

## Lactobacillus paracasei Lpc-37



### CHARACTERISTICS OF THE SPECIES

*Lactobacillus paracasei* is a Gram-positive, non-spore forming, homofermentative rod that is a common inhabitant of the human intestinal tract (1,2). *L. paracasei* strains are also found naturally in fermented vegetables, milk and meat. Strains of this species are used in many food products, including traditional fermented milks and cheese.

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



### 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 include it in the restored species *L. casei* with a neotype strain (3, 4). This proposal 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 paracasei* Lpc-37 has been genetically characterised and properly classified as *Lactobacillus paracasei* by independent labs using modern genotypic methods including 16S rRNA gene sequencing, PCR using species-specific primers, and electrophoretic whole-organism protein analysis (5).

*L. paracasei* Lpc-37 is a strain isolated from a dairy source and has been deposited in the American Type Culture Collection as SD5275.

### 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 (6-9).

*L. paracasei* 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. paracasei* Lpc-37 during screening by the EU-funded PROSAFE project.

The strain has been sold commercially for more than 15 years.

### 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 in order to confer a health benefit. This implies that a probiotic should survive passage through the GI tract and, according to some interpretations, transiently colonise 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. paracasei* Lpc-37 is very resistant to low pH conditions and shows moderate resistance to bile at the concentrations present in the duodenum.

Acid tolerance	++++ (80-90% 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)
Pepsin resistance	+ (<60% in 0.3% pepsin containing medium at pH 2 for 1h)
Pancreatin resistance	++++ (>60% survival in 0.1% pancreatin containing medium at pH 8 for 2h)

Table 1. Selected characteristics of *L. paracasei* Lpc-37 (internally generated data):  
++++ Excellent; +++ Very good; ++ Good; + Fair

### 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 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. paracasei* Lpc-37 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. paracasei* Lpc-37 (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 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 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 such an *in vitro* assay.

*L. paracasei* Lpc-37 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> : ++
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Selected characteristics of *L. paracasei* Lpc-37 (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. paracasei* Lpc-37 only produces L(+) lactic acid.

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

Internally generated data

### Human studies

*L. paracasei* Lpc-37 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 found in the placebo group two weeks after the cessation of antibiotic therapy (figure 2) (12).

### 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.

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.

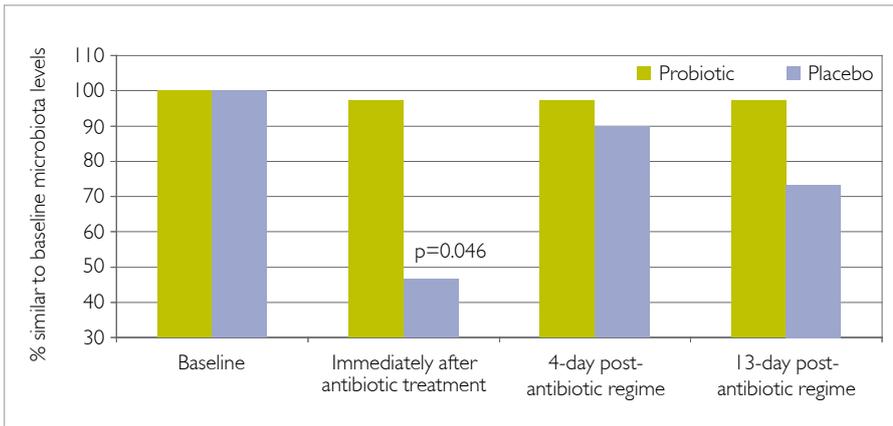


Figure 1. The probiotic mixture containing *L. paracasei* Lpc-37 protects the faecal microbiota from disruption by antibiotics, as indicated by the greater dissimilarity of the microbiota of the placebo group compared to the baseline microbiota composition (12).

