



SYNLAC Report

The Immunomodulatory Properties of *Lactobacillus plantarum* LP28 in Animals

SYNBIO TECH INC.



"Probiotic solutions for antibiotic free farming era"



In healthy animals, the gastrointestinal tract is the largest immune system and often referred to as gut-associated lymphoid tissue (or GALT) that contributes in excess of 70% of all immunocytes, where various immune mechanisms of protection throughout whole animal life evolve. At birth, the neonate's mucosal system is relatively undeveloped. A rapid development of the GALT occurs concomitantly with the development of digestive structures and functions. Dietary intake and colonization of intestinal flora accelerates development resulting in a functional immune system shortly after birth. The balance of intestinal flora can facilitate the development of lymphoid structures as a defense against pathogenic invasion. According to trials, in the absence of the microbiota, the animal intestine affects a low degree of intestinal cell differentiation and development of an immune system, resulting in a decrease in immune resistance to infection from pathogenic microorganisms or toxins and increased mortality; whereas, through the establishment and regulation of microbiota in the intestine, germ-free animals enhance the immune system and defense against infection. In addition, when more harmful bacteria than beneficial microbes colonize in animals, immunomodulation may develop, leading to increased morbidity. Therefore, the maintenance and balance of microbiota in the intestines of animals are a prerequisite for immune defense against diseases. This proves the importance of intestinal microbioflora within the entire immune system. With respect to strains associated with the immune system, immunologists discovered that *Lactobacillus plantarum* (*L. plantarum*) has immune modulation potential in 1960. This discovery has led to subsequent studies on the immune system related to lactic acid bacteria (LAB) derived from plant material.

L. plantarum is a common heterofermentative LAB from plant-origin sources, such as vegetables, cocoa beans, or fermented food (Hammes *et al.*, 2006). Since it lives under strict environmental circumstances, *L. plantarum* has a relatively large genome compared to other *Lactobacillus* spp., and can effectively convert nutrients or produce bacteriocine in order to facilitate survival in a barren environment. Consequently, its tolerance abilities are much higher than general LAB and *L. plantarum* is able to apply widely in different area (Daeschel and Fleming, 1984; Plumed-Ferrer *et al.*, 2008; Teusink *et al.*, 2005). In addition, plant-origin LAB is stereotyped as existing only in plant-material. Actually, this type of bacteria has been discovered in the intestines of mice, human beings, fish, swine, poultry, and even breast milk from sows (Butprom *et al.*, 2013; Rocío *et al.*, 2009).

Nevertheless, not all *L. plantarum* is capable of tolerating acid and bile, adhering to intestinal cells and modulating the immune system. In particular, some of them are unable to survive in low pH environments when passing through the stomach to the gastrointestinal (GI) tract (Barrlen *et al.*, 2009). Moreover, the beneficial effects of *L. plantarum* on immunomodulatory ability in animal and humans have been demon-

Fig. 1. Comparison of cytokine expression of IL-12p40 stimulated by co-culturing with LP28 originated from plant materials and other LAB screened from animals.

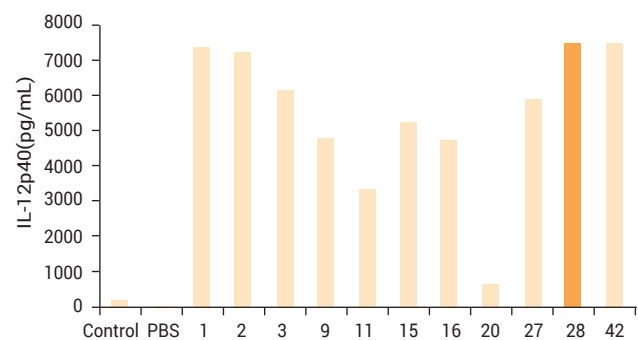
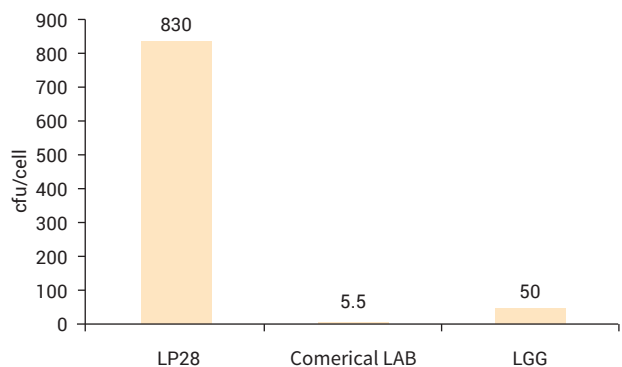


