CHARACTERISTICS OF THE GENUS

Bifidobacteria were discovered in 1899 in the faeces of breast-fed infants. This was of particular interest to scientists as these bacteria are typically the most abundant species present in the intestine of breast-fed infants and regarded as a primary reason for the infants’ greater resistance to disease.

Today bifidobacteria are broadly recognised for their key role in the human intestinal microbiota throughout life. A high proportion of bifidobacteria in the intestinal tract is considered beneficial to health.

Bifidobacterium sp. comprises Gram-positive, non-spore forming, anaerobic, pleomorphic bacilli that are dominant microbial residents of the colonic microbiota (1, 2).

SELECTION AND TAXONOMY

Bifidobacterium lactis was originally described by Meile et al. (3) and was recently re-classified as B. animalis subsp. lactis (4). In the interests of simplicity, Danisco refers to strains of this species as B. lactis.

B. lactis Bi-07 has been genetically characterised and properly classified as B. lactis by independent labs using modern genotypic methods, including 16S rRNA gene sequencing and PCR using species-specific primers (5).

B. lactis Bi-07 is of human origin and has been shown to grow well in milk.

B. lactis Bi-07 has been deposited in the American Type Culture Collection’s safe deposit as SD5220.

SAFE FOR CONSUMPTION

Bifidobacterium sp. has long been considered safe and suitable for human consumption with several published studies addressing its safety (6-10). Furthermore, Bifidobacterium lactis has been present in human food for decades and is listed in the Inventory of Microorganisms With Documented History of Use in Human Food (11). The European Food Safety Authority has also added the species to the Qualified Presumption of Safety list (12).

No harmful metabolic or toxigenic activities are associated with B. lactis. In addition to its long history of safe human consumption of the species, no acquired antibiotic resistance was detected in B. lactis Bi-07 during screening by the EU-funded PROSAFE project.

GASTROINTESTINAL PERFORMANCE

Resistance to acid and bile

According to the generally accepted definition of a probiotic, the probiotic microorganism should be viable at the time of ingestion in order to confer a health benefit. This implies that a probiotic should survive GI tract passage and, according to some interpretations, transiently colonise the host epithelium.

A variety of traits are believed to be relevant for surviving GI tract passage, the most important of which is tolerance both to the highly acidic conditions present in the stomach and to concentrations of bile salts in the small intestine.

In vitro studies have shown that B. lactis Bi-07 is extremely resistant to low pH conditions and survive the presence of bile at concentrations present in the duodenum.

Adhesion to intestinal mucosa

While adhesion is not a pre-requisite for a strain to elicit probiotic properties, interaction with the intestinal mucosa is considered important for a number of reasons. Binding to the intestinal mucosa may prolong the time a probiotic strain can reside in the intestine. This interaction with the mucosa brings the probiotic in close contact with the intestinal immune system, giving it a better opportunity to modulate the immune response. It may also protect against enteric pathogens by limiting their ability to colonise the intestine.

Currently, adherence is measured using two in vitro cell lines, Caco-2 and

| Acid tolerance | ++++ (>90% survival in hydrochloric acid and pepsin (1%) at pH 3 for 1h at 37°C) |
| Bile salt tolerance | +++ (>90% survival in 0.3% bile salt containing medium) |

Selected characteristics of B. lactis Bi-07 (internally generated data): ++++ Excellent; +++ Very good; ++ Good; + Fair
HT-29. While this is not a thorough test of the ability of probiotics to adhere to intestinal mucosa in the body, attachment to these cell lines is considered a good indicator of their potential to attach.

*B. lactis* Bi-07 has demonstrated very good adhesion to human epithelial cell lines (Caco-2) applied in *in vitro* studies.

<table>
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<tr>
<th>Adherence to human intestinal cells in vitro</th>
<th>HT-29: ++</th>
<th>Caco-2: +++</th>
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<tbody>
<tr>
<td>Selected characteristics of <em>B. lactis</em> Bi-07 (internally generated data):</td>
<td>++++ Excellent; +++ Very good; ++ Good + Fair</td>
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</table>

These findings were confirmed in a study that evaluated bacterial adhesion to Caco-2 cells, either by using radio-labelled bacteria and counting Caco-2-bound radioactivity (radioactive adhesion assay), or by quantifying Caco-2-bound bacteria with genus or species-specific primers via real-time PCR (non-radioactive adhesion assay).

