

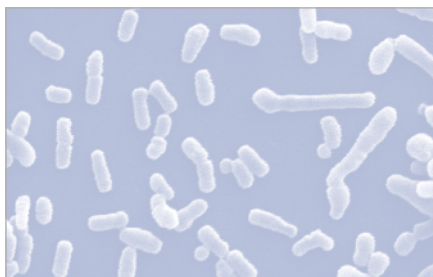
Bifidobacterium lactis BI-04



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 are 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 microflora 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-04 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-04 is of human origin.

B. lactis BI-04 has been deposited in the American Type Culture Collections safe deposit as SD5219.

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 the species *B. lactis*.

In addition to a long history of safe human consumption of the species, no acquired antibiotic resistance was detected in *B. lactis* BI-04 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 to confer a health benefit. Although not explicitly stated, this definition implies that a probiotic should survive GI tract passage and, according to some, colonize 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 found in the small intestine.

In vitro studies have shown that *B. lactis* BI-04 is extremely resistant to low pH conditions and survive the presence of bile at concentrations present in the duodenum.

Adhesion to intestinal mucosa

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 colonize the intestine.

Currently, adherence is measured using two *in vitro* cell lines, Caco-2 and HT-29.

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-04 (internally generated data):
++++ Excellent; +++ Very good; ++ Good; + Fair

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.

(>90% survival in 0.3% bile salt containing medium) BI-04 has demonstrated very good adhesion to human epithelial cell lines applied in *in vitro* studies.

Adherence to human intestinal cells <i>in vitro</i>	HT-29: +++ Caco-2: +++
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Selected characteristics of *B. lactis* BI-04 (internally generated data): +++++ Excellent; +++ Very good; ++ Good; + Fair

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 is used for thousands of years for 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 in quantity. 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.

Bifidobacterium sp. only produces L(+)-lactic acid.

L/D-lactic acid production	100/0
Molar ratio	Boehringer Mannheim/ R-Biopharm D-lactic acid/ L-lactic acid UV-method

Internally generated data

Human studies

B. lactis BI-04 was a component 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) (13).

A two-strain formulation containing *B. lactis* BI-04 in combination with an inulin-based prebiotic (Synergy, Orafit), was also investigated for its ability to beneficially modulate the intestinal microbiota in healthy elderly people. *B. bifidum* and *B. lactis* rRNA genes were measured

in faecal samples by real time PCR and in parallel with the cultivation technique. A significant increase in *B. bifidum* and *B. lactis* rRNA was observed while feeding the synbiotic formulation. This modification of the intestinal bifidobacterial population shows that the formulation can be of particular benefit to individuals with a more unbalanced gut ecosystem, which may be the cause of undesirable bacterial metabolism and a higher level of infections (14).

IMMUNOMODULATION

An immune system that functions optimally is an important safeguard against infectious and non-infectious diseases. The intestinal microbiota represent one of the key elements in the body's immune defence system.

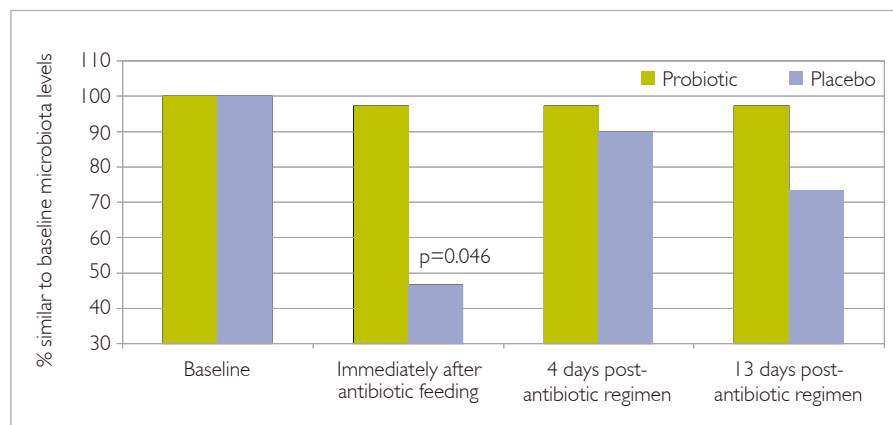


Figure 1. The probiotic mixture containing *B. lactis* BI-04 protects the faecal microbiota from disruption by antibiotics, as indicated by the greater dissimilarity of the microbiota of the placebo group compared to the baseline microbiota composition (13).

