**Lactobacillus paracasei 8700:2**

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Consumption of live lactic acid bacteria

Consumption of live lactic acid bacteria (LAB), included in fermented foods, has been a regular part of the human food intake for a long time. In fact, there are archaeological signs that humankind has used this technique from the beginning of time; it was presumably invented 1.5 million years ago by the early humanoids (Leakey 1993; Leakey 1995). Thus, humans have in this way consumed large numbers of live LAB throughout their entire history.

Fermentation is the simplest and often the safest way to preserve food, and before the Industrial Revolution, fermentation was applied just as much in Europe as it still is in many rural areas of the World. Thus, it could very well be that the human digestive tract evolved to adapt to a more or less daily supply of live LAB. This supply of live LAB ceased in many industrialized countries during the twentieth century, which eventually may have led to increased frequency of gastro-intestinal (GI) and immunological dysfunctions in urbanised humans.

When beneficial effects of certain types of live bacteria have been discussed, these types of bacteria have been gradually called “probiotics”. The original concept of probiotics implies that the balance between beneficial and harmful bacteria in the microbiota of the GI-tract can be positively affected by eating the right type of live microorganisms (Parker 1974; Fuller 1989). However, the concept of probiotics is today used more generally for describing live bacteria that after ingestion, exercise health beneficial effects beyond conventional nutrition. It is presupposed that these health beneficial effects have been scientifically proved.
Functional groups and taxonomically based taxa

Lactic acid bacteria

The bacteria performing the conversion of carbohydrates to carboxylic acids, mainly lactic acid in traditional fermented foods, are called lactic acid bacteria (LAB). Food microbiologists used the term early, and 1919 the Danish bacteriologist Orla Jensen tried to define key features of LAB, unaware of the fact that LAB is not forming a systematically defined group based on evolutionary relationships; instead it can be regarded as a functional group used by food microbiologists, aiming at those bacteria that occur and multiply spontaneously in traditional lactic acid fermented foods. Furthermore, it is understood that LAB are harmless to human health. Already 2002, it was shown in meta-analyses of published clinical trials that different kind of LAB can be used to prevent antibiotic associated diarrhoea (D’Souza et al. 2002) and shorten the duration of acute diarrhoeal illness in children (Huang et al. 2002).

From the taxonomic point of view, LAB means a relatively wide variety of different taxonomically based groups (taxa). The only absolute condition for organisms involved in lactic acid fermentation of food must be that the bacteria mainly produce lactic acid and that they are harmless to consume in high numbers, even for consumers with underlying sicknesses that may have weaken their immunological defence. The different kind of lactic acid producing bacteria frequently occurring in high numbers in traditional, spontaneously fermented foods belong to genera as Lactobacillus, Pediococcus, Weissella, Leuconostoc, Oenococcus, Lactococcus, and the species Streptococcus thermophilus (and similar species).

The genera Lactobacillus and Pediococcus belong to the family Lactobacillaceae which also includes the relatively new genera Paralactobacillus and Sharpea. They can all be included in the trivial expression “lactobacilli”.

Leuconostoc, Weissella and Oenococcus belong to the family Leuconostocaceae together with the genus Fructobacillus.

Lactococcus and S. thermophilus have from the phylogenetic point of view...
relatively little in common with *Lactobacillaceae* and *Leuconostocaceae* even if they all are included in the order of *Lactobacillales*.

**The species *Lactobacillus paracasei***

**Taxonomic considerations**

*Lactobacillus paracasei* is a bacterial species in the huge and relatively diverse genus of *Lactobacillus*, which comprises around 90 validly named species. *Lactobacillus paracasei* is a so called facultatively heterofermentative *Lactobacillus*, i.e. *L. paracasei* ferment hexoses exclusively to lactic acid, but can also ferment pentoses and/or gluconate, and then producing lactic and acetic acid (Kandler and Weiss 1986).