The study found that the Caco-2 cell adhesion activity of seven bifidobacterial strains, belonging to 5 different species, varied considerably. *B. lactis* Bi-07 was one of the two most adhesivest rains (13).

Up to date, there is limited knowledge available about the specific molecular mechanisms involved in the interaction process of the microbiota with the host. Two studies were conducted to investigate whether whole cells of *B. lactis* Bi-07 were able to capture human plasminogen on the cell surface. With two different techniques, a dose dependent human plasminogen-binding activity has been shown for *B. lactis* Bi-07.

The identification of five putative plasminogen-binding proteins among the cell wall fraction of *B. lactis* Bi-07, suggest that plasminogen binding to *B. lactis* is due to the concerted action of a number of proteins located on the bacterial cell surface. These findings represent a step forward in understanding the mechanisms involved in *Bifidobacterium*-host interaction (14,15).

**Inhibition of pathogens**

The protective role of probiotic bacteria against gastrointestinal pathogens is highly important to therapeutic modulation of the enteric microbiota. Probiotics are able to inhibit, displace and compete with pathogens, although these abilities are strain-dependent.

The probiotic strains' putative mechanisms of action against pathogenic microorganisms include the production of inhibitory compounds, competition with pathogens for adhesion sites or nutritional sources, inhibition of the production or action of bacterial toxins, ability to coaggregate with pathogens, and the stimulation of the immune system.

*In vitro* inhibition is usually investigated using an agar inhibition assay, where soft agar containing the pathogen is laid over colonies of probiotic cultures, causing the development of inhibition zones around the colonies. This effect may be due to the production of acids, hydrogen peroxide, bacteriocins and other substances that act as antibiotic agents and as competition for nutrients. It should be pointed out, however, that the extrapolation of such results to the *in vivo* situation is not straightforward. The assessment in the table below is based on such an *in vitro* assay.

*B. lactis* Bi-07 displayed *in vitro* inhibition of selected pathogens.

<table>
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<tr>
<th>Pathogen inhibition in vitro</th>
<th>Salmonella typhimurium: +</th>
<th>Staphylococcus aureus: +</th>
<th>Escherichia coli: +++</th>
<th>Listeria monocytogenes: +</th>
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<tbody>
<tr>
<td>Selected characteristics of <em>B. lactis</em> Bi-07 (internally generated data):</td>
<td>++++ Excellent; +++ Very good; ++ Good + Fair</td>
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</table>

Another *in vitro* study investigated how various bifidobacteria, including *B. lactis* Bi-07, and adhesive enteropathogens (*Salmonella enterica* serovar Typhimurium, *Yersinia enterocolitica*, and *Escherichia coli H10407*) compete for adhesion to Caco-2 cells. Two competition conditions were tested for each bifidobacterium-enteropathogen: displacement and exclusion.

In the displacement assay, the enteropathogen was added to the Caco-2 cell monolayer before the addition of the *Bifidobacterium*. In the exclusion assay, the bifidobacteria were added to the Caco-2 cell monolayer before the addition of the enteropathogen. At the end of both competition assays, the bifidobacterium and enteropathogen cells bound to Caco-2 cells were specifically quantified using real-time PCR. All the bifidobacteria strains showed strong displacement activity towards these enteropathogens.

In exclusion studies, the adhesive bifidobacterial strains excluded *Y. enterocolitica*. Only one strain of *B. bifidum* exerted exclusion activity towards *S. enterica* serovar Typhimurium, and no bifidobacteria strain excluded *E. coli H10407*. Enteropathogens excluded none of the bifidobacterium strains in the exclusion assays.

These results show the ability of *B. lactis* Bi-07 to compete with pathogens for epithelial monolayer adhesion, which plays a possible role in protecting against, or recovery from, pathogen colonisation (13).

**L/D lactic acid production**

Lactic acid is the most important metabolic end product of fermentation processes by lactic acid bacteria and other microorganisms. Lactic acid fermentation has been used for thousands of years in the production of fermented foods.

Due to its molecular structure, lactic acid has two optical isomers. One is known as L(+) lactic acid and the other; its mirror image, is D(-) lactic acid.

