

# *Pediococcus* Probiotics containing *Pediococcus acidilactici* 5051 strain - Interaction with gastrointestinal flora and mesenteric lymph nodes

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## Abstract

*Pediococcus acidilactici* has various strains. Different probiotic strains in the same genus or species have been found to have different characteristics regarding safety and efficacy. *Pediococcus acidilactici* 5051 (Scientific Name: *Pediococcus acidilactici* NRRL B-50517) is the most superior probiotics among all *Pediococcus* genus with respect to heat (high temperature) and low pH tolerance. *P. acidilactici* 5051 is certified by the Health Canada as a safe food ingredient. The safety of this probiotic strain had also been evaluated in cats, dogs and humans. *Pediococcus* probiotics using *P. acidilactici* 5051 was reported to prevent stress related digestive disorders in hospitalized dogs and cats. It is also known to help food digestion, reduce toxic compounds, and modulate cytokine balance.

Keywords: *Pediococcus*, Probiotics, 5051 strain, Intestinal flora, Immune modulation

Probiotics are live microorganisms that confer a benefit to host health if consumed in adequate amount 1). Anaerobic bacterial strains popular in commercial probiotic supplements such as *Bifidobacterium* and *Lactobacillus*, lack sufficient resistance to heat, oxygen exposure and acid environment 2)3). These characteristics raise concerns how many bacteria would survive in stomach acid and reach GI tracts live to have health functions. Therefore, reproducible results of commercial probiotics have been challenging.

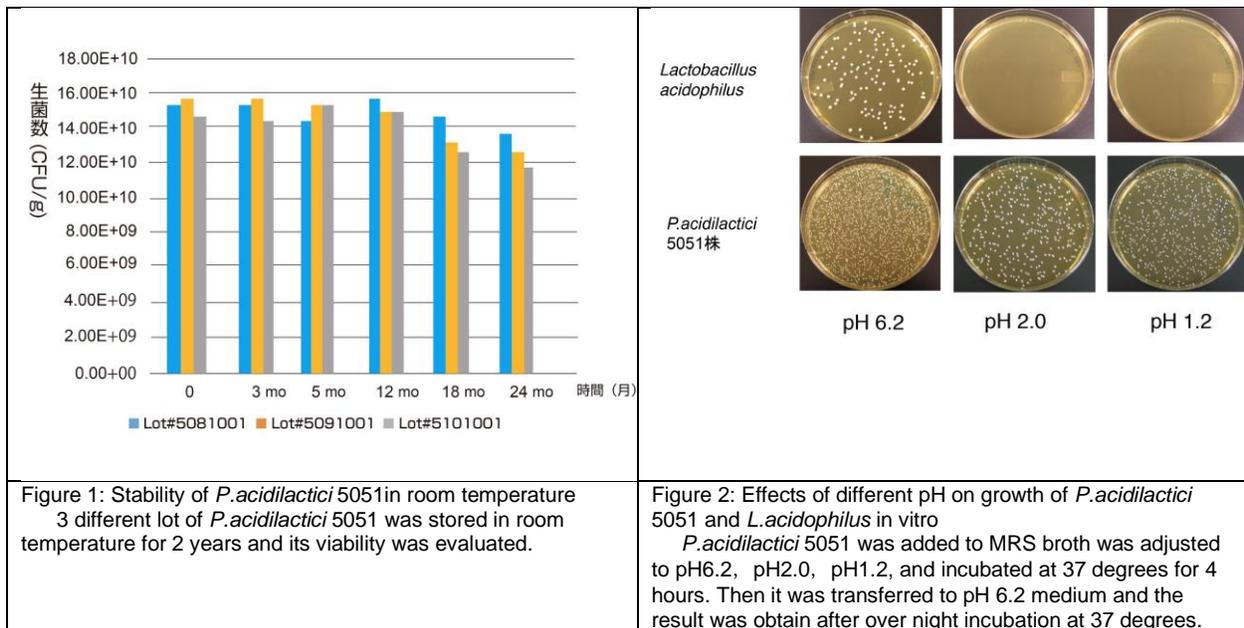
The ability of *Bacillus* spp to form endospores under the unfavorable conditions make it as naturally heat tolerable lactic acid bacteria. These endospores can maintain bacterial viability under the elevated temperature greater than 100°C. *Bacillus subtilis*, which is used in many Asian, can be a good candidate of probiotics products. Nevertheless, *Bacillus* spp contains deadly harmful pathogens like *B. anthracis* as anthrax (Madigan and Martinko, 2005), and *B. cereus* as food poisoning (Kotiranta et al, 2000). It exists potential dangers of transferring pathogenic genes from highly pathogenic *B. anthracis* or *B. cereus* into probiotic *B. subtilis*.