Fig. 2. Comparison of ability to adhere to intestinal epithelial cells with LP28 and other LAB



LP28 isolated from Taiwan Dasi tofu, has excellent gastric acid/bile salt tolerance and colon epithelial cell adhesion.

strated in many studies due to the structural characteristics of its cell wall which plays a crucial stimulatory role in inducing mucosal immune response in the GI tract (Rocío *et al.*, 2009). This study presented the immunomodulatory effects of *L. plantarum* LP28, screened by SYNBIOTECH using the SYNTEK® thorough system.

Cytokine Expression of Interleukin (IL)-12 p40

IL-12 p40 is a cytokine produced by macrophages, dendritic cells, or B lymphoblastoid cells that acts on T and natural killer (NK) cells in defense against foreign pathogens or harmful organisms. There are eleven strains assayed in cytokines expression. Consequently, the number 28 strain has better immune modulation ability in IL-12 p40 than do other LAB, and hereafter referred to as *Lactobacillus plantarum* LP28.

Adherence to Intestinal Epithelium

In many studies, probiotics have been known to confer healthy benefits to the host, especially through regulation of immunity. The most important mechanism of probiotic-mediated immunomodulatory effects is adherence to intestinal epithelial cells. As a result, probiotics colonize in the intestinal mucus layer and interact with immune cells, which are the portion of the immune system which stimulates protective response against infection, and prevents the uptake of antigens, microorganisms and other foreign materials. In terms of strain characteristics, the adhesion ability of plant-origin LAB is normally worse than LAB originated from animal or dairy products. However, LP28 screened from Dasi dried tofu in Taiwan has better adherence to intestinal epithelial cells in animals, and is even better than commercial strain *Lactobacillus rhamnosus* LGG (Fig. 2). This means that LP28 effectively colonize in animal intestine to stimulate gut associated lymphoid tissue (GALT), further effecting the modulation of immunity.

Fig. 3 Comparison of immunomodulatory ability of LP28 and other plant-origin LAB

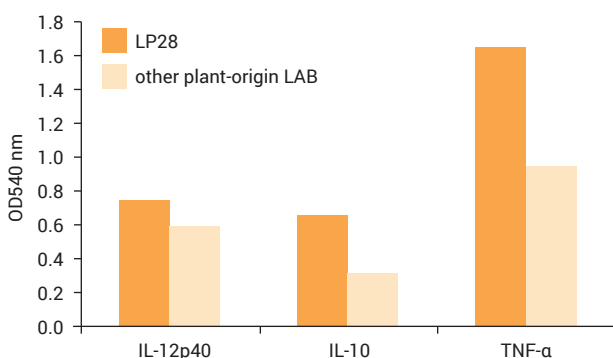
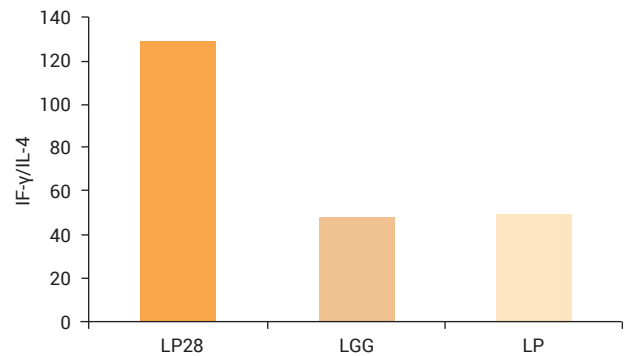


