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Probiotic News

Bacillus subtilis – Identification & Safety

3d rendered close up of Bacillus Subtilis bacteria by Sebastian Kaulitzki

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Bacillus subtilis is not an obscure or mysterious microbe. It is, instead, a very well-studied bacterium. It is the 'type' (original) species of the *Bacillus* genus[1] and it is viewed by microbiologists as a typical example of a Gram-positive bacterium and an endospore-producer. Consequently, *B. subtilis* attracts a lot of research and this is why it was one of the first organisms to have its full genome sequenced (more than ten years ago).

So what do research studies tell us about the safety of *B. subtilis* as a probiotic? Last year, three studies[2,3,4] were published on the safety of *B. subtilis*. Four strains were examined and none of these strains were found to have any pathogenic indications. The three studies involved researchers from Canada, France, Vietnam and the UK, including Dr Simon Cutting, Professor of Molecular Microbiology at Royal Holloway College, University of London.

Pathogenic genes

Specifically, the researchers tested for the presence of genes responsible for the production of various toxins, and other harmful substances such as haemolysin (blood cell disruption) and lecithinase (cell membrane disruption). No such genes were found.

The strains were also added to gut epithelial cells without causing harm, and were fed to various laboratory animals (mice, rats, guinea pigs, rabbits and piglets) with no adverse effects.

Antibiotic resistance

Another safety consideration for probiotic microbes is the presence of DNA that provides protection against the effects of antibiotics. Such antibiotic-resistant genes are found naturally in bacteria, but the question is whether they are resistant to antibiotics that are used in the treatment of humans and also whether they are the type of genes that can be transferred to other bacteria.

If such genes are transferrable to other bacteria, then they could be taken up by bacteria in the human gut flora and subsequently passed onto pathogens creating a new type of resistant pathogen.

All three of the 2008 safety studies tested their *B. subtilis* strains against a range of antibiotics. All the strains were sensitive (not resistant) to all the antibiotics important in medical treatment, as listed in a report of the European Food Safety Authority[5].

Accuracy of microbe identification

Given such data confirming the safety of *B. subtilis*, the major remaining question is whether a probiotic product contains the species it claims. Sanders *et al.*[6] reported on three studies that examined a total of seven *Bacillus* products and found that all of them were mislabelled as to the species contained. One product contained *Bacillus cereus* rather than *B. subtilis*, and this is significant because some strains of *B. cereus* can cause food poisoning.

The *B. subtilis* of Bio-Kult has been assessed independently by The National Collection of Industrial, Marine and Food Bacteria (NCIMB) (www.ncimb.com) and found to be 99.7% identical to genetic database records of *B. subtilis*. Anything over 99.0% is considered a species match. Furthermore, every batch of Bio-Kult is tested by an independent UKAS-accredited laboratory to ensure that it meets the label claims. Every pot of Bio-Kult contains exactly what the label states.

References

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Next page:
Peter Cartwright** BA (Hons) MA MSc **examines the case for multistrain probiotics.

Single-strain or Multistrain Probiotics?

Peter Cartwright BA (Hons), MA, MSc reviews the available studies to find out which works best.

It has been generally accepted that probiotic products intended for the benefit of more than one condition are likely to be more effective as multiple strains than single-strain products. "Different strains can be targeted toward different ailments and can be blended into one preparation" was the conclusion of a meeting of experts.[7]

There has, however, been little or no public research intended specifically to test this belief. This is due to two reasons:

a) Studying a multi-strain product is more complicated and more expensive than studying a single-strain

b) Most clinical studies are funded by companies with an interest in one specific strain only.

Timmerman *et al*[8] have overcome these limitations by carefully searching research literature and finding studies that have compared mono- and multi-strain probiotics.

