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Technical Bulletin – *Lactiplantibacillus plantarum* 276

Introduction

Lactiplantibacillus is a genus of Gram-positive, rod-shaped bacteria, commonly found in fermented foods, plants and the human gastrointestinal tract. *Lactiplantibacillus plantarum* has a long history of safe consumption in humans and has been used in food processing and fermentation for generations.

As a species Generally Recognized As Safe (GRAS), *Lactiplantibacillus plantarum* is often considered a probiotic bacteria for its ability to positively regulate the health of the immune system and GI function. Several strains have demonstrated reduced inflammation when administered to various inflamed tissues in both humans and animals (Duary et al., 2012; Ducrotté et al., 2012). In addition to anti-inflammatory characteristics, the *Lactobacillus* genera has well documented abilities to beneficially modulate the microbial community and decrease pathogens (Rokka et al., 2006; Tomusiak et al., 2015). Because of the ubiquitous nature, efficacy, and safety associated with these bacteria, *Lactobacillus* are commonly used as probiotics.

A panel of GRAS *Lactiplantibacilli* from the Arm & Hammer microbial library were assessed for their ability to decrease inflammatory markers in response to LPS stimulation. From this panel a specific strain (Lp276) demonstrated superior anti-inflammatory characteristics in an intestinal epithelial cell inflammation model and has been developed for probiotic use in humans.

Gastrointestinal Performance

Probiotics are live organisms that, when provided in sufficient numbers, elicit a health benefit. In order to provide a benefit, orally ingested bacteria must survive within the host digestive tract. Many traits are necessary for a strain to survive in the GI tract including the ability to tolerate the highly acidic conditions of the stomach and the levels of bile salts that exist in the intestine. It is also highly advantageous to adhere to the intestine. *In vitro* studies with *L. plantarum* 276 demonstrate this strain to be extremely resistant to low pH and able to survive in the presence of bile acids at concentrations that are present in the intestine (Table 1). Furthermore, *L. plantarum* 276 contains multiple fibronectin binding genes, a protein expressed by the intestinal epithelium, as well as mucus binding genes. The expression of both genes is a good indicator of the ability of *L. plantarum* 276 to bind intestinal tissues.

Consistent with this, *in vitro* cell culture binding assays demonstrated consistent cellular binding to Caco-2 cells.

Table 1. Acid and Bile Tolerance

Acid Tolerance	No loss of survival in medium containing hydrochloric acid at pH 3.0 for 1hr at 37C
Bile Tolerance	>97% survival in 0.3% bile salt containing medium

Immunomodulation

The immune system is an essential and complex physiological system dedicated to preventing and eradicating infection, removing noxious agents and maintaining homeostasis in the body. An optimally functioning immune system is vital for peak performance and development.

An intact intestinal barrier is the first line of defense in the immune system. Maintenance of the barrier becomes of critical importance as changes in intestinal permeability can allow antigens and pathogens to cross into the underlying tissue resulting in inflammation. *In vitro* studies measuring the Transepithelial Electrical Resistance (TEER) of human Caco-2 monolayers, a model of intestinal permeability, have demonstrated the ability of *L. plantarum* 276 to reverse the decreased intestinal barrier function induced by bacterial lipopolysaccharide (LPS) (Figure 1.).

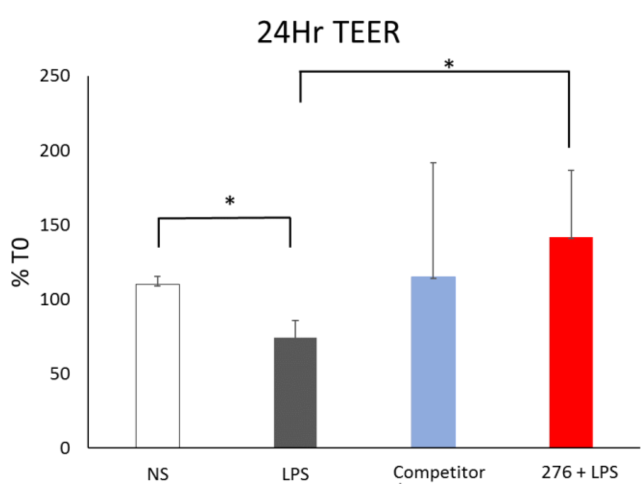

Figure 1. *L. plantarum* 276 protects intestinal barrier integrity as measured by transepithelial electrical resistance. Lipopolysaccharide (LPS) decreased barrier function. *L. plantarum* 276 reversed the loss of barrier function induced by LPS. * $P < 0.05$.

Another important characteristic of intestinal barrier function is the ability of the intestine to heal breaks in the barrier that occur daily due to physical and chemical assaults from the diet. *In vitro* studies measuring intestinal restitution demonstrate the ability of Lp276 to increase wound repair in intestinal epithelial monolayers (Figure 2).

