Protexin® health care

Probiotic News

Issue 1 October 2008

Bio-Kult powers forward following acquisition by Protexin Healthcare

Bio-Kult, the 14-strain probiotic supplement originally formulated by a team of doctors, nutritionists and scientists, has been sold by Cambridge Bioceuticals to Protexin.

Protexin is a global probiotic specialist based in Somerset, with 20 years' experience of manufacturing and marketing a wide range of probiotic supplements.

Protexin's recent purchase of Bio-Kult is a natural progression in the brand's growth and development in support of UK Health Care Practitioners. This is because Bio-Kult has always been produced by Protexin at its UK manufacturing facility, providing the manufacturing expertise from the start.

The takeover of Bio-Kult enables the brand to grow significantly, maximising its potential as a leading probiotic supplement. Jonathan Sowler, Commercial Director at Protexin comments:

"Bio-Kult has everything to gain by preserving its stand-alone brand identity within Protexin, yet reaping the marketing benefits of an association with Protexin's own globally-trusted brand."

Peter Cartwright BA (Hons), MA, MSc

Peter is the author of three books on intestinal health (including Probiotics for Crohn's and Colitis) and has 17 years experience in self-help and patient associations. He has recently completed his MSc in Microbiology.

Peter has been working closely with Protexin for several years. As well as presenting seminars worldwide for us he also writes regular reviews on many subjects. The first one we would like to share with you is his review of the evidence behind using *Bacillus subtilis* as a probiotic.

See over for a review by Peter Cartwright BA (Hons), MA, MSc, Bacillus subtilis; Transit through the gut & effect on Lactobacilli

Published by: Protexin, Matts Lane, Stoke sub Hamdon, Somerset, TA14 6QE, UK. Tel: +44 (0) 8707 665108 Email: info@bio-kult.com

Bacillus subtilis: Transit through the gut & effect on Lactobacilli

Peter Cartwright BA (Hons), MA, MSc **looks at the evidence behind using *Bacillussubtilis* as a probiotic**

Although *Bacillus subtilis* has been used as a probiotic for many years in parts of South-East Asia and Southern Europe, some observers have doubted that it could have any beneficial effect. The scepticism rests on the fact that the *B. subtilis* in probiotic products are in spore form, which is a dormant state, and it was hard to imagine the dormant spore having any influence on the human intestine. Furthermore, it had seemed unlikely that a spore would germinate into its normal active state (known as vegetative form) within the gut.

A study by Tam *et al* (2006)[1] helped to clarify how *B. subtilis* behaves in a mammalian gut.

Transit through the digestive tract

Bacteria of the genus *Bacillus* are Gram-positive rods that are commonly found in the soil. They form spores with a harder cell wall and a more rounded shape than the normal (vegetative) state. Spores are formed as a way of survival in adverse environments.

B. subtilis in vegetative form is very unlikely to survive stomach acid, but the spore should survive passage unharmed. But what happens during transit through the rest of the intestine?

Bacillus spore germination (into vegetative state) is usually triggered by the presence of nutrients, and in some *Bacillus* species germination is triggered by a low pH (acid environment). The authors argue that "spore germination should occur readily in the GIT" (gastrointestinal tract), and they point out that this was confirmed recently in another study[2].

The Tam *et al* (2006) study checked this result and explored further. By examining the intestines of mice fed special versions of *B. subtilis* spores (that expressed a gene only in vegetative form), it was possible to confirm that some of the spores were germinating (about 1-10%). This occurred in the jejunum (upper small intestine) of the mice. Furthermore, it appeared that these vegetative bacilli were able to colonise the gut temporarily, because spores were being excreted from the mouse up to 27 days after the last dose of bacilli.

B. subtilis bacteria were thought to be strict aerobes. Now it is known that, while they prefer aerobic metabolism, they can live anaerobically, although with a much slower metabolism. It is presumed, although not proven, that the vegetative bacilli start to sporulate as they reach the large bowel, as there is very little oxygen present and they find this environment less satisfactory. *B. subtilis* can sporulate in as little as four hours at human body temperature. Laboratory tests have shown that such bacteria can sporulate in anaerobic conditions.

A second part of the study by Tam *et al* (2006), examined human stool from 30 healthy volunteers. All of the volunteers were found to be carrying *bacillus* spores, at an average concentration of about 10,000 per gram of stool. This suggests that spores of bacilli are a normal part of the human GIT, and therefore the addition of *B. subtilis* as a probiotic is not conflicting with a natural process.

Effect of *Bacillus subtilis* on *Lactobacilus* species

A study by Hosoi *et al* (1999)[3] examined the effect of *Bacillus subtilis* on the gut microflora of mice. This was undertaken to clarify earlier conflicting evidence. Three studies had shown that the administration of *B. subtilis* increased the numbers of *Lactobacillus, Bifidobacterium* and *Streptococcus* species, but two other studies had not found this effect.

