

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.

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 (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 and PCR using species-specific primers as well as 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).

More specifically, *Lactobacillus paracasei* has been consumed in fermented milks and other food products for decades and is listed in the Inventory of Microorganisms With Documented History of Use in Human Food (10).

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 commercially sold for more than 15 years.

GASTRO-INTESTINAL PERFORMANCE

For health benefits to be obtained, probiotics must generally be able to survive and be active in the gastro-intestinal

tract. Among the properties considered important for probiotics is the ability to resist low pH and survive the presence of bile. *In vitro* studies have shown that *L. paracasei* Lpc-37 is able to resist conditions during intestinal passage (table 1).

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.

L. paracasei Lpc-37 showed strong adhesion to the applied human epithelial cell lines in *in vitro* studies. See table 1.

IMMUNOMODULATION

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

Probiotic bacteria with the ability to modulate certain immune functions may

Acid tolerance	80-89% 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)
L/D-lactate production molar ratio	100/0

Table 1. Selected characteristics of *L. paracasei* Lpc-37.

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.

By measuring the impact of probiotic bacteria during interaction with cytokine-expressing peripheral blood mononucleocytes (PBMCs), information is generated that is useful in determining the ability of each strain to contribute to the balance of 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)- α and interferon (IFN)- γ . The results were compared with *Lactobacillus plantarum*, a starter culture commonly used in the production of various fermented foods.

L. paracasei Lpc-37 was found to induce IL-10, TNF- α and IFN- γ to the same degree as *L. plantarum* (figure 1). However, *L. paracasei* Lpc-37 induced significantly higher PBMC excretion of IL-12, (figure 1) which is known to shift the immune system towards a so-called Th1 type of response; an anti-allergy response.

HUMAN STUDIES

The ability of *L. paracasei* Lpc-37 to stimulate specific immunity has been evaluated in a human study measuring primary immune reaction upon vaccination.

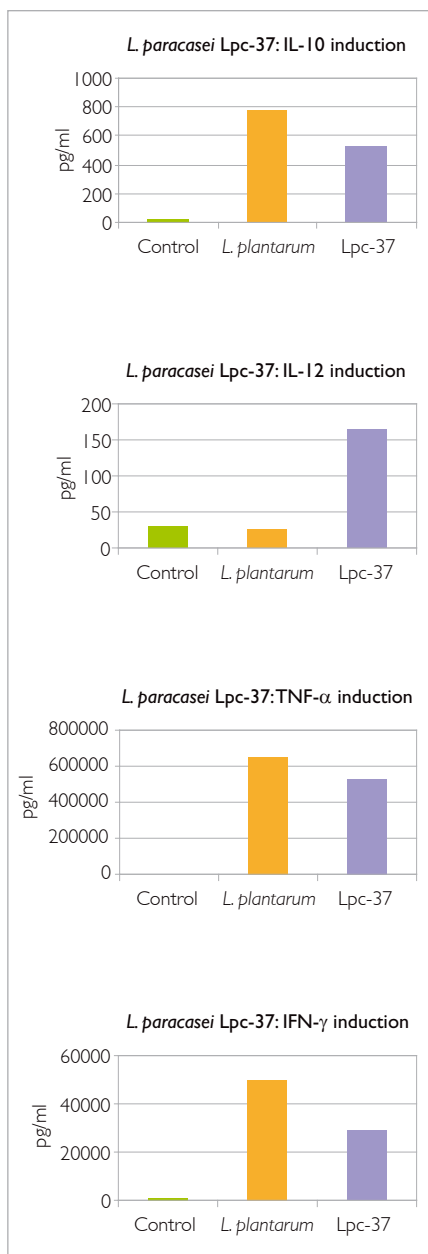


Figure 1. *In vitro* cytokine expression of *L. paracasei* Lpc-37.

Human volunteers were orally vaccinated (cholera vaccine was used as the vaccination model) and then 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.

The serum levels of specific IgA and IgM were not different from those in the placebo group.

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 (unpublished data, figure 2).

L. paracasei Lpc-37 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 3). In addition, the probiotic product still maintained bifidobacteria at significantly higher levels than that of the placebo group two weeks after the cessation of antibiotic therapy (figure 4), (11).