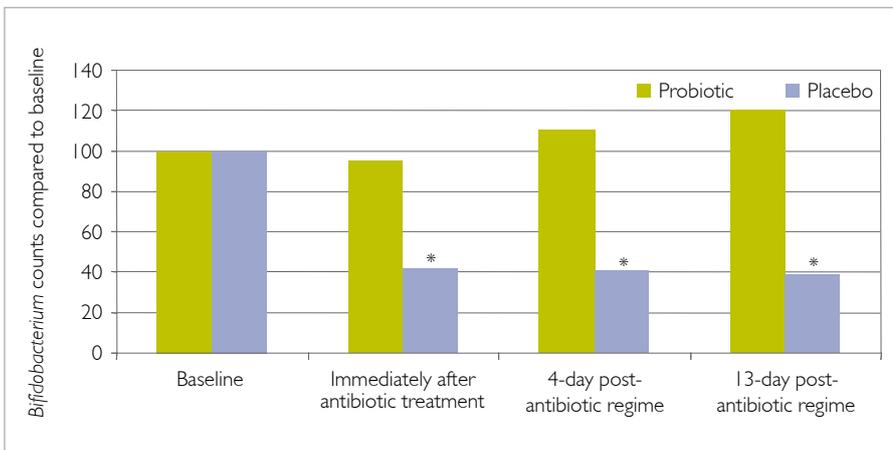


Figure 2. The probiotic mixture containing *L. paracasei* Lpc-37 promotes the maintenance of bifidobacteria levels in the faeces of antibiotic-consuming subjects during post-treatment (\*p=0.030) (13).

### 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.

*L. paracasei* Lpc-37 was investigated *in vitro* for its ability to induce the PBMC secretion of selected cytokines: interleukin IL-10, IL-12, tumour necrosis factor (TNF)- $\alpha$  and interferon (IFN)- $\gamma$ . The results were compared with *Lactobacillus plantarum* NCIMB8826 – a species commonly used as starter culture in the production of various fermented foods.

*L. paracasei* Lpc-37 was found to induce IL-10, (TNF)- $\alpha$  and (IFN)- $\gamma$  to the same degree as *L. plantarum* (figure 3). However, *L. paracasei* Lpc-37 induced significantly 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.

### Animal studies

*L. paracasei* Lpc-37 demonstrated an ability to modulate the immune system in an inflammation animal model, confirming its ability to contribute to a balanced immune system. Figure 4 demonstrates the percentage of protection from a chemically-induced intestinal inflammation. *L. paracasei* Lpc-37 exerts moderate but significant protection from the intestinal

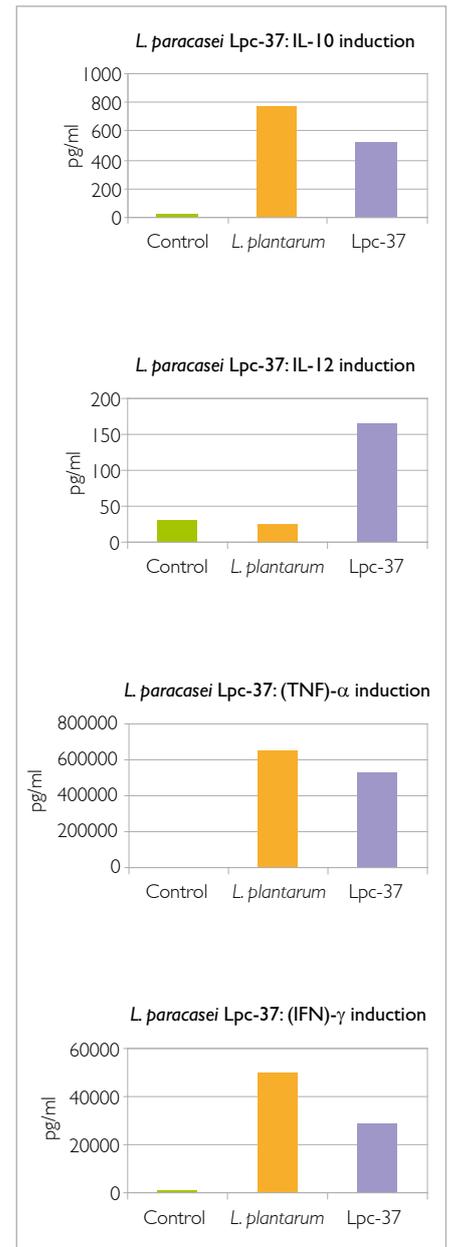


Figure 3. *In vitro* cytokine expression of *L. paracasei* Lpc-37 (internally created data).

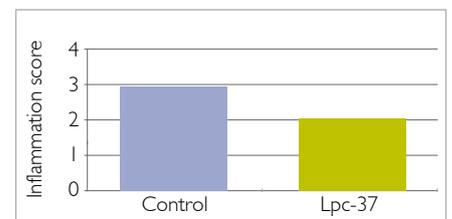


Figure 4. Colitis reduction in a mouse model. Inflammation score (internally generated data).

inflammation in this model, demonstrating its ability to interact with and balance the intestinal mucosal immune response (figure 4).

## Human studies

The ability of *L. paracasei* Lpc-37 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 the vaccination model. Then they received either a placebo (maltodextrin, n=20) or *L. paracasei* Lpc-37 (n=9).

Supplementation with *L. paracasei* Lpc-37 or the placebo started on day 0 and continued for 21 days. The subjects consumed two capsules a day with  $10^{10}$  CFU *L. paracasei* Lpc-37 or two capsules a 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 (immunoglobulins, IgA, IgG, IgM) were determined.