Fig. 4. Comparison of immunity indexes of LP28 and other LAB



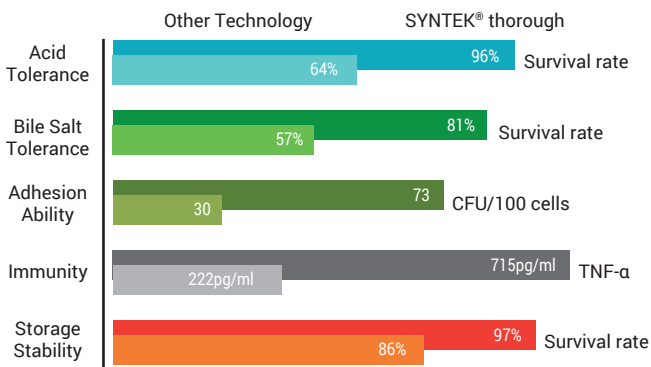
Immunomodulatory Ability

The cytokines of IL-12 p40, IL-10 and tumor necrosis factor (TNF)-α are involved in immunomodulation. IL-12, as mentioned above, can help T cell proliferation, promote the ability of the NK cell to kill foreign bacteria and viruses, and stimulate naïve T cell differentiating into TH1 cells that assist animals against pathogens and protozoa. IL-10 plays a major role in suppressing T cells and macrophages activities in order to assist in modulating antibodies and balancing the immune system. TNF-α is involved in inflammatory response in the immune system. When a virus or bacteria invade the body, causing the concentration of TNF-α to increase, TNF-α removes the infected cells through inflammatory response. Many LAB originated from plant materials have been shown to have potentially immune modulation ability, but not all strains have this property. In this study, LP28 has been shown to modulate the immune system more effectively than other LAB from plant materials (Fig. 3).

Modulation of Immune Response

Many probiotics have been shown to induce different cytokine profiles (TH1 or TH2) and might play an important role in maintaining the balance between the strength of TH-like and TH2-like response. With regard to cytokines, IL12 is produced by immune cells and activates naïve T cells to become like TH1 cells; meanwhile stimulation of macrophages and NK cells to secrete interferon (IFN)-γ results in decreased IL-4. IL-4 belongs to the TH2 immune system and triggers the differentiation of naïve immune cells to B cells, which is a humoral immune response. For example, as an allergy occurs, allergens induce the secretion of immunoglobulin E (IgE) in the serum, meaning that the TH2 immune response is higher than that of TH1. In Fig. 4, three LAB, including LP28, are able to modulate immunity through balancing TH1 and TH2 immune reaction. The results show that LP28 mainly modulates the TH1 response in terms of the balance of immunity and its performance was better than that

SYNTEK® thorough Optimizing Probiotic Efficacy



of other LAB. The mechanism of immunomodulation induced by LP28 top resist foreign pathogens may be through modulation of TH1 cytokine expression.

SYNBIO TECH INC. has been contributing research, development and manufacturing of lactic acid bacteria for decades. Moreover, more than two thousand lactic acid bacteria are deposited in our research center. Simultaneously, we developed SYNTEK® thorough which is an optimizing development system based on strain level in the production procedure. This system allows us provide better quality probiotic products with superior storage stability, acid/bile tolerance, adhesion ability, specific functions, etc. According to the different characteristics of each probiotic strain, we utilize the SYNTEK® thorough system to select effective strains applied in different fields. For example, LP28 screened from Dasi dried tofu has been selected and characterized based on its *in vitro* properties. In particular, LP28 expresses excellent immunomodulatory effects on the stimulation of the cytokines of the host and adherence to the intestinal mucus or epithelial cells, and thereby has been successfully applied to animal formulas for poultry, livestock, aquaculture and agriculture. SYNBIO TECH has integrated probiotic industrialization from research technology, manufacturing procedures and quality control to enhance product performance.

Reference

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