In humans, animals, plants and microorganisms, L(+) lactic acid is a normal intermediate or end product of the carbohydrate and amino acid metabolisms. It is important for the generation of energy under anaerobic conditions.

In the organs of humans and animals, the endogenous synthesis of D(-) lactic acid is very low. The isomer is normally
present in the blood of mammals at nanomolar concentrations and may be formed from methylglyoxal, which derives from lipid or amino acid metabolism.

*B. lactis* Bi-07 only produces L(+) lactic acid.

<table>
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<tr>
<th>L/D lactic acid production</th>
<th>Molar ratio</th>
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<tr>
<td>R-Biopharm D-lactic acid/</td>
<td>100/0</td>
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<tr>
<td>L-lactic acid UV-method</td>
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Internally generated data

**Human studies**

*B. lactis* Bi-07 was included in a five-strain formulation investigated for its ability to stabilise the intestinal microbiota during and after antibiotic therapy. In this human trial, the probiotic product was found to reduce the antibiotic-induced disturbance of the total microbiota population (figure 1). In addition, the probiotic product still maintained bifidobacteria at significantly higher levels than that of the placebo group even two weeks after the cessation of antibiotic therapy (figure 2) (16).

*B. lactis* Bi-07 was evaluated in a double-blind, placebo-controlled, randomised human clinical study as part of a three-strain formulation (also including *Lactobacillus reuteri* and *Lactobacillus acidophilus* NCFM®). A total of 243 children aged 12-36 months were recruited. During the 14-week intervention period, a statistically significant reduction in the incidence and episodic frequency of diarrhoea was recorded in the probiotic versus the placebo group (17).

*B. lactis* Bi-07 was evaluated in synbiotic nutritional supplements for 1-10 year old children (also including *Lactobacillus acidophilus* NCFM® and fructo-oligosaccharides) in two human clinical studies.

One multicentre, open, randomised, comparative study included acutely ill children aged 1-6 years who required antibiotic treatment for a bacterial infection. The children received either the synbiotic nutritional supplement (PS), a nutritional supplement without the synbiotic components (P) or a fruit-flavoured drink (D) with their medication.

Total energy intake, weight gain and faecal lactobacilli levels were significantly greater in the group that consumed the synbiotic formula (PS). This group also had the lowest rate of relapse or new bacterial infections, though these differences were not statistically significant. There were no significant differences in faecal bifidobacteria levels at the end of antibiotic therapy, although levels were higher in the PS group. There were also no significant differences among the groups in relation to the duration of illness or treatment. All three supplements were generally well tolerated.

Appropriate nutrition is particularly important for children during acute phases of illness to maximise energy and fluid intake and to improve the recovery process. The study results suggest that the use of nutritional supplements containing *B. lactis* Bi-07 is beneficial and safe in children undergoing antibiotic treatment (18).

The second study – a double-blind, randomised 4-month study – was conducted at 13 locations in Brazil, Mexico, Portugal, and Spain. The objective was to evaluate the incidence and duration of illness plus anthropometrics in children who received a nutritional supplement with or without synbiotics.

Children recruited for the study were 1 to 6 years old and underweight (as defined by a World Health Organization/National Center for Health Statistics chart (WHO/NCHS)), but otherwise healthy.
Overall, the incidence of sickness, number of sick days and antibiotic use were similar between the two groups. However, in the group consuming the synbiotic formula, subjects aged 3 to 5 years, who had at least one episode of sickness (p=0.47), experienced significantly fewer sick days. This suggests that the formula may help to reduce the duration of sickness in some children. The synbiotic group experienced a significant reduction in constipation across all ages.

All subjects experienced growth in relation to height, weight and weight/height-ratio. There were no differences in the growth rate of the synbiotic and control feeding groups.

Both supplements used in the study were well tolerated, and the overall incidence of adverse events was very low (19).

*B. lactis* Bi-07 was further evaluated in a multicentre, double-blind, placebo-controlled, randomised study with healthy toddlers, 12-34 months of age. The purpose of this study was to examine the safety of formulas containing probiotics and prebiotics as well as faecal microbiology and colonisation of the probiotic administered. Toddlers were divided at random into three groups: control, probiotic (containing *B. lactis* Bi-07) and synbiotic (containing probiotic and a fructo-oligosaccharide). *B. lactis* was detected significantly more frequently in the faeces of the probiotic (39%/41%) and synbiotic group (38%/50%) at day 7 and day 28, compared to the control group (1%/2%).

