at the National Institute of Advanced Industrial Science and Technology, International Organism Depository (AIST) in Japan. *Clostridium butyricum* FERM-BP 2789 is the current strain deposition number for CBM 588. The strain has not been modified genetically.

Miyarisan has sold preparations of CBM 588 in Japan and other Asian countries for both human and animal use since the 1960s with no adverse events or reports of allergenicity (Confidential Annex 2). Published *in vitro* and *in vivo* data and a long history of safe use in humans in Asian countries help support CBM 588 as a safe strain for use in food supplements in the EU (e.g. Confidential Annex 4; Fujita *et al* 1986; Fujita & Takashi, 1986, 1987; Imase *et al* 2008; Ito *et al* 1997; Kamiya *et al* 1997; Kitajo *et al* 1990, 1991; Kobashi *et al* 1983, 1976; Kobayashi *et al* 1976; Kurata *et al* 1988; Kuroiwa *et al* 1990a, 1990b; Maebashi *et al* 1998; Nakatsuji *et al* 1995; Okabayashi & Kobari 1994; Seki *et al* 2003; Shimbo *et al* 2005; Taguchi *et al* 1988; Takahashi *et al* 2000, 2004; Takeda *et al* 1976, 1983, Yuzawa et al 1987a, b, c; Zhang *et al* 1998).

In 2009, a preparation based on CBM 588 was approved in the EU as a zootechnical (functional group: gut flora enhancer) feed additive for fattening chickens and in 2011 for weaned piglets, minor avian and porcine species (Com. Reg. 903/2009; Com. Reg. 373/2011; EFSA 2009, EFSA 2011a).

IV-VIII Structured schemes – not relevant to CBM 588 (non-GMM)

IX. Structured Scheme IX: Anticipated intake/extent of use of the novel food

Historical and current consumption patterns of CBM 588 in non-EU countries were considered in order to derive appropriate daily intakes as food supplements in the EU. Daily intake of CBM 588 as a food supplement in the EU (Miya-Pro Tablets) is expected to lie within the range 3×10^5 – 1.35×10^8 CFU/day (1 Standard Miya-Pro Tablet to 3 Strong Miya-Pro Tablets per day) for healthy adults, over the age of 18 years. The highest daily intake is taken from the highest upper range value for Strong Miyarisan Tablets (Annex 6).

The effective and optimum daily intake of CBM 588 may vary between healthy adult individuals, but the appropriate daily intake is anticipated to provide physiological and functional benefits in the gut (e.g. claims subject to Regulation 1924/2006 may include improved gut transit time, improved faecal bulk and consistency, improved regularity and comfort of bowel movements and possibly reduction in risk factors associated with enteric disease).

Miya-Pro Tablets are not intended to replace other foods in the diet. Orally administered probiotic bacteria, including CBM 588, do not establish permanently in the gut (Sato & Tanaka 1997). CBM 588 does not supply significant dietary macro- or micro-nutrients and it does not replace any nutrients or foods in the diet.

X. Structured Scheme X: Information from previous human exposure to the novel food or its source

CBM 588 has never been used in the EU food chain. However, wild *Clostridium butyricum* strains are commensals in the gut of humans, and may colonise the colon of infants after birth (Finegold *et al* 2003; Sato & Tanaka 1997).

A recent UK study (Ghoddusi & Sherburn 2010) yielded presumptive *Clostridium butyricum* strains from 302 of 978 environmental samples tested (31%). The highest percentage of positive isolations came from soil, potato skins, Swede skin, yoghurt and cream. Early blowing by *Clostridium butyricum* of Grana cheese has been reported (Bottazzi 2001, cited by Quiberoni *et al* 2008).

In these respects *Clostridium butyricum* is not novel to the human gut, since it may be consumed with common food products and it forms a natural part of the gut flora of a significant proportion of human infants, children and adults.

CBM 588 is a wild strain of *Clostridium butyricum*, originally isolated from a soil sample in Nagano, Japan, in 1963. Miyarisan Pharmaceutical Co. Ltd. (Miyarisan) has sold preparations of CBM 588 for human use in Japan and other Asian countries for several decades, with Japanese product development and sales commencing in the 1960s. Confidential Annex 2 provides data of geographical markets and sales data for human preparations containing CBM 588.