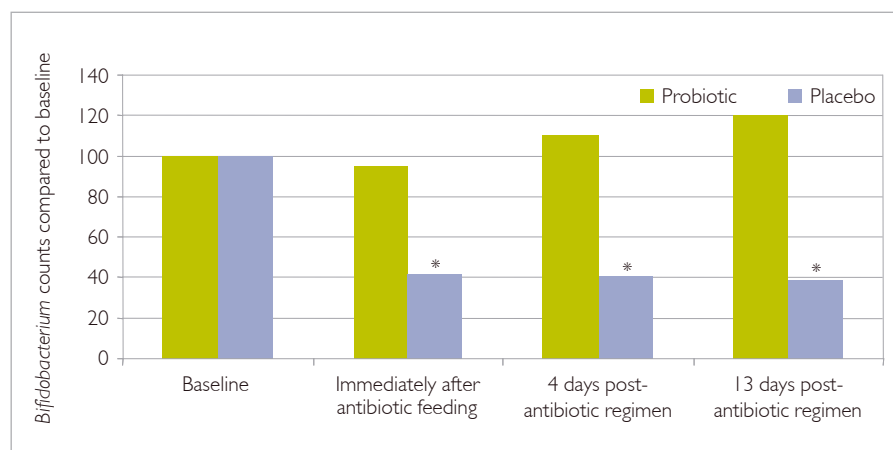


Figure 2. The probiotic mixture containing *B. lactis* BI-04 promotes the maintenance of bifidobacteria levels in the faeces of antibiotic-consuming subjects during post-treatment (*p=0.030) (13).

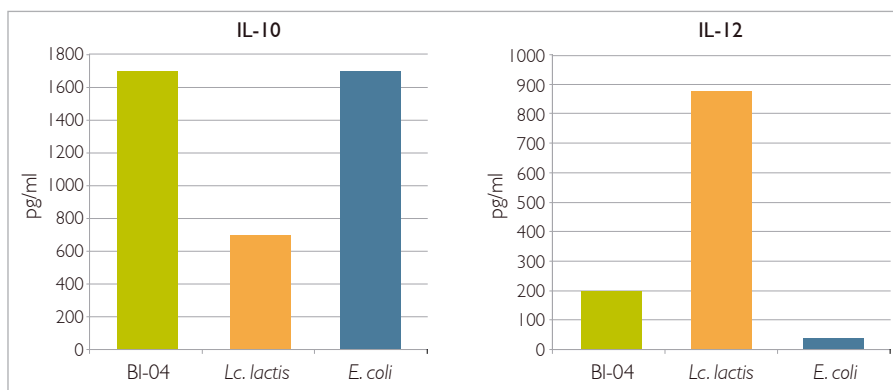


Figure 3. Induction of IL-10 and IL-12 by *Bifidobacterium lactis* BI-04 in PBMCs, compared with *Lactococcus lactis* and *Escherichia coli*. (15)

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-04 was investigated *in vitro* for its ability to induce the PBMC secretion of selected cytokines: interleukin (IL) IL-10 and IL-12. The results were compared with *Lactococcus lactis*, a

starter culture commonly used in the production of various fermented foods, and a strain of non-pathogenic *Escherichia coli*, a common member of the intestinal microbiota. IL-10 plays a key role in the control of inflammatory responses to intestinal antigens.

B. lactis BI-04 was found to induce IL-10, to a higher degree than *Lc. lactis* and IL-12 to a lesser degree, but still higher than *E. coli* (figure 3). This indicates anti-inflammatory properties (15).

In another study including *B. lactis* BI-04, it was investigated whether bacterial DNA is involved in the beneficial effects obtained by 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-04 stimulated secretion of IL-10 which exceeded IL-10

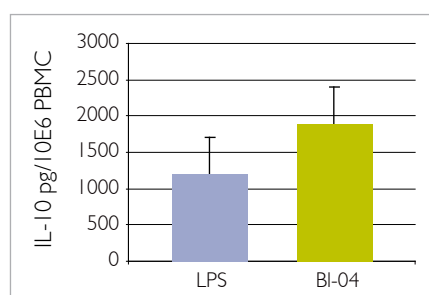


Figure 4. Cytokine response to bacterial DNA (cultured with PBMC) compared with the response to LPS (16).

levels induced by lipopolysaccharides (LPS) (figure 4) (16).

