Bacillus Probiotics

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SUMMARY

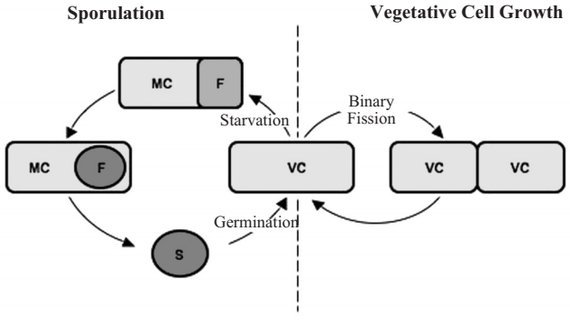
Bacillus species have been used as probiotics for at least 50 years with the Italian product known as Enterogermina® registered 1958 in Italy as an OTC medicinal specialty. The scientific interest in Bacillus species as probiotics though, has only occurred in the last 15 years and three principal reviews have covered the field (**1-3**).
Of the species that have been most extensively examined these are *Bacillus subtilis*, *Bacillus clausii*, *Bacillus cereus*, *Bacillus coagulans* and *Bacillus licheniformis*.
Spores being heat-stable have a number of advantages over other non-spore-formers such as *Lactobacillus* spp., namely, that the product can be stored at room temperature in a desiccated form without any deleterious effect on viability.
A second advantage is that the spore is capable of surviving the low pH of the gastric barrier (**4,5**) which is not the case for all species of *Lactobacillus* (**6**), so in principle a specified dose of spores can be stored indefinitely without refrigeration and the entire dose of ingested bacteria will reach the small intestine intact.
Spore probiotics are being used extensively in humans as dietary supplements (***Table 1***), in animals as growth promoters and competitive exclusion agents (***Table 2***) and lastly in aquaculture for enhancing the growth and disease-resistance of cultured shrimps, most notably the Black Tiger shrimp (*Penaeus monodon*) (***Table 3***).
This review will focus primarily on the use of spore products for human use. Interestingly, a number of *Bacillus* products are licensed as OTC products. Rather than describing specific products a short summary of the major *Bacillus* species used in commercial products will be summarised.

INTRODUCTION

Bacterial spores

Bacterial spores are produced in nature as a means to survive extreme environmental conditions enabling long-term survival in conditions that could otherwise kill vegetative bacteria (**7**). The decision to sporulate is very much dependant upon the decline in nutrients in the immediate vicinity of the live cell. Sensing this, the bacterium enters an irreversible program of development that results in the production of a spore some eight hours later (***Fig 1***) (**8**). Intrinsic to survival is the structure of the bacterial endospore, that contains, at its core, a condensed and inactive chromosome. Additional layers surround the spore, including a peptidoglycan-rich cortex and one or more layers of proteinaceous material referred to as the spore coat (**9**).

Figure 1 *The Sporulation Life Cycle*



A schematic showing the opposed life cycles of bacterial spore formers. Under conditions of nutrient starvation the growing, vegetative cell (VC) will undergo a series of morphological changes that create a forespore (F) within the mother cell (MC) of the sporangium. After approximately eight hours the spore (S) is released by lysis of the MC

Table 1 *Bacillus probiotics for human use*[a]