The type strain of *L. paracasei* is NCDO 151\(^T\) (= NCFB 151\(^T\); T = type strain).

The systematics of the two species *L. paracasei* and *L. casei* was under discussion for several years: It started with the loss of the original type strain of *L. casei*. *L. casei* was originally described by Orla-Jensen 1916, and referred by Kandler and Weiss in Bergey’s Manual of Systematic Bacteriology (Kandler and Weiss 1986). However, when the original type strain had been lost, Hansen and Lessel (1971) designated strain ATCC 393 as the neotype of *L. casei* (ATCC 393\(^T\)). Five subspecies were recognized within this restored species of *L. casei*: *Lactobacillus casei* subsp. *casei* (Hansen and Lessel, 1971; Orla-Jensen 1916), *Lactobacillus casei* subsp. *pseudoplantarum* (Abo-Elnaga and Kandler 1965; ATCC 25598\(^T\)), *Lactobacillus casei* subsp. *tolerans* (Abo-Elnaga and Kandler 1965; ATCC 25599\(^T\)), *Lactobacillus casei* subsp. *rhamnosus* (Hansen 1968; ATCC 7469\(^T\)) and *Lactobacillus casei* subsp. *alactosus* (Mills and Lessel. (1973; ATCC 27216\(^T\)). However, Johnson (1973) and Dellaglio *et al.* (1975) found high DNA:DNA homology between strains designated *L. casei* subsp. *casei* and *L. casei* subsp. *alactosus*. Furthermore, in Bergey’s Manual of Sytematic Bacteriology (Kandler and Weiss, 1986) *L. casei* subsp. *alactosus* was not mentioned. Though, it was commented that *L. casei* subsp. *casei* ATCC 393\(^T\) and strains of *L. casei* subsp. *rhamnosus* had low DNA:DNA homology to other *L. casei* strains. This was confirmed by Collins *et al.* (1989), and it was suggested that members of *L. casei* subsp. *alactosus*, *L. casei* subsp. *pseudoplantarum* and *L. casei* subsp. *tolerans*, and the majority of the tested *L. casei* subsp. *casei* strains should be given separate species status, and the names *Lactobacillus paracasei* subsp. *paracasei* (NCDO 151\(^T\)), and *L. paracasei* subsp. *tolerans* (ATCC 25599\(^T\)) were proposed (*L. paracasei* sp. nov.).

It was also proposed that *L. casei* subsp. *rhamnosus* should be elevated to species status, as *L. rhamnosus* sp. nov. (ATCC 7469\(^T\); Collins *et al.*, 1989).
However, the relevance of the type strain of *L. casei* subsp. *casei* (ATCC 393T), that had been selected as the neotype strain by Hansen and Lessel (1971), was questioned by Dellaglio et al. (1991); instead they suggested a new type strain for *L. casei* subsp. *casei* (ATCC 334), and suggested a rejection of the species name *L. paracasei*. But these suggestions were not approved by the Judicial Commission of the International Committee on Systematic Bacteriology (Wayne, 1994). However, Dicks et al. (1996) showed that the type strain, *L. casei* subsp. *casei* ATCC 393T, exhibited high DNA:DNA homology to the reference strain, *L. rhamnosus* ATCC 15820, that is the former type strain of "*Lactobacterium zeae*" (Kuznetsov 1959). On the basis of this, and results showing that *L. casei* subsp. *casei* ATCC 393T was separated from authentic *L. casei* and *L. paracasei* strains, Dicks et al. (1996) proposed that *L. casei* subsp. *casei* ATCC 393T and *L. rhamnosus* ATCC 15820 should be reclassified as members of *Lactobacillus zeae* nom. rev. (ATCC 15820T), that strain ATCC 334 should be designated the neotype strain of *L. casei* subsp. *casei*, and that the name *L. paracasei* should be rejected. Comparative sequence analyses of the genes coding for 16S rRNA have later shown that the type strains of *L. zeae*, *L. casei* (ATCC 393T), *L. paracasei* and *L. rhamnosus* were all different, and the variation in the 16S ribosomal DNA (rDNA) sequences was situated between the positions 69 and 100 (*Escherichia coli* numbering; Mori et al., 1997).