The three types of probiotic product used in the research were defined by Timmerman *et al* as follows:

- Monostrain – containing one strain of a certain species
- Multistrain – containing more than one strain of the same species or closely related species (e.g. *Lactobacillus acidophilus* and *Lactobacillus casei*)
- Multispecies – containing strains of different probiotic species that belong to one or preferentially more genera (e.g. *L. acidophilus, Bifidobacterium longum, Enterococcus faecium* and *Lactococcus lactis*)

The comparison studies

1. Zoppi *et al* [9] tested the effectiveness of six commercially available probiotic products for their effectiveness in reversing the adverse effects of an antibiotic on the gut microflora of children. Three of the probiotics were monostrain (*Saccharomyces boulardii, E. faecium and Lactobacillus rhamnosus*). Of the other three probiotics, one was a multistrain (containing three lactobacilli: *L. rhamnosus, L. acidophilus and Lactobacillus bifidus*) and the other two were multispecies. One of the multispecies contained two species (*L. acidophilus* and *Bifidobacterium bifidum*) and the other contained high numbers of nine species.

A total of 51 children received either the antibiotic alone (ceftriaxone) or in combination with one of the probiotics mentioned above. The *S. boulardii* left the microflora unchanged and the *E. faecium* did not correct dysbiosis. The *L. rhamnosus* "induced favourable alterations in the microflora, but these were less marked than those induced by the multistrain treatments".

Of the multistrain/multispecies probiotics, only the two multispecies preparations "significantly counteracted the increase in number of stools per day" caused by the antibiotic. "Only two probiotics, both multispecies preparations, were able to induce a statistically significant pH reduction", which "can be interpreted as a positive effect because an acidic environment inhibits the growth of pathogenic bacteria".

2. Perdigon *et al*[10] "tested the protective effect of milk fermented with either *L. acidophilus*, *L. casei* or a combination of both strains in mice challenged with *Salmonella typhimurium*". "The monostrain fermented milks failed to enhance resistance towards *S. typhimurium*". Only the "multistrain fermented milk was effective in preventing colonisation of *S. typhimurium* in liver and spleen."

Twenty-one days after the salmonella inoculation, all the multistrain-fed mice were alive, while only one-fifth of the monostrain-fed mice were alive. Among the control mice that received no probiotics, only one-fifth were alive; the same proportion as the monostrain-fed mice.

3. Paubert-Braquet *et al*[11] used mice orally infected with *S. typhimurium* to test the protective effect of milks fermented with different strains of *L. casei*, yoghurt, or a combination of both kinds of ferment. More protection was provided by the mixture of *L. casei* LAB-1 plus yoghurt, than each of the three monostrains, or the yoghurt alone.

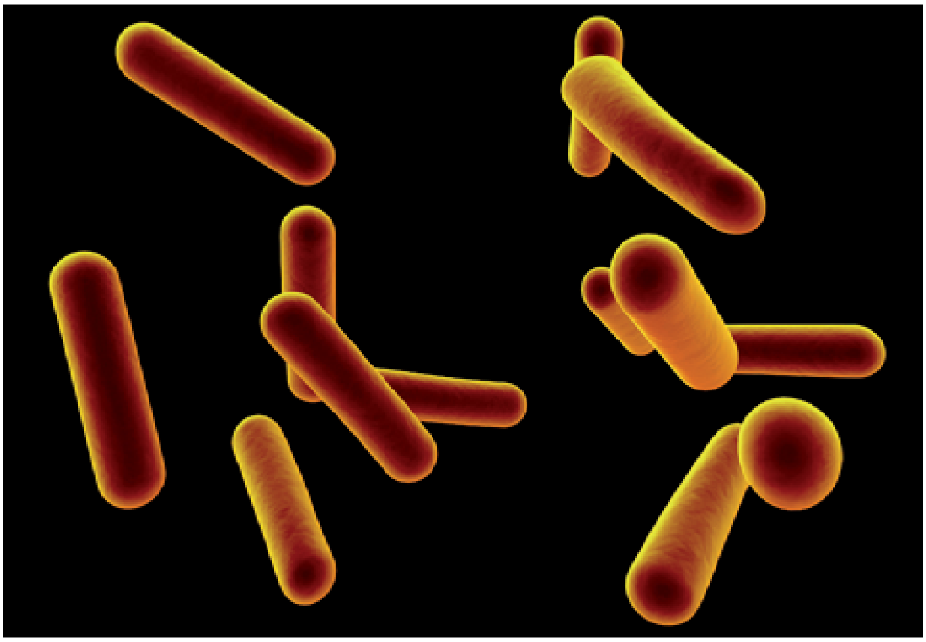
4. Lema *et al*[12] studied the efficacy of five species of lactic acid bacteria in reducing the faecal shedding of the food-borne pathogen of humans, *E. coli* O157:H7.