XI. Structured Scheme XI: Nutritional information on the novel food

The composition and production of Miya-Pro Tablets are described in Table 3, Figures 1-3 and Annexes 8 and 9. The probiotic strain supplied by Miya-Pro Tablets, CBM 588, is not intended to replace other foods in the diet. Probiotic bacteria do not establish permanently in the gut, and this includes CBM 588 (Sato & Tanaka 1997). Miya-Pro Tablets do not supply significant macro- or micronutrients, and they are not intended to replace any nutrients or foods in the diet. Viable spores of CBM 588 pass through the stomach, germinate in the anaerobic conditions of the small intestine and lower gut, undergo vegetative growth and are excreted as a mixture of vegetative cells, which die on exposure to air, and viable spores, which survive in faeces and soil (Kuroiwa *et al* 1990b; Sato & Tanaka 1997).

Subject to the provisions of the EU nutrition and health claims regulation (Regulation 1924/2006), CBM 588 is anticipated to provide physiological and functional benefits in the gut (e.g. claims may include improved gut transit time, improved faecal bulk and consistency, improved regularity and comfort of bowel movements and possibly reduction in risk factors associated with enteric disease).

CBM 588 does not have adverse physiological effects. Please refer to Structured Scheme XIII for further information on the safety of CBM 588.

XII. Structured Scheme XII: Microbiological information on the novel food

Miya-Pro Tablets contain viable spores of CBM 588, a non-GMM (non-Genetically-Modified Micro-organism), strain of *Clostridium butyricum*, originating from the soil. CBM has been described fully in previous sections and shown to be a non-pathogenic, non-toxigenic strain of established biochemical and genetic stability. For data on strain identity, safety, microbiological characterisation and biochemical, molecular and genetic stability please refer to CRL (2008, 2010), EFSA (2009, 2011a), Confidential Annexes 8, 12, 13, 14, and published references (Ikeda *et al* 1988; Maeda *et al* 1986; Nakanishi *et al* 2005, Wang *et al* 2000).

Classic toxicology tests are reported as published papers in the bibliography (Yuzawa *et al,* 1987a, 1987b, 1987c) and discussed in detail in Section XIII below.

A review of published literature indicates that CBM 588 has no adverse effects on the desirable properties of the intestinal flora of man and animals (Fujita *et al* 1986; Fujita & Takashi 1986, 1987; Imase *et al* 2008; Ito *et al* 1997; Kamiya *et al* 1997; Kitajo *et al* 1990, 1991; Kobayashi *et al* 1976; Kurata *el al* 1988; Kuroiwa *et al* 1990a, 1990b, 1995; Maebashi *et al* 1998; Nakatsuji *et al* 1995; Okabayashi & Kobari 1994; Seki *et al* 2003; Shimbo *et al* 2005; Taguchi *et al* 1988; Takahashi *et al* 2000, 2004; Takeda *et al* 1976; Yamazaki *et al* 1996).

XIII. Structured Scheme XIII: Toxicological information on the novel food

In a series of GLP (Good Laboratory Practice) studies, Yuzuwa *et al* (1987a, b, c) investigated the toxicity of CBM powder, the dried fermentation concentrate of CBM 588. CBM powder contains ≥1 x 10^9 colony-forming units of CBM 588/g.

Yuzawa *et al* (1987b) investigated the acute toxicity of CBM powder in rats. A high dose of 5,000 mg/kg body weight was given orally to rats. No animals, however, showed any signs of toxicity or died during 14 days of observation. There were no changes attributable to the administration of the test powder in body weight gain or clinical signs. The results indicate that the acute toxic dose of CBM powder is exceeds 5,000 mg/kg.

Yuzawa *et al* (1987b) also investigated the mutagenicity of CBM powder by *in vitro* reverse mutation and chromosomal aberration tests. The reverse mutation (Ames) tests were carried out on 5 strains of *Salmonella typhimurium* (TA 98, 100, 1535, 1537, 1538) and *Escherichia coli* WP2 *uvrA* with and without S9 activation. No increases in revertant colonies were observed at concentrations of CBM powder ranging from 156 to 5,000 μg/plate. The chromosomal aberration test was performed on cultured Chinese hamster cells (CHL/IU). The test was carried out at 4 concentrations of CBM powder: 70, 140, 280 and 560 μg/ml by the direct method; and at 1,250, 2,500, 5,000 and 10,000 μg/ml by the activation method. No increases in chromosomal aberration were observed in those concentrations by either method. These results indicate that CBM 588 has no mutagenicity or clastogenicity effects.