The Judicial Commission of the International Committee on Systematics of Bacteria has come to the conclusion that *Lactobacillus paracasei* should remain as name of the species, with the type strain *Lactobacillus paracasei* subsp. *paracasei* NCDO 151T (Tindall, 2008).

Well-known probiotic strains as *L. casei* “Shirota” (Yakult, in the product Yakult) and *L. casei* “defensis” (Danone, in the product Actimel) should thus presumably be designated *L. paracasei* instead of *L. casei*.

**Characteristics**

*L. paracasei* differs from many other *Lactobacillus* spp. in the following points:

1) *L. paracasei* grows very well in cheese during ripening.
2) *L. paracasei* is relatively resistant to heat.
3) *L. paracasei* has comparably high proteolytic activity.

The species *L. paracasei* is frequently present on human gastro-intestinal (GI) mucosa of healthy individuals (Molin *et al.* 1993; Ahrné *et al.* 1998), but is also often dominating the spontaneous, secondary bacterial-flora in semi-dry cheese, especially if the cheese has been manufactured with pasteurised milk (Antonsson 1991; Antonsson *et al.* 2001; Antonsson *et al.* 2003).

It has been shown that different strains of heat killed, whole cells of *L. paracasei* were more efficient in triggering the production of the regulatory
cytokin IL-12 in human blood mononuclear cells (monocytes) than were cells of *Lactobacillus plantarum* or *Lactobacillus rhamnosus* (Hessle *et al.* 1999).

Different strains of *L. paracasei* has for long been used as probiotics in a wide range of different probiotic products, marketed in many countries. The most well known strains are *L. paracasei* strain “Shirota” (labelled “casei” by the manufacturer, Yakult) and *L. paracasei* strain “Immunitas/Defencis” (labelled “casei” by the manufacturer, Danone). These particular strains of *L. paracasei* have shown to have health beneficial effects.

The bacterial strain *Lactobacillus paracasei* 8700:2

The *L. paracasei* strain 8700:2 (= DSM 13434) (Antonsson *et al.* 2002) has been isolated from healthy human colonic mucosa (Ahrné *et al.* 1998). The strain is growing quickly in milk and thrives in cheese and in yoghurt.

*L. paracasei* 8700:2 can be defined and identified by restriction endonuclease analysis (REA) of total chromosomal DNA by the use of relatively frequently cutting restriction enzymes such as *Eco*RI and *Cla*I, and traditional agarose gel electrophoresis (Johansson *et al.* 1995; Vásquez *et al.* 2004). *L. paracasei* 8700:2 has a relatively close genomic similarity to “*L. casei*” ATCC 334 and the former type strain of *L. casei* subspecies *pseudoplantarum* (DSM 20008) (Vásquez *et al.* 2004). The genomic relationship between *L. paracasei* 8700:2 and *L. paracasei* 02A (= DSM 13432), and the relationships to type strains and a battery of reference strains of the *L. paracasei/casei* complex have been scrutinised (Vásquez *et al.* 2004).

*L. paracasei* 8700:2 (= DSM 13434), has primarily been selected on the basis of the ability to grow in Swedish semi-hard cheese during storage and beneficially contribute to the sensory quality during ripening (Antonsson *et al.* 2002). However, the strain has also probiotic potential as it after administration in cheese to healthy volunteers could be re-isolated from faeces of the consumers (Antonsson 2001). Irrespectively of what strain that is used as probiotics, a condition must be that the bacterium survives and remains active during the passage through the gastrointestinal tract. The ability of *L. paracasei* 8700:2 to survive the passage through the human gastro-intestinal tract when administrated in cheese has been proved (Antonsson 2001).