The two monostrain preparations contained *L. acidophilus* or *E. faecium*, and the two multispecies preparations contained either two species (*L. acidophilus and E. faecium*) or five species (*L. acidophilus, E. faecium, L. casei, Lactobacillus fermentum* and *Lactobacillus plantarum*).

L. acidophilus by itself did not affect the amount of *E. coli* O157:H7 shed. *E. faecium* did reduce the shedding, but the five species multistrain preparation performed significantly better.

5. Van Es and Timmerman[13] compared the protection given by probiotics to rats challenged by *Salmonella enteritidis*. The salmonella innoculation was a sublethal dose; none of the rats died and no signs of disease were seen. One monostrain was tested, along with two multistrains (one with two species of Lactobacilli and one with three species of Lactobacilli), plus two multispecies probiotics. The multispecie preparations consisted of one with four species (three Lactobacillus and one Lactococcus) and one with five species (the four species above, plus *L. plantarum*).

The results showed that weight gain was highest for the four-species multispecies probiotic preparation.



3d rendered close up of bacteria by Sebastian Kaulitzki

Summary of the studies

Timmerman *et al*[8] summarised the above studies as follows: "The studies described indeed provide evidence for multistrain probiotics being more effective than monostrain probiotics. The use of multispecies preparations, containing multiple strains of more than one genus, could even be more effective than that of multistrain probiotics." The reviewers also pointed out that such benefits work both with fermented and freeze-dried products.

Possible mechanisms involved in multispecies probiotics

The mechanisms by which probiotic bacteria have their positive health effects are not known, although there is a wide range of possible explanations. The same can be said of the mechanisms of multispecies probiotics.

Timmerman *et al*[8] describe examples of how such mechanisms may operate:

- "*Streptococcus thermophilus* are oxygen scavengers and create anaerobic conditions that could enhance growth and survival of strict anaerobes like bifidobacteria".
- "The presence of *L. rhamnosus or Lactobacillus delbrueckii* subsp. *bulgaricus* more than doubled the adhesion of *Bifidobacterium animalis* to human intestinal mucus.
- There may be a "greater variety of antimicrobial capacities associated with mixed preparations, such as production of weak organic acids, bacteriocins, hydrogen peroxide, coaggregation molecules (blocks the spread of the pathogen) and/or biosurficants (inhibit adhesion)" and the stimulation of the host to produce more sIgA [antibodies secreted into the intestinal lumen and mucus which have antibacterial properties].
- "Addition of typical yoghurt bacteria particularly *L. delbrueckii* subsp. *bulgaricus* will enhance the growth of the probiotic strains", such as *L. acidophilus* and Bifidobacterium species. These species normally do not grow well in milk because they lack protein-degrading enzymes, but they can use amino acids and peptides released by the bulgaricus bacterium.
- Lactobacilli are "able to produce bifidogenic growth factors in the form of extracellular polysaccharides (EPS). EPS may protect the microorganism against anti-microbial factors because it surrounds the bacterial cell as a capsule or is secreted into the extracellular environment as slime."
- The enhanced effect of the multistrain of *L. casei* and *L. acidophilus* in the Perdigon *et al* study is thought to be due to the *L. casei* activating the adaptive immune system, while *L. acidophilus* induces an innate immune response.

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