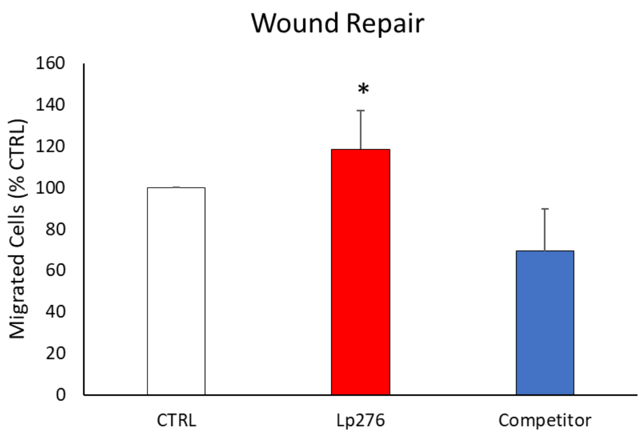

Figure 2. *L. plantarum* 276 increased intestinal epithelial cell migration in wounded IEC-6 monolayers over a 24hr period compared to untreated control cells. * $P < 0.05$.

The GI tract is the largest immune organ in the body. The cells of the GI tract must constantly surveil the contents of intestine, differentiating innocuous agents and organisms from potential pathogens that might enter the body. When appropriate, the immune system must be able to elicit a targeted immune response to restrict pathogens while not inducing inflammatory disorders that can result in collateral damage to healthy tissues in the body. Mounting evidence demonstrates the ability of probiotics to modulate the immune system through multiple pathways such as stimulating the immune response or regulating the production and function of anti- inflammatory immune mediators (Ducrotté et al., 2012, van Hemert et al., 2010).

Consistent with the role of certain probiotics as potential immune mediators, *in vitro* assays with *L. plantarum* 276 demonstrated an increase in the expression of pro- inflammatory cytokine IL-6 and IL-8 in Caco-2 cells (Figure 3). Localization assays demonstrate that while IL- 8 is secreted to both the luminal and basolateral compartments, IL-6 is primarily secreted to the lumen of the intestine. IL-8 is a chemoattractant cytokine and the production by the epithelial cells is likely responsible for the increase in wound repair (Figure 2) as well attracting immune cells to the intestinal mucosa.

IL-6 is a potent activator of immune cells to respond to an infection. The localization of IL-6 to the intestinal lumen, in the presence of a tightened epithelial barrier, sequesters the immune activation signal away from the effector cells thereby allowing for activation of the immune response only if the barrier is broken.

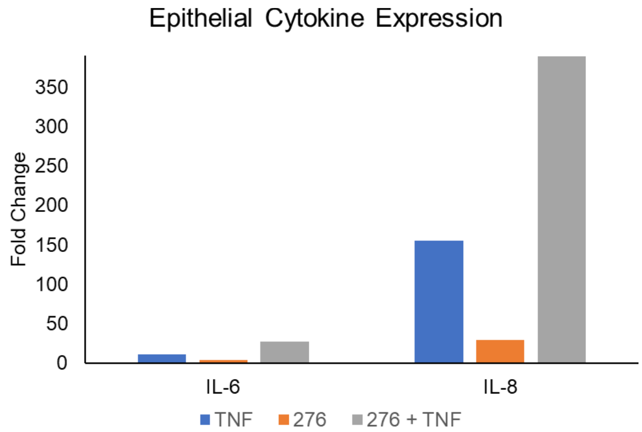

Figure 3. *L. plantarum 276* increases the expression of epithelial cytokines genes during in inflammatory stimulus. When added in combination with tumor necrosis factor α (TNF- α), *L. plantarum* 276 elevated the expression of inflammatory IL-6 and chemoattractant IL-8.

In circulating peripheral blood mononuclear cells (PBMC), *L. plantarum* 276 demonstrated a concomitant decrease in proinflammatory IL-12 and an increase in the expression of anti-inflammatory cytokine IL-10 and the regulatory transcription factor FoxP3 (Figure 4). The expression of these inflammatory mediators suggests that Lp276 has the ability to regulate the immune system at a systemic level as well as at the level of the intestinal epithelium.

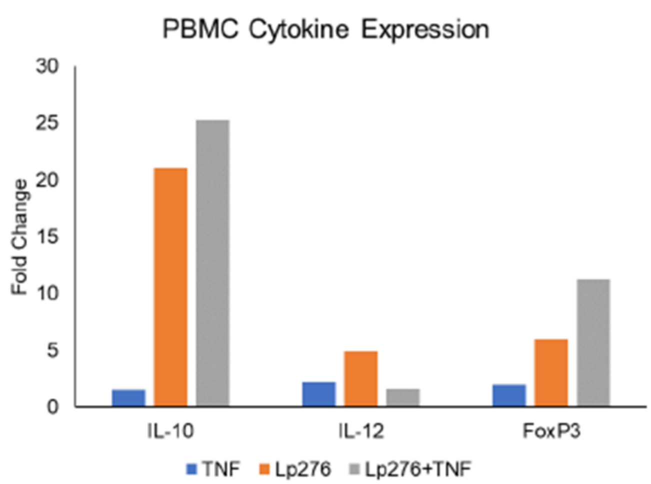

Figure 4. *L. plantarum* 276 increased the expression of anti- inflammatory Il-10 and the regulatory transcription factor foxp3 while concomitantly decreasing inflammatory IL-12 in PBMC during an inflammatory challenge.

Taken together, the abilities of *L. plantarum* 276 to maintain the epithelial barrier function, modulate intestinal wound repair and regulate the expression of inflammatory cytokines during periods of immune stimulus demonstrate the aptitude of this strain to beneficially regulate health.

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