



ORIGINAL ARTICLE

# *In vitro* importance of probiotic *Lactobacillus plantarum* related to medical field



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## KEYWORDS

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**Abstract** *Lactobacillus plantarum* is a Gram positive lactic acid bacterium commonly found in fermented food and in the gastro intestinal tract and is commonly used in the food industry as a potential starter probiotic. Recently, the consumption of food together with probiotics has tremendously increased. Among the lactic acid bacteria, *L. plantarum* attracted many researchers because of its wide applications in the medical field with antioxidant, anticancer, anti-inflammatory, antiproliferative, anti-obesity and antidiabetic properties. The present study aimed to investigate the *in vitro* importance of *L. plantarum* toward medical applications. Moreover, this report short listed various reports related to the applications of this promising strain. In conclusion, this study would attract the researchers in commercializing this strain toward the welfare of humans related to medical needs.

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## 1. Introduction

The human digestive system contains approximately four hundred different bacterial species and its abundance differs between individuals. Among them few probiotic *Lactobacillus*

species namely, *Lactobacillus acidophilus*, *Lactobacillus pentosus*, *Lactobacillus brevis*, *Lactobacillus lactis*, *Lactobacillus amylovorus*, *Lactobacillus casei*, *Lactobacillus bulgaricus*, *Lactobacillus fermentum*, *Lactobacillus plantarum* and *Lactobacillus rhamnosus* specifically produce extracellular proteins, exopolysaccharides, bacteriocins and lipoteichoic acids which influence the health and physiology of the host by interacting with the epithelial cells and enhance the host immune system (Sanchez et al., 2010). *Lactobacillus* strains are recognized as safe for consumption because of their presence in food and their role in the gut defense mechanism. Of the *Lactobacillus* strains, *L. plantarum* is a Gram positive, short-rod, micro-aerophilic, acid-tolerant, non-spore forming,

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non-respiring, low G + C content, hetero-fermentative group of lactobacilli with a range of applications in the food industry as a starter culture and preservatives (Arasu et al., 2013). It is a non-spore forming bacterium which produces organic acids such as acetic acid, succinic acid and lactic acid as major metabolites. The antibacterial, antifungal and probiotic properties of LAB strains have been widely studied (Rejiniemon et al., 2015). *L. plantarum* grow under low buffering capacity in the stomach and other complex bile salt secretions in humans and other mammals. Besides applications in the food industry, *L. plantarum* has wide applications in the pharma industry by contributing significantly to human medicine without contributing to any side effects. Recently, *L. plantarum* has been applied in medical fields for the treatment of various chronic and cardiovascular diseases such as Alzheimer's, Parkinson's, diabetes, obesity, cancer, hypertension, urogenital complications, liver disorders, etc. (Woo et al., 2014). The present study aimed to investigate the in vitro importance of *L. plantarum* related to the medical field.

## 2. Materials and methods

### 2.1. Isolation of *Lactobacillus* strains

Novel *L. plantarum* was isolated from the silage (Arasu et al., 2013). For the isolation process, the silage sample was serially diluted and spread on de Man–Rogosa (MRS) agar and incubated at 37 °C for three days. Morphological, biochemical and physiological characteristics of the strains were examined by following the reported literature. Phenotypic characteristics were studied using API 50CHB kits. The growth and fermentation pattern of the strains under various sugars were determined by following the standard method.

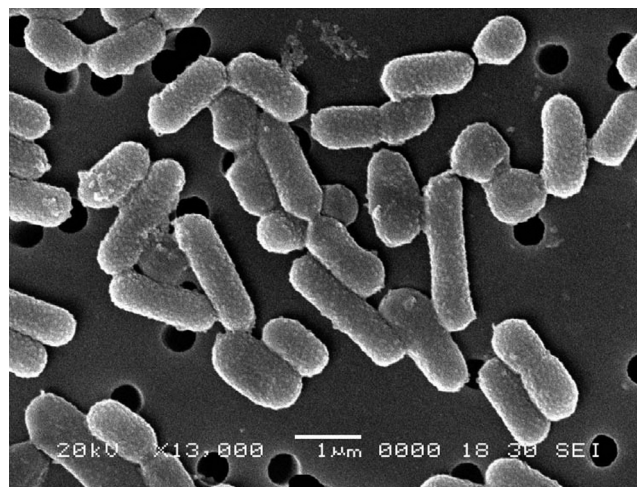
### 2.2. Importance of *L. plantarum*

The in vitro application of the *L. plantarum* was reported by many researchers. Besides the applications of these strains in our lab, the importance of these strains reported by other researchers was summarized in this report.