Certain strains of *Bacillus* can survive in high heat conditions; *Bacillus* confers heat resistance through spore formation. Since spore forming bacteria show resistant to alcohol disinfection, high temperature steam or low concentration of sodium hypochlorite, they are undesirable to manufacturers because of the time and cost associated with additional quality control procedures. Therefore, in many countries, manufacturers would not like to use *Bacillus* in their facilities. *Bacillus* spp contains deadly harmful pathogens like *B. anthracis* as anthrax 4) and *B. cereus* as food poisoning 5) in the same genus. It exists potential dangers of transferring pathogenic genes from highly pathogenic *B. anthracis* or *B. cereus* into probiotic *B. subtilis*.

*Pediococcus acidilactici* (*P. acidilactici*) is recognized as GRAS in the US, as its safety can be proven from the long history of usage in human foods. *P. acidilactici* is a beneficial bacteria naturally existing in

human and animal gastrointestinal (GI) tracts 7). Unlike *Bacillus*, *P.acidilactici* does not have clinical reports indicating it as a widely infectious pathogen. Unlike *B.antracis* and *B.cereus*, *P.acidilactici* does not have pathogenic bacteria in the same genus. *Pediococcus acidilactici* 5051 strain (Scientific Name: *Pediococcus acidilactici* NRRL B-50517) is the most superior probiotics among *Pediococcus* genus with respect to heat and low pH resistance characteristics. It is a plant based, non-spore forming probiotics that can survive through oxygen exposure, heat and stomach acid 8). It has history of usage for more than 10 years in humans, dogs, cats, rabbits, birds and horses in Japan, confirming that *P.acidilactici* 5051 has no indication of being an infectious pathogen. Safety evaluations had been also performed in rats, dogs and humans. Recently, *P.acidilactici* 5051 was certified as a food ingredient by the Health Canada.

*P.acidilactici* 5051 can survive up to 2 years when stored in room temperature (Figure1). When treated by 85 degrees of heat, about 97% of live probiotic bacteria were detected after 2 minutes and 90% after 10 minutes. While many probiotic strains have difficulty to go through stomach acid live, *P.acidilactici* 5051 can survive even in pH1.2. (Figure 2). After administered to rats and dogs, *P.acidilactici* 5051 was detected in their feces 9). *P.acidilactici* 5051 is a natural probiotic strain that had not been genetically engineered. Among non-spore forming probiotics, probiotics that has such superior characteristic as *P.acidilactici* 5051 is very rare.



In diarrhea or vomiting cases, probiotics are often administered together with antibiotics. Since most probiotics are gram positive bacteria, they will be killed by antibiotics and thus lose viability. *P.acidilactici* 5051 has certain tolerance to antibiotics and is able to administer together. When *Pediococcus* probiotics, which contains *P.acidilactici* 5051 and *Saccharomyces boulardii* (*S.boulardii*), was treated by different concentration of Chloramphenicol or Tetracycline in liquid culture broth, significant amounts of live probiotics were detected (Figure 3).