Yuzawa *et al* (1987c) investigated the sub-acute toxicity of CBM powder in young (6 months old) beagle dogs. Four groups of dogs were given gelatin capsules orally at doses of 0 (5 males, 5 females), 80 (3 males, 3 females), 400 (3 males, 3 females) and 2,000 (5 males, 5 females) mg CBM powder/kg body weight/day, respectively, for 5 weeks, followed by a 5-week recovery period involving 4 control dogs (0 mg/kg/day group, 2 males & 2 females) and 4 dogs receiving 2,000 mg/kg body weight/day (2 males & 2 females). Kennel staff observations included general health, daily food and water intake, and weekly body weights. Dogs were subjected to full veterinary clinical examinations, blood (haematology, biochemistry) and urinalysis and opthalmological examinations 1 week before study start, after 5 weeks on trial, and at the end of the 5-week recovery period. All dogs were sacrificed at the end of the administration or recovery period, respectively, and subjected to detailed gross and histopathological examinations. No animals died during the test and recovery periods. A few dogs receiving the highest dose of the powder showed occasional loose stools, but there were no clear dose-response effects. There were no changes attributable to the administration of the test powder in body weight gain, water consumption, ophthalmological examination or urinary parameters. Food consumption was slightly decreased in females receiving the powder at any dose. The mean cell haemoglobin and relative monocyte ratios were significantly higher in dogs receiving the highest dose than in the control dogs. Other than these changes, there were no haematological effects.

Male dogs receiving the intermediate dose showed decreased thymus weight and slightly increased kidney weight. However histopathological examination revealed no pathological lesions corresponding to the organ weight changes. In dogs allowed to recover for 5 weeks, there were no pathological changes attributable to the CBM powder. These results suggest that the maximum NOEL (No Observed Effect Level) oral dose of CBM powder orally is 2,000 mg/kg bodyweight/day.

Yuzawa *et al* (1987a) investigated chronic toxicity of CBM powder in SPF rats of the Fischer 344 strain. Four groups of young rats, 5½ weeks old at study start, received diets containing 0, 500, 5,000 or 50,000 mg CBM powder/kg diet for a period of 12 months. Each of the four groups consisted of 20 males and 20 females. There were no changes attributable to the feeding of the test compound in body weight gain, health, clinical observations, food consumption or ophthalmological examination. No deaths occurred and no behavioural abnormalities were observed. Haematological examination revealed increased platelet and white blood cell counts (WBC) in rats fed 5,000 or 50,000 mg CBM powder/kg diet. Blood biochemistry revealed increased blood glucose in rats fed 50,000 mg CBM powder/kg diet. Male rats fed 5,000 or 50,000 mg CBM powder/kg feed showed a decrease in GOT, GPT, uric acid, total protein, albumin and LDH. Female rats fed 5,000 or 50,000 mg CBM powder/kg feed showed a decrease in calcium. These changes, however, were too slight to indicate any functional changes of the organs. Males fed 50,000 mg CBM powder/kg diet showed increased urine volume and decreased kidney weight. Both males and females fed CBM powder showed decreased liver weight. Macroscopic and microscopic pathological examinations revealed no differences between the untreated and treated rats. There were no pathological findings observed at any dose in any organ. The highest dose affected no specific organs. Only slight changes in clinical parameters were observed. From the above results, the maximum NOEL oral dose of CBM powder was estimated to be 5,000 mg/kg diet in rats (male 241 mg/kg body weight/day, female 288 mg/kg body weight/day).

The safety factor of CBM 588 extrapolated to humans is calculated to be in excess of 100, as described below.

In the above chronic study by Yuzawa *et al* (1987a) the lowest No Observed Effect Level (NOEL) of CBM powder, containing ≥1 x 10^9 CBM 588/g 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 delivers ≥1 x 10^9 CFU CBM 588 per g, hence 14.46 g delivers 14.46 x 10^9 CFU, a NOEL of 1.45 x 10^{10} CFU/60 kg adult/day. Using the highest number tablets ingested (3 Strong Miyarisan Tablets per 60 kg adult per day), the likely highest CBM 588 intake is calculated as 1.35 x 10^8 CFU/adult/day (calculated from the upper value of the range for 9 tablets in Annex 6). This divisible by the NOEL for a 60 kg human results in a figure of 107. This implies a safety factor for human use of CBM 588 of over 100-fold.

As previously stated, CBM 588 is a non-pathogenic strain that conforms to the type strain of *Clostridium butyricum* (Ikeda *et al* 1988; Nakanishi *et al* 2005). Hence the type strain of *Clostridium butyricum* may be considered as a traditional counterpart as a baseline to facilitate toxicological assessment. Sato & Tanaka (1996, 1997) tracked CBM 588 through the intestinal tract of rats and characterised its behaviour *in vivo* as a typical cycle of spore germination, vegetative cell multiplication and excretion of mainly vegetative cells, with a small percentage of viable spores.