BENEFIT SUMMARY

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

- Well suited for intestinal survival
 - High tolerance to acid and bile are present in the intestine
 - Strong adhesion to intestinal cell lines
- A 5-strain formulation including *L. paracasei* Lpc-37 was found to reduce antibiotic-induced microbiota disturbance and maintain bifidobacteria levels
- Immune modulation
 - *L. paracasei* Lpc-37 may improve specific immune response, as demonstrated in a human clinical study (not yet published)
 - *L. paracasei* Lpc-37 may have an influence on immune regulation, as demonstrated through induction of IL-12 *in vitro*

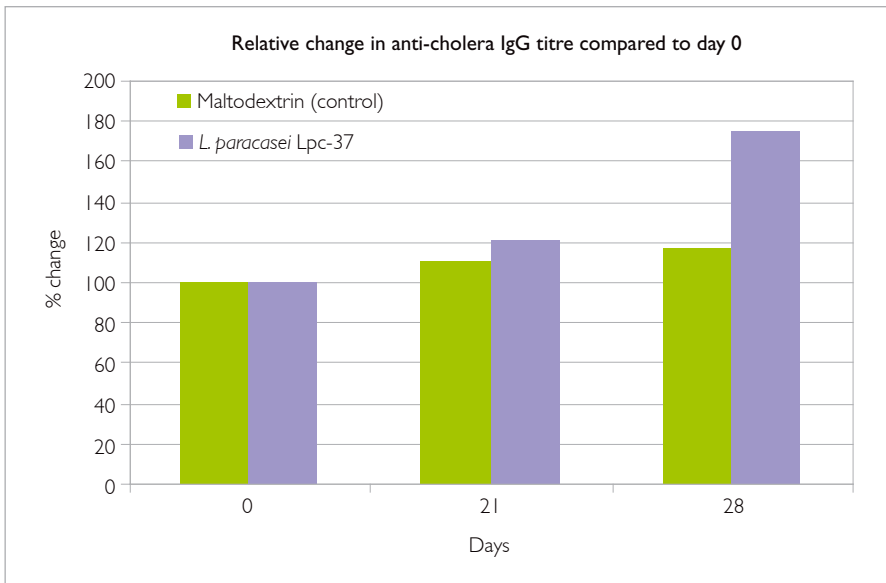


Figure 2. Relative change in specific IgG titre in orally vaccinated humans after supplementation with *L. paracasei* Lpc-37.

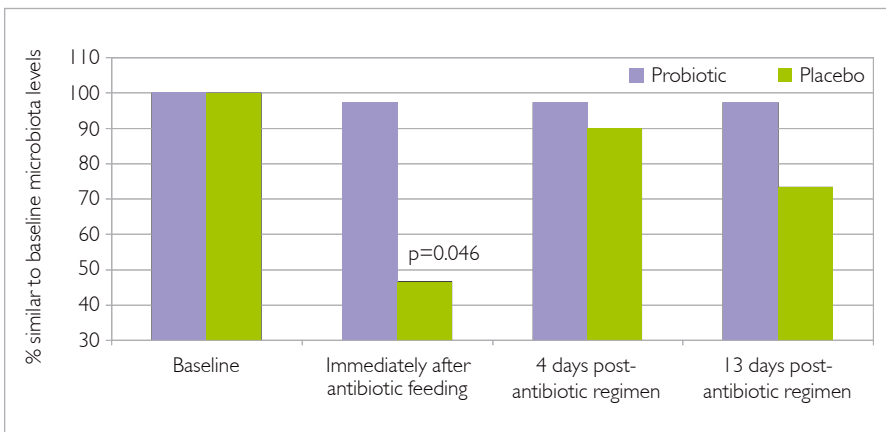


Figure 3. The probiotic mixture containing *L. paracasei* Lpc-37 protects the fecal microbiota from disruption by antibiotics, as indicated by greater dissimilarity of microbiota of the placebo group, compared to the baseline microbiota composition.

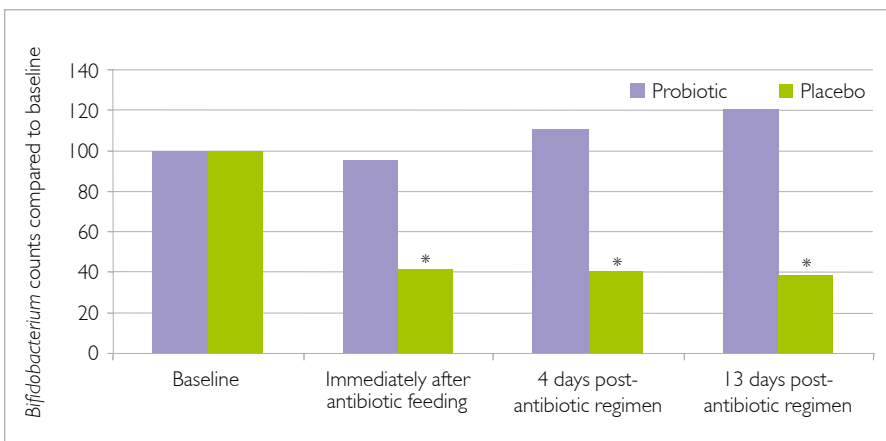


Figure 4. The probiotic mixture containing *L. paracasei* Lpc-37 promotes the maintenance of bifidobacteria levels in the faeces of antibiotic-consuming subjects during post-treatment (*p=0.030).

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Danisco A/S

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