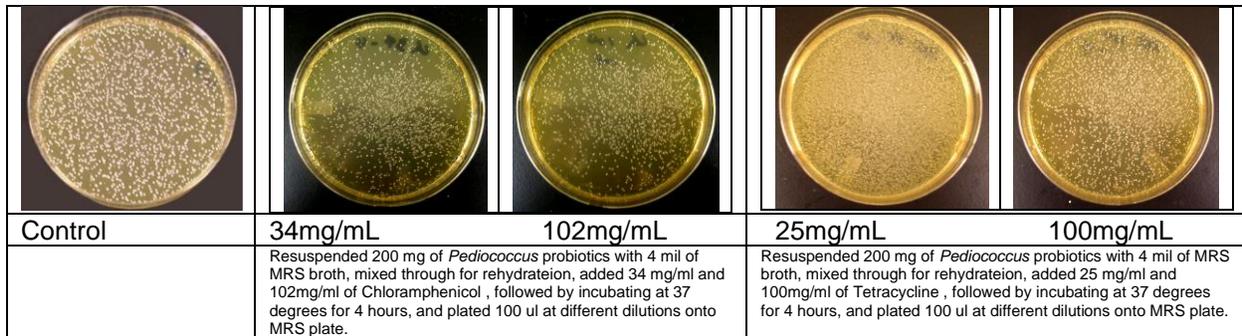


Figure 3: Effects of Chloramphenicol and Tetracycline on the growth of *Pediococcus probiotics*

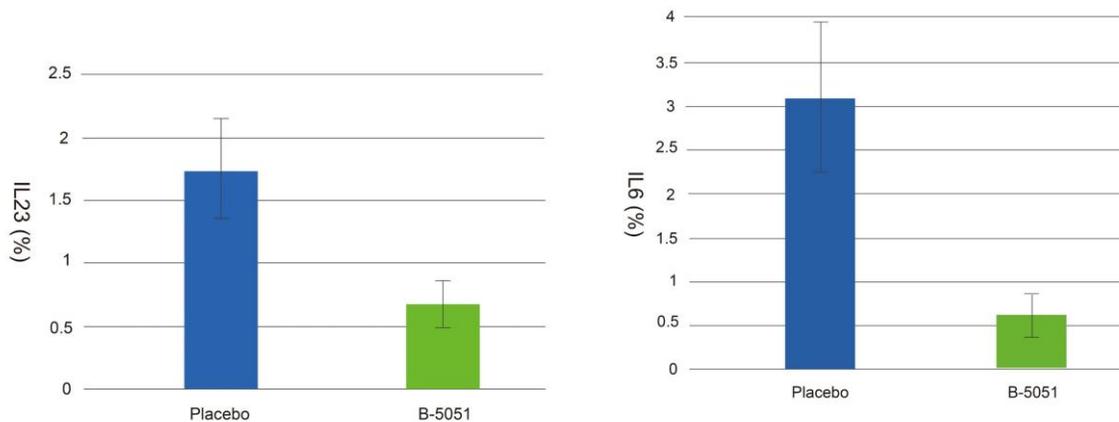
Previous studies have demonstrated that *P.acidilactici* 5051 increased immunity against parasitic diseases like coccidiosis 10). Separate study with mitogen-treated assays demonstrated increases in T-cell and B-cell proliferation in rats fed with *P.acidilactici* 5051 (Table 1). Similar immune responses results were also described in horses fed with *P.acidilactici* 5051. These results demonstrated that *P.acidilactici* 5051 is able to stimulate cellular and humoral immune responses in rats and horses 11)12). Activation of lymphocyte may possibly be occurred due to activation of macrophage. There are two ways for macrophage activation; Classical activation, which promote inflammation (activation by Interferron  $\gamma$ ) and Alternative pathway activation (activation by IL-4 or IL-13). Macrophage is considered to have antiparasitic, anti-inflammatory, and tissue repair effects 13). In a rat study, *P.acidilactici* 5051 demonstrated to increase macrophage in a dose dependent manner (Lin JJ, unpublished).