Brand	*Manufacturer*	*Comments/References*
Bactisubtil®	Produced by Marion Merrell (Levallois- Perret, France) but also by Hoechst and then Aventis Pharma following merger acquisitions. Also cited as being produced by Casella-Med, Cologne, Germany	Capsule carrying 1 x 10^9 spores of *Bacillus cereus* strain IP 5832[b] (ATCC 14893) [n.b., originally deposited as *B. subtilis*].
Biosporin®	(1) Biofarm, Dniepropetrovsk, Ukraine	Biosporin® is a mixture of two strains of living antagonistic bacteria *B. subtilis* 2335 (sometimes referred to as *B. subtilis* 3) and *B. licheniformis* 2336 (ratio is 3:1). Originally isolated from animal fodder
	(2) Garars, Russia	There are a number of versions of this products produced in different countries including a recombinant form, Subalin
Biovicerin®	Geyer Medicamentos S. A. Porto Alegre, RS, Brazil http://www.geyermed.com	*B. cereus* strain GM Suspension of 10^6 spores ml^{-1}
Bispan®	Binex Co. Ltd, Busan, S. Korea - www.bi-nex.com	Tablet carrying spores (1.7 x 10^7) of *B. polyfermenticus* SCDc
Domuvar	BioProgress SpA, Anagni, Italy - www.giofil.it	Vial carrying 1 x 10^9 spores of *Bacillus clausii* in suspension, labelled as carrying *B. subtilis*. No longer marketed
Enterogermina®	Sanofi Winthrop SpA, Milan, Italy - www.automedicazione.it	Vial (5 ml) carrying 1 x 10^6 spores of B. clausii in suspension. At least four different strains of *B. clausii* present and product originally labelled as carrying *B. subtilis*
Flora- Balance	Flora-Balance, Montana, USA - www.flora-balance.com	Capsules labelled as carrying *B. laterosporus* BODc but containing *Brevobacillus laterosporus* BOD
Sustenex®	Ganeden Biotech Inc., Ohio, USA - www.sustenex.com	B. coagulans GanedenBC[30] This is a patented strain that has GRAS approval in the USA
Lactipan Plus	Istituto Biochimico Italiano SpA, Milan, Italy	Capsule carrying spores of *Bacillus subtilis* labelled as carrying 2 x 10^9 spores of *Lactobacillus sporogenes*[c]
Lactospore	Sabinsa Corp., Piscataway, NJ, USA - www.sabinsa.com	Labelled as *Lactobacillus sporogenes*[c] but contains B. coagulans 6-15 x 10^9 g^{-1}
Medilac-Vita	Hanmi Pharmaceutical Co. Ltd., Beijing, China www.hanmi.co.kr	B. subtilis strain RO179 (at 10^8 g^{-1}) in combination with *Enterococcus faecium*
Nature's First Food	Nature's First Law, San Diego, CA, USA http://www.rawfood.com	42 species listed as probiotics including: *B. subtilis, B. polymyxa*[c], *B. pumilus* and *B. laterosporus*[c]
Neolactoflorene	Newpharma S.r.l., Milan, Italy	Mixture of lactic acid bacteria inc. *L. acidophilus, B. bifidum* and *L. sporogenes*[c] *L. sporogenes* at 3.3 x 10^5 CFU g^{-1} whose valid name is *B. coagulans* and is mislabelled as a strain of *B. subtilis*
Primal Defense™	Garden of Life®, Palm Beach, Florida, USA www.gardenoflife.com/	*B. subtilis*

a This list is likely incomplete and excludes Vietnamese products that are shown in Table 4
b contains the same strain used in the now discontinued animal feed product Paciflor
c not recognised as a *Bacillus* species (www.bacterio.cict.fr)

Together these protect the spore from UV radiation, extremes of heat (typically up to 80-85oC in most species), exposure to solvents, hydrogen peroxide and enzymes such as lysozyme (**7**). The spore itself is dehydrated and if exposed to appropriate nutrients will germinate, a process taking just a few minutes, allowing water to enter the spore, breakage and removal of the spore coats, and outgrowth and resumption of vegetative cell growth (***Fig 1***) (**10**).

Depending on species, spores are spherical or ellipsoidal in shape, between 0.8-1.4 μm in length, have a negative surface charge and are moderately hydrophobic. Spore forming bacteria commonly fall under two genera, *Bacillus* and the strictly anaerobic *Clostridia* although a surprisingly large number of other, lesser-known, genera include spore formers.

B. clausii

B. clausii spores are used in the product Enterogermina® which is registered as an OTC medicinal supplement. Unusually, spores (2 X 10^9) are suspended in 5ml of water and 2-3 vials are taken each day with the aim of preventing infantile diarrhoea. The suspension of spores in water is thought to enhance delivery of spores to the

Table 2 *Bacillus probiotics for veterinary use*[a]