The current study tried to clarify this matter by feeding the mice different diets to see if that influenced the effectiveness of *B. subtilis* in increasing the numbers of lactic acid bacteria.

The results of the Hosoi *et al* (1999) study were that the effects of *B. subtilis* on gut flora were influenced by the diet consumed by the mice. For example, mice fed on an egg-white diet had a reduction in numbers of lactobacilli in their faeces, but there was no reduction if *B. subtilis* spores were added to the mice (by intubation) at a similar time.

This effect was examined in greater detail by in vitro studies whereby a mouse-originated *Lactobacillus* was mixed with *B. subtilis* and different nutrients. *Lactobacillus* numbers increased when the nutrients were sugars such as sucrose, glucose, maltose and fructose, but not with polysaccharides such as cornstarch, soluble starch or microcrystalline cellulose.

It was not clear why *B. subtilis* increased the numbers of *lactobacilli* in the gut of mammals or what role sugars played in the process.

The same team of researchers continued their studies and published a follow-up paper [4]. In this study, the researchers co-cultured *B. subtilis* with each of three *Lactobacillus* species (an *L. reuteri* and an *L. acidophilus* from human intestine, and an *L. murinus* from a rat). They found that the *B. subtilis* spores improved the growth of L. reuteri and *L. murinus*, but not the *L. acidophilus*.

Confirmation by another research team

A different research team has confirmed the growth effect of *B. subtilis* on *lactobacilli*. This team [5] is from China, while Hosoi *et al* are from Japan. Strain *B. subtilis* MA 139 was fed to pigs and compared with a control (basal diet) and an antibiotic diet. The *bacillus* diet had a significantly better feed conversion rate compared with the control and was similar to the conversion rate of the antibiotic diet.

By the twenty-eighth day of feeding, the lactobacilli counts in the *B. subtilis* diet group were significantly increased (about nine-fold greater) compared with the control diet (P=0.02).

The Chinese team were not able to explain how *B. subtilis* increases lactobacilli numbers. They suggested two possibilities:

- decreased oxidation-reduction potential caused by the germination of spores in the intestine (a process which has been shown to benefit the growth of lactobacilli in another study)
- Production of antimicrobials (e.g. aminocumain A and bacteriocin).

In summary, the best evidence suggests that, within mammals, *B. subtilis* spores pass safely through the stomach, a proportion germinate in the jejunum, replicate for a few rounds, possibly form attached colonies, and then probably re-sporulate in the large intestine. In this way, the vegetative cells have a chance of influencing the small intestine and possibly parts of the large intestine.

Two independent research teams have shown that *Bacillus subtilis* stimulates the growth of *Lactobacillus species* in the gut of animals. These results support three earlier studies that showed this effect. The different animals involved in these studies were mice, pigs and chickens. The mechanisms of this effect are unknown, although various ideas have been put forward. There is some evidence that the pro-lactobacillus effect occurs in an environment of sugars, of which there should be plenty in the small intestine of humans.

References

1. *Tam, N.K.M., Uyen, N.Q., Hong, H.A., Duc, L.H., Hoa, T.T., Serra, C.R., Henriques, A.O. & Cutting, S.M. (2006) The Intestinal Life Cycle of Bacillus subtilis and Close Relations. Journal of Bacteriology 188: 2692-2700.*
2. *Casula, G. & Cutting, S.M. (2002) Bacillus Probiotics: Spore Germination in the Gastrointestinal Tract. Applied and Environmental Microbiology 68: 2344-2352.*
3. *Hosoi, T., Ametani, A., Kiuchi, K. & Kaminogawa, S. (2000) Improved growth and viability of lactobacilli in the presence of Bacillus subtilis (natto), catalase, or subtilisin. Canadian Journal of Microbiology 46: 892-897.*
4. *Hosoi, T., Ametani, A., Kiuchi, K. & Kaminogawa, S. (1999) Changes in fecal microflora induced by intubation of mice with Bacillus subtilis (natto) spores are dependent upon dietary components. Canadian Journal of Microbiology 45: 59-66.*
5. *Guo, X., Li, D., Lu, W., Piao, X. & Chen, (2006) Screening of Bacillustrains as potential probiotics and subsequent confirmation of the in vivo effectiveness of Bacillus subtilis MA139 in pigs. Antonie van Leeuwenhoek 90: 139-146*

Published by: Protexin, Matts Lane, Stoke sub Hamdon, Somerset, TA14 6QE, UK. Tel: +44 (0) 8707 665108 Email: info@bio-kult.com