No significant differences were found in faecal concentrations of bacteroides, streptococci or total bifidobacteria. There was no statistically significant difference across formula groups in the number and kind of formula-related adverse effects (21).

These studies provide further evidence of the safe, beneficial use of *B. lactis* Bi-07 in child nutrition.

**IMMUNOMODULATION**

An immune system that functions optimally is an important safeguard against infectious and non-infectious diseases. The intestinal microbiota are a key element in the body’s immune defence system.

Probiotic bacteria with the ability to modulate certain immune functions may improve the response to oral vaccination, shorten the duration or reduce the risk of certain types of infection, or reduce the risk of or alleviate the symptoms of allergy and other immune-based conditions.

Modulation of the immune system is an area of intense study in relation to the Danisco probiotic range. The goal is to understand how each strain contributes to the maintenance and balance of optimal immune function. The immune system is controlled by compounds known as cytokines. Cytokines are hormone-like proteins made by cells that affect the behaviour of other cells and, thereby, play an important role in the regulation of immune system functions.

**In vitro studies**

In vitro assays are widely used to define the cytokine profiles of probiotics and, thereby, determine their immunological effects. By measuring the impact of probiotic bacteria during interaction with cytokine-expressing peripheral blood mononucleocytes (PBMCs), information is generated that can help determine the ability of each strain to contribute to balanced immune health.

*B. lactis* Bi-07 was investigated in *vitro* for its ability to induce the PBMC secretion of selected cytokines: interleukin IL-10 and IL-12. The results were compared with a strain of *Lactococcus lactis* and a strain of non-pathogenic *E. coli*. Similar to Lc. lactis, *B. lactis* Bi-07 induced moderate amounts of IL-10. However, *B. lactis* Bi-07 induced higher PBMC excretion of IL-12 (figure 3). This is known to shift the immune system towards a so-called Th1 type of response which plays a key role in, for example, warding off tumours and viruses and the anti-allergy response (21).

In another study including *B. lactis* Bi-07, it was investigated whether bacterial DNA is involved in the beneficial effects obtained from probiotic treatment. Peripheral blood mononuclear cells (PBMC) from healthy donors were incubated with pure DNA from probiotic strains before and after probiotic ingestion. Cytokine production was analysed in culture supernatants. It was shown that the DNA of *B. lactis* Bi-07 stimulated the PBMC from healthy donors were obtained from probiotic treatment.

Peripheral blood mononuclear cells (PBMC) from healthy donors were incubated with pure DNA from probiotic strains before and after probiotic ingestion. Cytokine production was analysed in culture supernatants. It was shown that the DNA of *B. lactis* Bi-07 stimulated the PBMC excretion of IL-10, exceeding the IL-10 levels induced by lipopolysaccharides (LPS) (figure 4) (22).

These results indicate the IL-10 inducing effect of whole cells differs from that of DNA. It also suggests that, even when cells die and their DNA is released, they may modulate the immune system.

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**Figure 3.** Induction of IL-10 and IL-12 by *B. lactis* Bi-07 in PBMCs, compared with *Lactococcus lactis* and *Escherichia coli* (21).
Animal studies

*B. lactis* Bi-07 has demonstrated an ability to modulate the immune system in an inflammation animal model, confirming its ability to contribute to a balanced immune system. The graph below demonstrates the percentage of protection from a chemically-induced intestinal inflammation. *B. lactis* Bi-07 exerts moderate but significant protection from the intestinal inflammation in this model, demonstrating its ability to interact with and balance the intestinal mucosal immune response (figure 5) (21).

*Candida* yeasts are usually present in most people but uncontrolled overgrowth, e.g. due to medication or underlying disease can lead to candidiasis, a fungal infection (mycosis) of any of the *Candida* species. Candidiasis thereby encompasses infections that range from superficial, such as oral thrush and vaginitis, to systemic and potentially severe diseases.