*L. paracasei* 8700:2 has the capability to degrade oligofructose and long-chain inulin (Makras *et al.* 2005). *L. paracasei* 8700:2 grows rapidly on both oligofructose and inulin, with lactic acid as the main metabolic end-product (Makras *et al.* 2005). In a comparison between the ability of six different
probiotic strains to grow and multiply on nine different commercially carbohydrates (saccharides or fructooligosaccharides) with purported prebiotic properties, *L. paracasei* 8700:2 fermented seven of the tested carbohydrates while for example *Lactobacillus rhamnosus* GG were unable to ferment any of the tested prebiotics (Saulnier *et al.* 2008). *L. paracasei* 8700:2 was the only one of the tested probiotic strains that was able to degrade all the test-fructans.

*L. paracasei* 8700:2 can adhere to HT-29 cells via mannose-sensitive mechanisms (Rask *et al.* 2013).
Health effects

Antagonistic *in vitro* effects

*L. paracasei* 8700:2 has been shown *in vitro* to possess strong antagonistic properties against *Salmonella enterica* subsp. *enterica*, and an “intermediate” antagonistic activity against *Helicobacter pylori*, *Shigella sonnei* and *Escherichia coli* (Hütt *et al.* 2006).

Animal models

*Translocation*

Translocation, i.e. the passage of viable bacteria through the epithelial mucosa into the *lamina propria* and then to the mesenteric lymph nodes and possibly other tissues (Berg and Garlington, 1979), was reduced in rats with colitis by treatment with *L. paracasei* 8700:2 (Osman *et al.* 2004). The colitis was induced by giving the rat 5 % (w/v) dextran sulphate sodium (DSS) dissolved in drinking water for 7 days. Samples were collected on the 7th day for examination of the bacterial translocation. The total translocation to the mesenteric lymph nodes and the translocation of *Enterobacteriaceae* to the liver decreased significantly when the colitis rats were treated with *L. paracasei* 8700:2.

*Mitigation of Enterobacteriaceae*

The viable count of *Enterobacteriaceae* in the colon decreased in rats with DSS-induced colitis by treatment with *L. paracasei* 8700:2 (Osman *et al.* 2004).

Also, pretreatment with *L. paracasei* 8700:2 in an acute liver injury model where the injury had been induced by D-galactosamine decreased the viable count of *Enterobacteriaceae* in the colon (Osman *et al.* 2005).
**Multiple sclerosis**

Multiple sclerosis (MS) is a Th1 cell-mediated chronic inflammatory disease of the central nervous system. Treatment with *L. paracasei* 8700:2 in a mouse model for experimental autoimmune encephalomyelitis (EAE), mimicking MS, prevented and delayed the onset of the clinical signs of EAE compared to control mice (Lavasani et al. 2010). In contrast, treatment with *Lactobacillus paracasei* PCC 101 or *Lactobacillus delbrueckii* subsp. *bulgaricus* DSM 20081 had no effect on the disease development.

*L. paracasei* 8700:2 induced CD4⁺CD25⁺Foxp3⁺ regulatory T cells in mesenteric lymph nodes and enhanced production of serum TGF-β1 (Lavasani et al. 2010).

**Cortical bone loss**

With the aim to determine if probiotics can protect from ovariectomy-induced bone loss, mice were given *L. paracasei* 8700:2 in the drinking water for 6 weeks, starting 2 weeks prior before ovariectomy (Ohlsson et al. 2014). It was found that *L. paracasei* 8700:2 decreased the serum levels of the resorption marker C-terminal telopeptides and the urinary fractional excretion of calcium after ovariectomy, and reduced the expression of the two inflammatory cytokines, TNF-α and IL-1β, and increased the expression of osteoprotegerin (OPG) which is a potent inhibitor of osteoclastogenesis, in cortical bone. The probiotic treatment also improved the frequency of regulatory T cells in bone marrow (Ohlsson et al. 2014). The authors conclude that “treatment with *L. paracasei* 8700:2 prevents ovariectomy-induced cortical bone loss”, and the findings “indicate that the probiotic treatment alter the immune status in bone resulting in attenuated bone resorption” (Ohlsson et al. 2014).

