

Bifidobacterium breve Bb-03



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 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

B. breve Bb-03 has been genetically characterised and properly classified as *B. breve* by independent labs employing modern genotypic methods, including 16S rRNA gene sequencing and PCR using species-specific primers.

Originally isolated from an unknown source, the strain has been deposited in the American Type Culture Collection as SD5206.

SAFE FOR CONSUMPTION

Bifidobacterium sp. has long been considered safe and suitable for human consumption with several published studies addressing its safety (3-7).

More specifically, *B. breve* is listed in the *Inventory of Microorganisms With Documented History of Use in Human Food* (8) and the Qualified Presumption of Safety list of the European Food Safety Authority.

No acquired antibiotic resistance was detected for *B. breve* Bb-03 during screening by the EU-funded PROSAFE project.

GASTROINTESTINAL PERFORMANCE

Resistance to acid and bile

According to the generally accepted definition of a probiotic, a 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 passage through the GI tract and, according to some, colonize the host epithelium.

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

In vitro studies have shown that *B. breve* Bb-03 is tolerant 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. 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. breve Bb-03 has demonstrated very good adhesion to human epithelial cell lines (HT-29) 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. breve* Bb-03 (internally generated data): +++++ Excellent; +++ Very good; ++ Good; + Fair

Acid tolerance	+ (<60% survival in hydrochloric acid and pepsin (1%) at pH 3 for 1h at 37°C)
Bile salt tolerance	+ (<60% survival in 0.3% bile salt containing medium)

Selected characteristics of *B. breve* Bb-03 (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. For thousands of years, lactic acid fermentation has been used 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 carbohydrate and amino acid metabolism. 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, derived 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

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 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 (10).

As yet, no case of antibiotic resistance transfer has ever been identified and

<i>Bifidobacterium breve</i> Bb-03 antibiogram	
Amoxicillin	S
Ampicillin	S
Ceftazidime	R
Chloramphenicol	S
Ciprofloxacin	R
Clindamycin	S
Cloxacillin	R
Dicloxacillin	I
Erythromycin	S
Gentamicin	R
Imipenem	R
Kanamycin	R
Neomycin	R
Nitrofurantoin	R
Penicillin G	S
Polymixin B	R
Rifampicin	R
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.

reported for the lactic acid bacteria used in foods and feed.

B. breve Bb-03 is vancomycin sensitive. The antibiotic susceptibility patterns for *B. breve* Bb-03 are summarised in table 1.

BENEFIT SUMMARY

Based on the data generated, the strain's key attributes can be summarised as follows:

- *B. breve* Bb-03 is well suited to intestinal survival
 - Tolerant of acid and bile
 - Adheres to intestinal HT-29 cell lines

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