The increased incidence of *Candida* infections and their increasing resistance to antifungal antibiotics provides a strong impetus for new research efforts to explore the use of probiotic bacteria for the prophylaxis and therapy of candidiasis. *B. lactis* Bi-07 has been evaluated in a *Candida* infection model for its capability to protect immunodeficient mice from lethal candidiasis (23). Here the strain was found to reduce the level of *Candida* colonisation in all parts of the gastrointestinal tract and significantly reduce the incidence and severity of candidiasis. Furthermore, the study showed higher total levels of IgA, IgG and IgM and an improved specific antibody response. In addition, *B. lactis* Bi-07 induced a stronger cell-mediated immune response against *Candida*. As a result, the lethality of the candidiasis was significantly reduced in both adult and new-born mice.

A further study has evaluated the capacity of two *B. lactis* strains (*B. lactis* Bi-07, called *B. infantis* in the study, and another *B. lactis* strain) to protect two types of immunodeficient mice from oro gastric candidiasis and systemic candidiasis of endogenous origin. It was seen that both bifidobacteria prolonged the survival of *Candida albicans*-colonised adult and neonatal mice.

Mice diassociated with *C. albicans* and *B. lactis* Bi-07 or the other *B. lactis* strain had significantly fewer *C. albicans* in their stomachs and intestines compared with mice monoassociated with *C. albicans*.

The presence of either of the two *B. lactis* strains in the alimentary tract reduced the incidence of disseminated candidiasis in mice. Less systemic candidiasis of endogenous origin in mice was detected in mice colonised with *B. lactis* Bi-07 rather than the other strain of *B. lactis*.

Immune responses were evaluated as immunoglobulins in the sera of mice either monoassociated with one of the bifidobacteria or *C. albicans* or dissociated with one of the bifidobacteria and *C. albicans*.

Both bifidobacteria affected the production of antibodies to *C. albicans*, but the effects were different for the two mouse types and the two bifidobacteria strains. Despite these differences, both mouse types monoassociated with *B. lactis* Bi-07, but not the other *B. lactis*, had increased serum IgG, IgA, and IgM compared with sera from germ-free (GF) controls. Additionally, in both types of mice diassociated with *C. albicans* and *B. lactis* Bi-07, the levels of IgG, IgA, and IgM were higher compared to the GF control sera.

The two bifidobacteria strains also suppressed weight loss associated with *C. albicans* infection.

The results show that *B. lactis* Bi-07 can provide important protection against candidiasis in immunodeficient mice and that different strains of the same species show quantitative and qualitative differences in their possible biotherapeutic effects (24).

Another study has looked at the effect of prior colonisation with probiotic bacteria on the antibody responses of immunodeficient mice subject to alimentary tract colonisation with *C. albicans*. Although the probiotic bacteria did not induce a vigorous antibody response to their own antigens, the study demonstrated that they altered the antibody response to *C. albicans* in mice.

The authors observed mixed immunomodulatory effects from the probiotic bacteria.

The probiotic strains induced antibody responses to some *C. albicans* antigens but inhibited antibody responses to others. However, the data indicate that probiotic bacteria (such as *L. acidophilus* NCFM® and *B. lactis* Bi-07), which effectively prolonged the survival of immunodeficient mice colonised with *C. albicans* (23), also strongly stimulated the production of antibodies to *C. albicans* antigens.

These results suggest that commensal bacterial flora should be considered an important component of the humoral
immune system in protecting against candidiasis. They also demonstrate that the presence of certain probiotic bacteria can enhance or suppress antibody responses to antigens administered via the mucosal surfaces of the alimentary tract (25).

**Human studies**

The ability of *B. lactis* Bi-07 to stimulate specific immunity has been evaluated in a human study measuring primary immune reaction following vaccination. Human volunteers were orally vaccinated using cholera vaccine as a vaccination model. In addition, they received either a placebo (maltodextrin) or *B. lactis* Bi-07. Supplementation with *B. lactis* Bi-07 or the placebo started on day 0 and continued for 21 days. The subjects consumed two capsules per day with 10^10 CFU *B. lactis* Bi-07 or two capsules per day with maltodextrin (control). On day 7 and 14, the subjects received the oral vaccine. Blood samples were collected on day 0, 21 and 28, and antigen-specific antibodies IgG (immunoglobulin G) were determined.