Brand	*Animal*	*Manufacturer*	*Comments*
AlCare™	Swine	Alpharma Inc., Melbourne, Australia www.alpharma.com.au/alcare.htm	*B. licheniformis* (NCTC 13123) at 10^9- 10^{10} spores kg^{-1}. This is a non-bacitracin producing strain. Not licensed in the EU
BioGrow®	Poultry, calves and swine	Provita Eurotech Ltd., Omagh, Northern Ireland, UK. http://www.provita.co.uk	Listed as containing spores of *B. licheniformis* (1.6×10^9 CFU g^{-1}) and B. subtilis (1.6×10^9 CFU g^{-1})
BioPlus® *2B*	Piglets[a], chickens, turkeys for fattening[c]	Christian Hansen Hoersholm, Denmark http://www.chbiosystems.com	Mixture (1/1) of *B. licheniformis* (DSM 5749) and *B. subtilis* (DSM 5750) at 1.6×10^9 CFU g^{-1} of each bacterium. EU approved[a]
Esporafeed Plus®	Swine	Norel, S.A. Madrid, Spain	1×10^9 *B. cereus* (CECT 953). Not licensed in the EU
Lactopure	Poultry, calves and swine	Pharmed Medicare, Bangalore, India http://www.pharmedmedicare.com	Labelled as *Lactobacillus sporogenes*[b] but contains B. coagulans
Neoferm BS 10	Poultry, calves and swine	Sanofi Sante Nutrition Animale, France	2 strains of *B. clausii* (CNCM MA23/3V and CNCM MA66/4M). Not licensed in the EU
Toyocerin®	Calves, poultry, rabbits and swine. Possible use also for aquaculture	Asahi Vet S.A., Tokyo (Head Off.), Japan http://www.asahi-kasei.co.jp	*B. cereus* var toyoi (NCIMB-40112/CNCM-1012) at a minimum concentration of 1×10^{10} CFU g^{-1} mixed with maize flour (4% by weight) and calcium carbonate (90% by weight). Licensed in the EU[a]

a authorised for unlimited use by the EU.
b not recognised as a *Bacillus species* (www.bacterio.cict.fr)

Table 3 *Bacillus probiotics for aquaculture*[a]

Brand	*Manufacturer*	*Comments*
BaoZyme-Aqua	Sino- Aqua Corp., Kaohsiung, Taiwan www.sino-aqua.com	*B. subtilis* strains Wu-S and Wu-T at 10^8 CFU g^{-1}, product also contains *Lactobacillus* and *Saccharomyces* spp
Biostart®	Microbial Solutions, Johannesburg, South Africa and Advanced Microbial Systems, Shakopee, MN, USA	Mixture of: *B. megaterium*, *B. licheniformis*, *Paenibacillus polymyxa* and two strains of *B. subtilis* (**45**)
Liqualife®	Cargill, Animal Nutrition Division www.cargill.com	Undefined *Bacillus* species
Promarine®	Sino-Aqua company Kaohsiung, Taiwan www.sino-aqua.com	Carries four strains of *B. subtilis*
Sanocare	INVE Technologies	
Sanolife	Dendermonde,	
Sanoguard	Belgium - www.inve.com	Various *Bacillus* species

a This shows just a selection of registered products from international companies.
In shrimp-producing countries the number of 'local' products is substantial, for example, in Vietnam over 30 different products are sold

mucosa and demonstrates the versatility of spore formulations. The product carries four antibiotic resistant strains of *B clausii* that are recommended for use with antibiotics (**11-13**). Although the initial scientific studies used to register this product in 1958 are obscure, clinical trials have subsequently been performed demonstrating efficacy. The product was originally labelled as carrying spores of *B subtilis* but subsequent studies have identified the species as *B clausii* (**12,13**). This product is not specifically referred to as a probiotic but claims to enhance the body's immune system following germination of the spores in the small intestine.

B. coagulans

This species is often labelled, incorrectly, as *Lactobacillus sporogenes* which is an unrecognised species name. The origin of this species for use in probiotics stems from India where a number of manufacturers produce *B. coagulans* as a food ingredient for export and relabelling in Europe and the US. *B. coagulans* secretes a bacteriocin, Coagulin, which has activity against a broad spectrum of enteric microbes (**14**) and the organism has been shown to have beneficial effects on urinary tract infections (**15**). More recently one strain, labelled as GanedenBC[30] has been granted self-affirmed GRAS status by

the FDA in the US. It is being used in a number of products such as Sustenex and is also being incorporated into foods where spores can survive the mild heat-treatments used to sterilise foods.

B. subtilis and B. licheniformis

B. subtilis has been extensively studied at a genetic and physiological level so it is interesting that it is in use as a probiotic. Numerous probiotic products are labelled as carrying *B. subtilis* and in part, this probably results historically from a certain carelessness in assuming that most aerobic spore formers are *B. subtilis*. Accordingly, numerous products claiming to carry *B. subtilis* have been shown to carry other species (***Tables 1,4***) (***Fig 2,3***). However, *B. subtilis* var. Natto is worthy of comment. This bacterium is used in the fermentation of soybeans that is used to prepare the Japanese staple known as *Natto*. Natto carries as many as 10^8 viable spores per gram of product and for decades health benefits have been associated with consumption of Natto, including anti-cancer properties and stimulation of the immune sys-