Supplementation with *L. paracasei* Lpc-37 resulted in relatively higher, but not statistically significant, induction of specific IgG than in the control group. This may indicate the stimulation of specific immunity by *L. paracasei* Lpc-37 (figure 5). The stronger increase in specific serum IgM from day 0 to day 21 was not significant. The decrease in serum IgM after day 21 showed a trend for being different from the change in the control group. Because under normal conditions IgM is "replaced" by IgG, the observed sequence of events indicates a higher specific immune response on supplementation with *L. paracasei* Lpc-37 compared with a standard vaccination. Earlier induction of specific IgM followed by an earlier induction of IgG leads to a simultaneous decrease in IgM. Changes in the levels of IgA were no different from those of the control group (figure 5) (13).

*L. paracasei* Lpc-37 was the main probiotic component in a double-blind, placebo-controlled, randomised cross-over study with 15 healthy adults and 15 patients with atopic dermatitis (AD).

The purpose of the study was to elucidate the effect of a probiotic drink con-

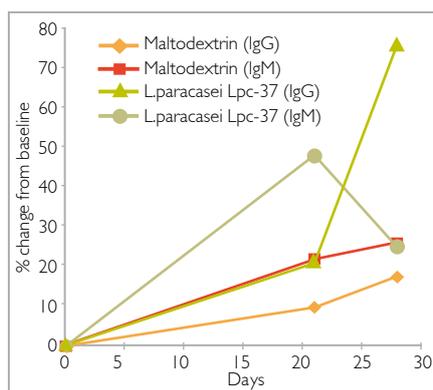


Figure 5. Relative change in specific IgG and IgM titre in orally vaccinated humans after supplementation with *L. paracasei* Lpc-37 (13).

taining a combination of the probiotics *L. paracasei* Lpc-37, *Lactobacillus acidophilus* 74-2 and *Bifidobacterium animalis* subsp. *lactis* DGCC 420 (*B. lactis* 420) on clinical and immunological parameters and microbiology in faeces. The SCORAD (Scoring Atopic Dermatitis) system was used for assessing the severity (i.e. extent, intensity) of atopic dermatitis.

High levels of *L. paracasei* and *B. lactis* were present in faeces after supplementation, whereas *L. acidophilus* marginally increased. In patients, the SCORAD tended to decrease by 15.5% ( $P = 0.081$ ). Few differences were observed in the expression of lymphocyte subsets resulting from probiotic intervention. The phagocytic activity of monocytes and granulocytes was significantly increased in healthy subjects.

This study reveals that probiotics have a differing modulatory effect on peripheral immune parameters in healthy subjects and patients with AD. It also shows that *L. paracasei* Lpc-37 is able to colonise the intestine transiently (14).

## 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 be-

fore 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. paracasei*, pediococci and leuconostoc, is due to intrinsic factors related to the composition of their cell wall. It is not due to any transmissible elements (15). Through PCR testing, *L. paracasei* Lpc-37 has been found to be 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. paracasei* Lpc-37 are summarised in the table below.

<b><i>Lactobacillus paracasei</i> Lpc-37 antibiogram</b>	
Amoxicillin	S
Ampicillin	S
Ceftazidime	R
Chloramphenicol	I
Ciprofloxacin	R
Clindamycin	S
Cloxacillin	S
Dicloxacillin	S
Erythromycin	S
Gentamicin	R
Imipenem	R
Kanamycin	R
Neomycin	R
Nitrofurantoin	R
Penicillin G	S
Polymixin B	R
Rifampicin	S
Streptomycin	R
Sulfamethoxazole	R
Tetracycline	S
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 $\geq 64\mu\text{g/ml}$ )	

## BENEFIT SUMMARY

Based on the data generated supporting *L. paracasei* Lpc-37 strain qualities, the following health-related attributes can be summarised:

- Long history of safe use
- Well-suited for intestinal survival
  - High tolerance to gastrointestinal conditions (acid and bile)
  - Transient colonisation after consumption
  - Very good adhesion to intestinal cell lines

- Gastrointestinal health and well-being
  - A five-strain formulation including *L. paracasei* Lpc-37 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
  - May improve innate immune response when used in a three-strain combination, as demonstrated in a human clinical study
  - *L. paracasei* Lpc-37 may have an influence on immune regulation, as demonstrated through induction of IL-12 *in vitro*

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**Danisco A/S**

Edwin Rahrsvej 38  
DK-8220 Brabrand, Denmark  
Telephone: +45 89 43 50 00  
Telefax: +45 86 25 10 77  
info@danisco.com  
www.danisco.com

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