Supplementation with *B. lactis* Bi-07 resulted in higher specific IgG induction than in the control group. This indicates the stimulation of specific immunity by *B. lactis* Bi-07 (figure 6) (26).

The impact of a combination of *B. lactis* Bi-07 and another Danisco probiotic strain on respiratory health was investigated in a study of 326 Chinese children. The formulation significantly reduced the incidence and duration of upper respiratory infection symptoms and antibiotic use, indicating improved efficacy compared to a placebo or the single strain alone (27, 28).

**ANTIBIOTIC RESISTANCE PATTERNS**

Antibiotic susceptibility patterns are an important means of demonstrating the potential of an organism to be readily inactivated by the antibiotics used in human therapy.

Antibiotic resistance is a natural property of microorganisms and existed before antibiotics became used by humans. In many cases, resistance is due to the absence of the specific antibiotic target or is a consequence of natural selection.

Antibiotic resistance can be defined as the ability of some bacteria to survive or even grow in the presence of certain substances that usually inhibit or kill other bacteria. This resistance may be:

- **Inherent or intrinsic**: Most, if not all, strains of a certain bacterial species are not normally susceptible to a certain antibiotic. The antibiotic has no effect on these cells, being unable to kill or inhibit the bacterium.

- **Acquired**: Most strains of a bacterial species are usually susceptible to a given antibiotic. However, some strains may be resistant, having adapted to survive antibiotic exposure. Possible explanations for this include:
  - A mutation in the gene coding for the antibiotic’s target can make an antibiotic less effective. This type of antibiotic resistance is usually not transferable.
  - A resistance gene may have been acquired from a bacterium.

Of the acquired resistances, the latter is of most concern, as it may also be passed on to other (potentially pathogenic) bacteria.

Much concern has arisen in recent years regarding vancomycin resistance. Vancomycin-resistant enterococci are a leading cause of hospital-acquired infections and are refractory to treatment. The transmissible nature of genetic elements that encode vancomycin resistance in these enterococci is an important mechanism of pathogenicity.

Resistance to vancomycin in certain lactobacilli, pediococci and leuconostoc is due to intrinsic factors related to the composition of their cell wall. It is not due to any transmissible elements (29).

As yet, no case of antibiotic resistance transfer has ever been identified and reported for the lactic acid bacteria used in foods and feed.

*B. lactis* Bi-07 is vancomycin-sensitive.

The antibiotic susceptibility patterns for *B. lactis* Bi-07 are summarised in the table below.

**Bifidobacterium lactis Bi-07 antibiogram**

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<tr>
<td><strong>S</strong></td>
<td>Susceptible (minimum inhibitory concentration ≤ 4µg/ml)</td>
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<td><strong>I</strong></td>
<td>Intermediate (minimum inhibitory concentration = 8 to 32µg/ml)</td>
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<td><strong>R</strong></td>
<td>Resistant (minimum inhibitory concentration ≥ 64µg/ml)</td>
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Figure 6. Relative change in specific IgG titre in orally vaccinated humans after supplementation with *B. lactis* Bi-07 (26).
BENEFIT SUMMARY

Extensive in vitro and in vivo studies support the health-enhancing probiotic properties of B. lactis Bi-07. These attributes can be summarised as follows:

- Long history of safe use
- Well-suited to intestinal survival
  - High tolerance to gastrointestinal conditions (acid and bile)
  - Strong adhesion to intestinal cell lines
- Gastrointestinal health and well-being
  - A five-strain formulation including B. lactis Bi-07 was found to maintain and more rapidly restore microbiota after antibiotic treatment
- Beneficial modulation of immune functions
  - May improve specific immune response, as demonstrated in a human clinical study
  - Proven in an animal model to protect against inflammation and balance the intestinal mucosal immune response
- Significant reduction in the incidence and severity of intestinal candidiasis in an animal model
- B. lactis Bi-07 in combination with another Danisco probiotic strain reduced the incidence and duration of upper respiratory infection symptoms and antibiotic use in children
- Several human studies provide evidence of the safe, beneficial use of B. lactis Bi-07 in child nutrition

REFERENCES

(Publications on B. lactis Bi-07 in bold)


