

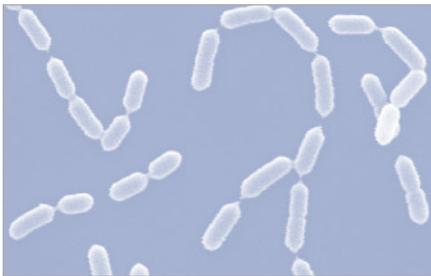
Lactobacillus casei Lc-11



CHARACTERISTICS OF THE GENUS

L. casei is a common inhabitant of the human intestinal tract (1) and is also found naturally in fermented vegetables, milk and meat. Strains of this species are used as starter cultures in many fermented food products, including traditional fermented milks and cheese.

Selected strains of this species are also used in probiotic foods and dietary supplements.



Lactobacillus casei is a Gram-positive, non-spore forming, homofermentative rod.

SELECTION AND TAXONOMY

Bacterial taxonomy is in dynamic development as new technologies continue to differentiate closely-related taxonomic groups.

This is particularly true for the *L. casei/paracasei* group. Here research in DNA homology and typing has led to several proposals to reject the species *L. paracasei* and to include it in the restored species *L. casei* with a neotype strain (2, 3). Many taxonomists strongly endorse these proposals for revised nomenclature (4). It has, however, not been confirmed by the Judicial Commission of the International Committee on

Systematic Bacteriology. Consequently, *Lactobacillus casei* today is restricted to strains ATCC 393 and NCFB 173, while almost all other "*Lactobacillus casei*" strains, are properly named *Lactobacillus paracasei* subsp. *paracasei*.

Lactobacillus casei Lc-11 has been genetically characterised and properly classified as *L. casei*.

The strain was originally isolated from a dairy source and has been deposited in the American Type Culture Collection as SD5213.

SAFE FOR CONSUMPTION

Lactic acid bacteria have long been considered safe and suitable for human consumption. Very few instances of infection have been associated with these bacteria and several published studies have addressed their safety (5-9).

L. casei is listed in the *Inventory of Microorganisms With Documented History of Use in Human Food* (10). The European Food Safety Authority has also included the species on its Qualified Presumption of Safety list (11).

In addition to a long history of safe human consumption of the species, no acquired antibiotic resistance was detected in *L. casei* Lc-11 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 *L. casei* Lc-11 is very resistant to low pH conditions and survives the presence of bile at the 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

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 *L. casei* Lc-11 (internally generated data):
++++ Excellent; +++ Very good; ++ Good; + Fair

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.

L. casei Lc-11 has demonstrated excellent adhesion to human epithelial cell lines (Caco-2) 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 *L. casei* Lc-11 (internally generated data): +++++ Excellent; +++ Very good; ++ Good; + Fair

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 immunoglobulin A.

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 as well as competition for nutrients.

It should be pointed out, however, that extending such results to the *in vivo* situation is not straightforward.

The assessment in the table below is based on an *in vitro* assay.

L. casei Lc-11 displayed *in vitro* inhibition of selected pathogens.

Pathogen inhibition <i>in vitro</i>	<i>Salmonella typhimurium</i> : ++
	<i>Staphylococcus aureus</i> : ++
	<i>Escherichia coli</i> : +++++
	<i>Listeria monocytogenes</i> : ++

Selected characteristics of *L. casei* Lc-11 (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.

L. casei 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, including *L. casei*, pediococci and leuconostoc, is due to intrinsic factors related to the composition of their cell wall. It is not due to any transmissible elements (12). Through PCR testing, *L. casei* Lc-11 has been confirmed as

being free of *Enterococcus*-like vancomycin-resistant genes.

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

The antibiotic susceptibility patterns for *L. casei* Lc-11 are summarised in table 1.

<i>Lactobacillus casei</i> Lc-11 antibiogram	
Amoxicillin	S
Ampicillin	S
Ceftazidime	R
Chloramphenicol	R
Ciprofloxacin	R
Clindamycin	I
Cloxacillin	I
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	R
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.

BENEFIT SUMMARY

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

- *L. casei* Lc-11 is well suited for intestinal survival and functionality
 - Highly tolerant of acid and bile
 - Adheres strongly to intestinal Caco-2 cell lines
 - Inhibits common pathogens

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