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Lactobacillus paracasei 8700:2

Consumption of live lactic acid bacteria (probiotics)

Consumption of live lactic acid bacteria (LAB) included in lactic acid fermented foods has been a regular part of the food intake of humans for a long time. In fact, there are archaeological indications that mankind has used this technique from the beginning of time and it was presumably invented 1.5 million years ago by the early humanoids (Leakey 1993; Leakey 1995). Lactic acid fermentation is the simplest and often the safest way of preserving food, and before the Industrial Revolution, lactic acid fermentation was applied just as much in Europe as it still is in Africa. Thus, it could very well be that the human gastro-intestinal (GI) tract evolved to adapt to a more or less daily supply of live LAB. This supply ceased in industrialized countries during the twentieth century, which might have led to GI problems, and to immunologic dysfunction.

When beneficial effects of certain types of live bacteria have been discussed, these types of bacteria gradually have been called "probiotics". The original concept of probiotics implies that the balance between beneficial and harmful bacteria in the microflora of the GI-tract can be positively affected by eating the right type of living micro-organisms (Parker 1974; Fuller 1989). However, the concept probiotics is today used more generally in order to describe live bacteria that exercise health beneficial effects after ingestion, i.e. probiotics = living micro-organisms, which upon ingestion in certain numbers, exert health benefits beyond inherent basic nutrition.

The species *Lactobacillus paracasei*

Lactobacillus paracasei is a bacterial species in the huge and relatively diverse genus of *Lactobacillus*, which comprises about 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* has been under discussion for some time: It started with the loss of the original type strain of *L. casei*. Hansen and Lessel (1971) then designated strain ATCC 393 as the neotype of *L. casei* (ATCC 393^{T}); the species was originally described by Orla-Jensen (Orla-Jensen 1916; referred by Kandler and Weiss, 1986). Five subspecies were recognized within this species: *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*, and 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); it was suggested that members of *L. casei* subsp. *alactosus*, *L. casei* subsp. *pseudoplantarum* and *L. casei* subsp. *tolerans*, and the majority of *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 393^T), 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*. 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 393^T, 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 393^T was separated from authentic *L. casei* and *L. paracasei* strains, Dicks *et al*. (1996) proposed that *L. casei* subsp. *casei* ATCC 393^T and *L. rhamnosus* ATCC 15820 should be reclassified as members of *Lactobacillus zeae* nom. rev. (ATCC 15820^T), 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 393^T), *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 151^T (Tindall, 2008).

Well-known probiotic strains as *L. casei* "Shirota" (Yakult, in the product Yakult) and *L. casei* "Immunitas/Defensis" (Danone, in the product Actimel) should thus presumably be designated *L. paracasei*.

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 heat resistant.
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 several health beneficial effects.

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) and patent has been granted (possessor of all rights is Probi AB, Lund, Sweden). 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 endonculease 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 gastro-intestinal tract. The ability of *L. paracasei* 8700:2 when administrated in cheese to survive the passage through the human gastro-intestinal tract has been proved (Antonsson 2001).

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

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).

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 7^{th} 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). The counts of *Enterobacteriaceae* in the colon also decreased in an acute liver injury model, where the injury was induced by D-galactosamine, by pre-treatment with *L. paracasei* 8700:2 (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). 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-*beta*1 (Lavasani *et al*. 2010).

Safety

The species *L. paracasei* is present in cheese as it multiplies spontaneously during the ripening process and often reach numbers around 10^{7} CFU per g of cheese.

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).

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