Total Number of <i>P.acidilactici</i> 5051 and <i>S.boulardii</i>	T- cell mitogen (ConA)*	B- cell mitogen (LPS)*
Control	23.34±6.53	10.0±1.33
1x10 <sup>9</sup>	34.27±3.08	42.51±8.23
1x10 <sup>10</sup>	91.14±31.65	31.53±5.09

Table 1: Effects of rats fed with *P.acidilactici* 5051 and *S.boulardii* on proliferation of B-cell and T-cell

Analysis of cell subsets of mitogen response to concanavalinA (Con A, 0.4, 0.8 or 1.6  $\mu$ g/well, Sigma ) or lipopolysaccharide (LPS, 1.25, 2.5, 5.0  $\mu$ g/well, Sigma) were carried out as described by Babu et al 14).

Studies with human subjects have also been investigating potential immune effects of *P.acidilactici* 5051; healthy human volunteers who had daily *P.acidilactici* 5051 for 3 months showed decrease body fat percentage as well as decrease in pro-inflammatory biomarkers, IL-6 and IL-23, compared to human volunteers who were given placebo (Figure 4). Adults with metabolic syndrome have elevated levels of inflammatory cytokines IL-6 and TNF  $\alpha$  15, 16). Similarly, women with metabolic syndrome have higher levels of IL-23 compared to women without clinical evidence of syndrome 17). In probiotic group, inflammatory cytokines IL-6 and IL-23 were decreased compared to placebo group. Body fat was also decreased by 0.86±0.42% compared to 0.28±0.19% in placebo group 18). In this study, anti-inflammatory cytokine IL-10 increased compared to placebo group. These results indicate that *P.acidilactici* 5051 may stimulate macrophage, suppress inflammatory cytokines, and increase anti-inflammatory cytokines.



\*: P=0.0068, t=3.0194, placebo mean=1.714±0.377 (SEM);  
*P.acidilactici* 5051 mean = 0.648±0.137 (SEM)

\*: P=0.0295, t=2.4239, placebo mean=3.058±0.867 (SEM);  
*P.acidilactici* 5051 mean= 0.612±0.221 (SEM)

Figure4: Effect of *P.acidilactici* 5051 on pro-inflammatory cytokines

A randomized, double-blind placebo-controlled pilot study of *P.acidilactici* 5051 in humans. This study was conducted by Sylvana Institute and Rotary Club of Frederick on the basis of Helsinki declaration. Without changing diet, *P.acidilactici* 5051 (4 billion CFU) was administered to 30 volunteers for 12 weeks. IL-6 and IL-23 were measured by Milliplex (St.Louis, MO).

Chronic enteropathy includes Inflammatory Bowel Disease (IBD), Non specific enteropathies (NEP), that show chronic digestive disorders but are not diagnosed as IBD, and Diet reactive bowel disease. In When *Pediococcus* probiotics (*P.acidilactici* 5051 and *S.boulardii*) were administered IBD and NEP dogs, veterinarians were able to reduce or completely terminate steroid or immune-suppressors. There was no relapse of symptoms after the reduction of these treatment drugs 19). *S.boulardii* that is contained in *Pediococcus* probiotics is reported to have effects on antibiotic-associated diarrhea and recurrent *Clostridium difficile* intestinal infections 20). *S. boulardii* was also described to enhance mice intestinal immune responses to fight against *Clostridium difficile* toxin A related diarrhea 21).

Immune mediated chronic diseases such as chronic digestive disorders or Immune Mediated Hemolytic anemia (IMHA) in dogs and cats often require long term administration of steroid or immune-suppressors. However, long term, multi-drug regimens cause severe side effects to dogs and cats. It is desirable to reduce or terminate those treatment drugs if possible. Unfortunately, it is not always easy as the recurrences of symptoms are often observed when the amounts of drugs are reduced. *Pediococcus* probiotics may be able to restore inherited anti-inflammatory mechanism by normalizing intestinal bacterial flora in dogs and cats.

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