Figure 2 *Biosubtyl and Biosubtyl DL*

Typical Vietnamese products, in this case, Biosubtyl that carries spores of *B. cereus* IP 5832 and Biosubtyl DL carrying a mixture of *B. cereus* IP5832 and *Lactobacillus acidophilus*. Neither product is labelled properly nor carries the stated dose

Figure 3 *Natto*

Natto is normally consumed as a fermented soybean product either hot or cold. In this example it is sold as a snack with dried soybeans coated with a fine white powder of *B. subtilis* var. Natto, the active ingredient required for the taste and texture of Natto

Table 4 *Vietnamese Bacillus OTC products licensed for human use*

Brand	***Manufacturer***	***Comments***
Bio-Acimin	Viet-Duc Pharmaceutical Co. Ltd., Hanoi	Labelled as containing *B. subtilis*, *L. acidophilus*, *S. faecalis* but *B. subtilis* is *B. cereus* at 10^7/g
Bibactyl	Tediphar Corporation (TEDIPHARCO), Ho Chi Minh City, Vietnam	Sachet (1g) carrying 10^7-10^8 spores of *B. subtilis*
Bidisubtilis	Bidiphar. Binh Dinh Pharmaceutical and Medical Equipment Company, 498 Nguyen Thai Hoc, Qui Nhon, Vietnam	Labelled sachets carrying 1 x 10^6 spores of *B. cereus* but mislabelled as *B. subtilis*
Biosubtyl (***Fig 2***)	Biophar Company, Da lat, Vietnam	Sachet (1 g) carrying 10^6-10^7 of *B. cereus* spores mixed with tapioca. Product labelled as *B. subtilis*. The strain is closely related by 16S rRNA analysis to IP 5832 used in Bactisubtil®
Biosubtyl DL (***Fig 2***)	IVAC, 18 Le Hong Phong, Da Lat, Vietnam	Sachets (1g) carrying 10^7-10^8 cfu of *B. subtilis* and *L. acidophilus*
Biosubtyl I and II	Biophar Company, Nha Trang, Vietnam	Sachet (1g) carrying 10^6-10^7 of *B. pumilus* spores mixed with tapioca. Product labeled as *B. subtilis*
Pastylbio	Pasteur Institute of Ho Chi Minh City, Vietnam	Sachets (1g) carrying 10^8 spores of *B. subtilis*
Subtyl	Mekophar, Pharmaceutical Factory No. 24, Ho Chi Minh City, Vietnam	Capsule carrying 10^6-10^7 spores of a *B. cereus* species termed *B. cereus* var vietnami. Product labeled as carrying *B. subtilis*
Biobaby	ILdong Pharm Co., Ltd 60-1, SinKeonji-Dong, Ansung-Si, Kyong Ki-Do Korea	Each gram of granules contains: *Lactobacillus sporogenes* 5.0 x 10^7 cfu; *Clostridium butyricum* 1.0x10^7 cfu; *B.* 3.0x10^6; Thiamine Nitrate 0.3 mg; Riboflavin 0.2 mg; Ascorbic Acid 5.0 mg; Nicotinamide 0.1 mg; Dibasic calcium photphate 20.0 mg; Dried yeast 50.0 mg
Ildong Biovita	ILdong Pharm Co., Ltd 60-1, SinKeonji-Dong, Ansung-Si, Kyong Ki-Do, Korea	Each gram of granules contains: *Lactobacillus sporogenes* 5.0 x 10^7 cfu; *Clostridium butyricum* 1.0x10^7 cfu; *B. subtilis* 3.0x10^6; Thiamine Nitrate 0.3 mg; Riboflavin 0.2 mg; Ascorbic Acid 5.0 mg; Nicotinamide 0.1 mg; Dibasic calcium photphate 20.0 mg; Dried yeast 50.0 mg

tem (**16**). A serine protease known as Nattokinase is secreted from vegetative cells of *B. subtilis* var Natto and has been shown to reduce blood clotting by fibrinolysis (**17,18**).
There are several important points here; firstly, the serine protease that is named Nattokinase is in fact produced by all strains of *B. subtilis* but in the Natto strain it is produced at higher levels. Second, it cannot be ruled out that health benefits ascribed to Natto require consumption of both soybeans and bacteria, rather than just the bacterium. In any event, Nattokinase has GRAS status as an enzyme produced from a bacterium in the US and is purified and sold as a dietary supplement worldwide.
B. subtilis and *B. licheniformis* are used together in two products, Biosporin and BioPlus® 2B. BioPlus® 2B is used in animal feed while Biosporin is licensed as a medicine in the Ukraine and Russia. Biosporin is sold in glass vials that must be reconstituted in water before consumption. The two *Bacillus* strains, *B. subtilis 2335* and *B. licheniformis 2336* are well characterised and a number of clinical studies have been used to demonstrate probiotic effects (**19-23**). In particular, *B. subtilis 2335* has been shown to produce the antibiotic Amicoumacin with *in vitro* activity against *Helieobacter pylori* (**24**). In the case of BioPlus® 2B this animal feed product has also been extensively studied with numerous efficacy studies completed resulting in the registration of this product as a feed supplement in Europe (**25**). It remains unclear whether there is any added benefit in the combined use of the two species.