EFSA (2009, 2011a) reviewed the safety of CBM 588 and concluded that the strain is safe for animals, consumers of food products from animals fed CBM 588, industrial workers/users (with appropriate protection against dust in the case of feed additive formulations), and the environment. Specifically, EFSA (2009, 2011a) considered that the CBM 588 lacks the genetic determinants of relevant toxins and virulence factors, does not harbour acquired or transferable antibiotic resistance, and relevant toxicological studies do not raise concerns in relation to consumer safety. The classic toxicological studies reported above (Yuzawa *et al,* 1987a, 1987b, 1987c) provide a NOEL (No Observed Effect Level) which is more than 100 times the maximum envisaged dose of CBM 588 when used as an EU food supplement in healthy adults. Additionally, CBM 588 has been used without adverse effects for close to 5 decades in Asian populations (since 1963), including age ranges from infants to geriatrics, with health status ranging from healthy to immune-compromised, severely-ill hospitalized patients.

Evaluation of allergenicity and pathogenicity potential of CBM 588

The European Community defines twelve classes of food products that are potential allergens such as cereals containing gluten; crustaceans; eggs; fish; peanuts; soybeans; milk (including lactose); nuts; celery; mustard; sesame seeds; sulphur dioxide and sulphites (Dir. 2003/89). Food allergies related to food consumption affect as many as 8% of young children and 2% of adults in westernised countries (Cianferoni *et al* 2009). Food allergy varies according to culture, population, and geography. In Russia, Estonia and Lithuania, citrus fruits, chocolate, apple, hazelnut, strawberry, fish, tomato, egg and milk were the most common self-reported allergens, whereas in Sweden, Denmark, tree nuts, apple, pear, kiwi, stone fruits, and carrot were most common (Cianferoni *et al* 2009). In 2009, the EU received 127 notifications of failure to list potential allergens on food product labels, out of a total of 3,322 food safety alerts (RASFF 2010). This was 3.8% of the total, but does not reflect allergic reactions in the EU populations. Microbial proteins, in keeping with food proteins and other potential allergens derived from foods and food ingredients, may provoke allergic reactions in sensitive individuals. However allergies to microbial proteins, particular to fungal species, are mostly due to respiratory and not oral routes of exposure (EFSA 2010b).

In Japan, adverse events (AE) and adverse drug reactions (ADR) applicable to pharmaceutical preparations of CBM 588 are monitored in accordance with International Conference Harmonisation (ICH) guidelines in relation to Good Pharmacovigilance Practice (GVP). For practical reasons, OTC tablets and "quasi drugs" (approximately equivalent to EU food supplements) are included in Miyarisan's GVP processes. For OTC tablets and "quasi drugs" consumer feedback and reports (i.e. not associated with prescriptions of pharmaceutical preparations of CBM 588, which are administered under stricter medical supervision) are recorded and investigated by Miyarisan using the same standard operating procedures governed by GVP. Confidential Annex 2 contains examples of the common GVP documents used to monitor medical use of CBM 588 as well as consumer feedback and reports. For each report received, firstly the report is recorded for statistical purposes, then the details are passed to the Miyarisan GVP department, who decide/evaluate what further actions are required. To date, no AE, ADRs or adverse effects on human health have been associated with the consumption of CBM 588 (Confidential Annex 2).

Despite mean annual sales of around 200,000 packs each of Strong Miyarisan and Miyarisan tablets to Japanese consumers since 2005, the incidence of complaints and consumer feed back reports is very low, less than 0.1%. Additionally, Miyarisan has conducted formal pharmacovigilance (GVP) in Japan since 1995, and there have been no adverse events associated with the use of CBM 588, despite current annual prescriptions of over 1 million for pharmaceutical preparations containing CBM 588. Pharmaceutical preparations of CBM 588 are used in more sensitive population cohorts, e.g. hospitalized patients or outpatients with medical conditions under the care of physicians, and ranging in age from infants to the elderly. The lack of adverse events to CBM 588 in the general healthy adult population and in more sensitive population cohorts supports the safety of CBM 588 for the intended use in healthy, adult EU consumers. Further details are supplied in Confidential Annex 2.

Taking into account the data from the toxicological tests and the pharmacovigilance monitoring the allergenicity and pathogenicity potential of products containing CBM 588 is considered to be low.

5. Evaluation and conclusion by applicant

This application contains the necessary data requirements in accordance with Regulation (EC) Nº 258/97 and Commission Recommendation 97/618/EC on novel foods, to support the safety of Miya-Pro tablets (containing viable spores of CBM 588, *Clostridium butyricum* MIYAIRI 588 [FERM BP-2789]) for human consumption, based on:

* Microbial techniques that establish CBM 588 strain identity, characteristics and safety,
* A demonstrable, long, safe history of CBM 588 use in human populations in Asian countries,
* *In vitro* & *in vivo* toxicology testing of CBM 588, with extrapolation of safety data to humans.