These results provide further evidence of anti-inflammatory properties of *B. lactis* BI-04 and also indicate the possible role of pure bacterial DNA in probiotic efficacy.

Animal studies

In line with the results above, *B. lactis* BI-04 has further 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-04 has considerably reduced colitis symptoms, securing significant protection from the intestinal inflammation in this model. This demonstrates its ability to interact with and beneficially balance the intestinal mucosal immune response (figure 5) (15).

Human studies

The ability of *B. lactis* BI-04 to stimulate specific immunity has been evaluated in a human study measuring primary immune reaction upon vaccination. Human volunteers were orally vaccinated using cholera vaccine as a vaccination model. In addition to this, they received either a placebo (maltodextrin) or *B. lactis* BI-04. Supplementation with *B. lactis* BI-04 or the placebo started on day 0 and continued for 21 days. The subjects consumed two capsules per day with 10^{10} CFU

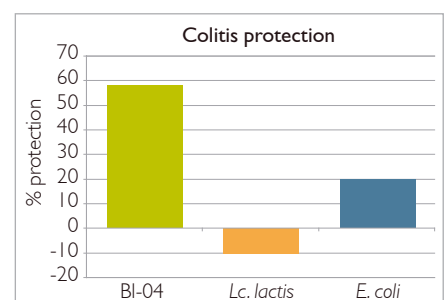


Figure 5. Percentage of protection in an acute murine model of inflammation (TNBS) (15).

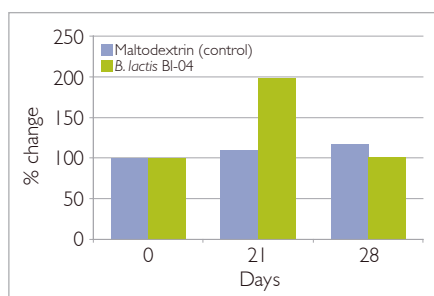


Figure 6. Relative change in specific IgG titre in orally vaccinated humans after supplementation with *B. lactis* BI-04.

B. lactis BI-04 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-04 resulted in faster IgG induction than in the control group. This indicates stimulation of specific immunity by *B. lactis* BI-04 (figure 6, unpublished data).

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 efficient. 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, as vancomycin-resistant enterococci are a leading cause of hospital-acquired infections and are refractory to treat-

ment. 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 (17).

As of today, 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-04 is vancomycin sensitive. The antibiotic susceptibility patterns for *B. lactis* BI-04 are summarised in table 1.

BENEFIT SUMMARY

Extensive *in vitro* and *in vivo* studies support the health-enhancing, probiotic properties of *B. lactis* BI-04. Following is a summary of these attributes:

- Long history of safe use
- Well-suited for intestinal survival
 - High tolerance to gastrointestinal conditions (acid and bile)
 - Strong adhesion to intestinal cell lines
- Gastrointestinal health and well-being
 - A 5-strain formulation including *B. lactis* BI-04 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 (manuscript submitted)
 - May influence immune regulation, as demonstrated by the induction of IL-10 *in vitro*
 - Proven in an animal model to protect against inflammation and balance the intestinal mucosal immune response

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<i>Bifidobacterium lactis</i> BI-04 antibiogram	
Amoxicillin	S
Ampicillin	S
Ceftazidime	S
Chloramphenicol	I
Ciprofloxacin	R
Clindamycin	I
Cloxacillin	S
Dicloxacillin	S
Erythromycin	I
Gentamicin	R
Imipenem	R
Kanamycin	R
Neomycin	R
Nitrofurantoin	R
Penicillin G	S
Polymixin B	R
Rifampicin	S
Streptomycin	R
Sulfamethoxazole	R
Tetracycline	R
Trimethoprim	R
Vancomycin	S
S = Susceptible (minimum inhibitory concentration $\leq 4\mu\text{g/ml}$)	
I = Intermediate (minimum inhibitory concentration = 8 to 32 $\mu\text{g/ml}$)	
R = Resistant (minimum inhibitory concentration $\geq 64\mu\text{g/ml}$)	

Table 1.

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Regarding Health Claims, users should conduct their own legal investigations into national demands when marketing and selling a consumer product containing the probiotic described in this technical memorandum.



First you add knowledge...