B. cereus
B. cereus is a known human pathogen that is the cause of mild food poisoning due to the production of up to three enterotoxins and one emetic toxin (**26**). Not all strains of *B. cereus* carry enterotoxin genes yet a number of *B. cereus* probiotics have been shown to carry the enterotoxin genes (**27**) and one product, Paciflor, used in animal feed has been withdrawn from use in the EU (**28**). Despite this, *B. cereus* products are still being used for example, Toyerocin®, an animal feed product is registered for use in Europe (**29**) and Bactisubtil® as a registered as OTC products for human use. Interestingly, the strain of *B. cereus* used in Bactisubtil® known as IP5832 is the same as that in the withdrawn animal product Paciflor®.

HOW DO SPORE PROBIOTICS WORK?

Bacillus species are often considered soil organisms since spores can readily be retrieved from soil. However, attempting to isolate vegetative bacteria from soil is more problematic and it now seems likely that spores are designed to survive transit across the gastric barrier of animals that ingest them. This view originates from studies that show that spores of *B. subtilis* can germinate in the small intestine, grow and proliferate and then resporulate (**30,31**). Peristalsis ensures that spores are shed in faeces resulting in their accumulation in the soil. An intestinal habitat of spore formers helps explain why spores can be found in the gut of insects, animals and humans (**4,32,33**). Numerous studies have shown that germinating spores can elicit potent immune responses in the GI-tract of mouse models and this immune stimulation may be the underlying reason why spores exert a probiotic effect (**34**). One of the most informative, yet least recognised studies was one examining the effect of orally administered bacteria on the development of the gut-associated lymphoid tissue (GALT) in infant rabbits (**35**). In these studies, *B. subtilis* was shown to be of greater importance than other commensal bacteria in GALT development. Of course, other properties such as the secretion of antimicrobials such as Coagulin, Amicoumacin and Subtilisin may also further provide a probiotic effect by suppressing growth of competing microbes as well as enteric pathogens. Studies showing efficacy are less easy to distil yet a few convincing examples are as follows. In a poultry model *B. subtilis* spores were shown to suppress infection with pathogenic *Salmonella enterica* (**36**), *Clostridium perfringens* (**36**) and *E. coli* (**37**). A mouse model has been used to show suppression of *Citrobacter rodentium* (a model for the traveller's diarrhoea pathogen, ETEC) by administration of *B. subtilis* spores (**38**).

SAFETY

Two spore formers, *B. anthracis* and *B. cereus* are known as human pathogens. The former requires no elaboration while the use of *B. cereus* appears to be a cause for concern on a case-by-case basis. The safety of *Bacillus* species has been extensively reviewed elsewhere (**3,39-43**) and most incidences of illness associated with *Bacillus* appear to result for opportunistic infections or miss-diagnosis. Extensive animal studies including acute and sub-chronic toxicity testing as well as *in vitro* studies have now been performed on a number of species, including *B. subtilis* var. Natto (**44**), *B. indicus* (**44**), *B. coagulans* (**45**) and *B. subtilis 2335* (**46**) and *B. licheniformis 2336* (**46**). All appear to show no indicators of adverse effects.

PRODUCTION

It is generally assumed that bacteria are most easily produced in liquid growth using a bioreactor and for many bacteria including *Lactobacillus spp.* this appears true. In recent work using a batch-fed process a maximum yield of 2 X 10^{10} spores/ml of medium could be obtained for *B. subtilis* but no higher (**47**). The authors of this work concluded that spore-forming efficiency in liquid medium is a self-limiting process and possibly subject to feedback regulation. By contrast, solid medium is almost

exclusively used in Vietnam for spore production where proprietary vegetable-based media can generate over 100-1000 times greater spore yields.