**Human trial**

**Anti-oxidative activity**

The impact on the antioxidative activity markers of blood in asymptomatic *Helicobacter pylori*-colonized persons of a product containing a mixture of *L. paracasei* 8700:2, *Lactobacillus fermentum* ME-3 and *Bifidobacterium longum* 46, together with Raftilose, has been evaluated in a randomized, double-blind
placebo-controlled study (Hütt et al. 2009). After consumption of the product for three weeks the total antioxidative status of serum increased in $H.\ pylon$-positive subjects, while the ratio between oxidized and reduced glutathione decreased.

**Cell-mediated immunity**

In a blind placebo-controlled study, the effect of a daily intake for 2 weeks of *L. paracasei* 8700:2 on the innate and acquired immune system was investigated *in vivo* (Rask et al. 2013). Blood lymphocyte subsets were quantified by flow cytometry (FACS) and the expression of activation and memory markers was determined. The strain was also examined for its capacity to be phagocytosed by human peripheral blood mononuclear cells (PBMCs). Intake of *L. paracasei* 8700:2 tended to expand the NKT cell population. Also, the phagocytic activity of granulocytes towards *Escherichia coli* was increased. (Rask et al. 2013).

**Common cold infections**

In combination with the strain *Lactobacillus plantarum* HEAL9 (=DSM 15312), *L. paracasei* 8700:2 was supplemented daily to 272 subjects for a 12-week period in a randomised, parallel, double-blind placebo controlled study with the intention to clarify if the treatment product could reduce the risk of common cold episodes, number of days with common cold symptoms, frequency and severity of symptoms, and cellular immune response in common cold infections (Berggren et al. 2010). It was shown that the incidence of acquiring common cold episodes was reduced, and so was the number of days with common cold symptoms. Also, the pharyngeal symptoms were reduced, and the proliferation of B lymphocytes was counteracted (Berggren et al. 2010).

In another randomized, double blind and placebo controlled study with the same mixture of *L. plantarum* HEAL9 and *L. paracasei* 8700:2 (ProbiDefendum), including totally 310 subjects with increased risk for common cold infection (Busch et al. 2013). It was concluded from the study results that “the daily intake of the probiotic dietary supplement ProbiDefendum over a period of 12 weeks efficiently alleviated symptoms of common cold and the duration of cold episodes” (Busch et al. 2013).
Safety

The species *L. paracasei* is present in cheese as it multiplies spontaneously during the ripening process and often reaches numbers around $10^7$ CFU per g of cheese. Thus the species has been eaten alive in high numbers by innumerable numbers of humans over an immense period of time without any empirically found hazards.

The strain *L. paracasei* 8700:2 has been evaluated in the EU funded PROSAFE project (Vankerckhoven *et al.* 2008). The identity of the strain was confirmed and no acquired antibiotic resistance could be detected (PRO SAFE report on strain *Lactobacillus paracasei* 8700:2).
References


Hansen, P.A. (1968). Type strains of *Lactobacillus* species. A report by the taxonomic subcommittee on lactobacilli and closely related organisms. American Type Culture Collection, Rockville, Maryland.


For further information please contact Probi AB who owns the commercial rights [http://probi.se/en](http://probi.se/en)


Tindall, B. J. (2008). The type strain of Lactobacillus casei is ATCC 393, ATCC 334 cannot serve as the type because it represents a different taxon, the name Lactobacillus paracasei and its subspecies names are not rejected and the revival of the name ‘Lactobacillus zeae’ contravenes Rules 51b (1) and (2) of the International Code of Nomenclature of Bacteria. Opinion 82. *International Journal of Systematic and Evolutionary Microbiology* 58: 1764–1765.