Recent innovations: functional foods

In recent work pigmented *Bacillus* species have been characterised and the pigment has been shown to be due one or more carotenoids (**48**). Yellow, orange, red and pink *Bacillus* species can be easily obtained from soil, river and pond sediments as well as from the intestinal tracts of animals (**33,49,50**). This includes a red pigmented *Bacillus megaterium* (**51**) a pink pigment found in some isolates of *Bacillus firmus* (**52**), and red pigment found in *Bacillus atrophaeus* (**53,54**). A variable yellow-orange pigmentation has been found in a number of species including, *B. indicus* (**55**), *B. cibi* (**50**), *B. vedderi* (**56**), *B. jeogali* (**49**), *B. okuhidensis* (**57**), *B. clarkii* (**58**), *B. pseudofirmus* (**58**) and *B. firmus* (**59**). The carotenoids are found in the vegetative cell as well as in the spore and they help protect spores from UV radiation. It is no surprise that *Bacillus* species found in aquatic environments and the animals that inhabit these environments are often rich in carotenoids. Carotenoids are of nutritional value and used as dietary supplements. When used as supplements the recommended daily allowance of carotenoids is often quite high (e.g., 800 mg/day for β-carotene). The reason for this is that carotenoids are rapidly degraded in the stomach which raises questions over their nutritional value. Spore carotenoids though appear to be gastric stable and studies currently in progress are designed to establish the uptake of spore carotenoids using *in vitro* and *in vivo* models (SM Cutting, unpublished data). It is apparent that carotenoid-rich spores could be used commercially as dietary supplements providing a source of carotenoids as well as conferring probiotic properties.

A further development with spore probiotics is that they can survive mild heat treatments used to sterilise food. In principle, spores could be added to beverages and foods yet retain their probiotic properties. Indeed, such probiotic foods have already entered the market with 'Activate Muffins' containing GanedenBC^{30} launched by Isabella's Health Bakery in the USA in 2008.

CONCLUSIONS

The use of spores of *Bacillus* species as probiotic dietary or food supplements is expanding rapidly with increasing number of studies demonstrating immune stimulation, antimicrobial activities and competitive exclusion. The single and most important advantage of these products is that they can be produced easily and the stability of the finished product can be assured, further they can be incorporated into everyday foods in spore form. Studies are showing that these bacteria are able to grow within the intestinal tract and possibly be considered temporary residents. This is important because it shows that these bacteria are not foreigners but rather may exert a unique symbiotic relationship with their host.

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Appendix 1

Approved products in Europe and the USA

Bacillus products that have been formally approved in the West are few. Numerous authors routinely cite *B. subtilis* as having GRAS (Generally Regarded as Safe) status but this is incorrect. Nattokinase, the proteolytic enzyme that is purified from *B. subtilis* var Natto does carry GRAS status as a microbially produced enzyme but not the bacterium. In 2008 *B. coagulans* strain GanedenBC^{30} was the first *Bacillus* strain to be given self-affirmed GRAS approval. In Europe, for approval, for use as a supplement a case must be made based on prior use. The application is first made by authorities in the host country and then assessed by a EU committee. To date, *B. subtilis* has been approved for use as a supplement in Italy and the UK. *B. clausii*, used in the medicinal OTC product Enterogermina® (***Fig 4***) and *B. cereus IP5832* (Bactisubtil®) are registered as OTC products with specific claims regarding the prevention of childhood diarrhoea.

Figure 4 *Enterogermina®*

This is a licensed OTC product containing 2 X 10^9 of GMP-produced spores of *B. clausii* in 5ml of water. 2-3 vials are consumed per day to help prevent gastroenteritis in infants and children

Appendix 2

The Vietnamese market

In SE Asia, notably, Vietnam, where no concept of dietary supplements exists, *Bacillus* products are licensed with the Ministry of Health as *medicinal supplements* (***Table 4***) (***Fig 2,3***) with claims ranging from prevention of rotavirus infection (infant diarrhoea) and food poisoning to immune stimulation. It is unclear whether their approval requires formal clinical trials but in any event these products are easily obtained and often used as the first line of defence against enteric infections both prophylactically but more often therapeutically. The use of *Bacillus* probiotics in Vietnam is more developed than in any other country and the reason for this is unclear. There is also intense interest in using heat-stable *Bacillus* spores in aquaculture and it is not uncommon for shrimp farms to use products produced for human use. Western companies are currently focusing their attention on Vietnam as a site for the manufacture of *Bacillus* probiotics where companies such as Nam Khoa Co. Ltd and Nanogen Biopharma can provide ISO 9002 certification as well as GMP compliance.

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