## 3. Results and discussion

### 3.1. Identification and characterization of *L. plantarum* strains

The fermented food is commonly known for the presence of *Lactobacillus* strains. Besides protecting the nutritional quality, *Lactobacillus* strains were used for protecting the fermented food from various fungal pathogens. Many beneficial species of *Lactobacillus* strains were derived from the fermented food and other silage samples. The routine microbiological identification methods and 16S rRNA gene amplification followed by sequencing identified the strain as *L. plantarum* (Arasu et al., 2013). The physiological and biochemical identification study showed that the strain was catalase negative, Gram positive (Fig. 1). This novel strain was tolerant to a different range of salts especially NaCl and bile salts, pH of 4.0–8.0, temperatures of 28–45 °C, and with optimum cell growth at a temperature of 37 °C and pH 7.0 respectively. Similar to the literature the identified strain



**Figure 1** Micro-morphological image of *Lactobacillus plantarum* strain.

survived various biological barriers such as low pH, lytic enzymes, and bile salts in the upper GI tract (Vijayakumar et al., 2015). Carbohydrate assimilation test concluded that the strain was able to utilize a wide range of sugars especially monosaccharide's and disaccharides respectively. Moreover, the production of extracellular enzymes such as amylase and protease was to its advantage. The above mentioned results were commonly observed in *L. plantarum* strains.

The bile salt tolerance level of the *Lactobacillus* strains was induced by the expression of proteins such as GshR4, Cfa2, Bsh1, OpuA, and AtpH (Hamon et al., 2011). Besides the tolerance level, the antagonistic properties of the novel *Lactobacillus* strains are important to prevent the spreading of the intestinal infections. In general, the probiotic *Lactobacillus* strains exhibited significant antimicrobial activity against various GI tract pathogens. The antimicrobial properties of the strains were mainly related to the secretion of the extracellular metabolites such as lactic acid, acetic acid, succinic acid, and bacteriocins.

### 3.2. Importance *L. plantarum* strains

Literature claimed that *L. plantarum* strains were widely studied for their applications in the medical field (Table 1). Especially, these strains were reported to possess the down regulation of the risk of cardiovascular diseases (Ahren et al., 2014), produce pro-inflammatory cytokines in the intestinal epithelial cells (Murofushi et al., 2015), produce varied concentrations of exopolysaccharide with anticancer property (Wang et al., 2014), reduce kidney stones (Sasikumar et al., 2014), enhance splenocytes in dendritic cells (Ku et al., 2014) and reduce the cholesterol level in the adipose tissue (Li et al., 2014). Recently, Ilavenil et al. (2015) claimed that the phenyl lactic acid recovered from *L. plantarum* promotes adipogenic activity in 3T3-L1. Interestingly, *L. plantarum* significantly induces mucosal, humoral and cellular immune responses (Shi et al., 2014) and protects against symptoms of irritable bowel syndrome (Stevenson et al., 2014). It inhibited the production of pro-inflammatory cytokines such as NF-κB and suppresses atherosclerotic plaque inflammation

**Table 1** Medical and pharmacological applications of *Lactobacillus plantarum*.

Key finding	References
This study showed that <i>L. plantarum</i> together with blueberries significantly reduced hypertension and blood pressure. Therefore, this strain might be used for the down regulation of the risk of cardiovascular diseases	Ahren et al. (2014)
The exopolysaccharides obtained from <i>L. plantarum</i> significantly decreased the production of pro-inflammatory cytokines in the intestinal epithelial cells in a RP105/MD1-dependent manner	Murofushi et al. (2015)
This study reveals that the cell bound exopolysaccharide isolated from <i>L. plantarum</i> 78010 showed significant anticancer activity	Wang et al. (2014)
This study demonstrated that the <i>L. plantarum</i> expressing oxalate decarboxylase gene significantly degrades calcium oxalate in the kidney, thus protects the kidney from stones	Sasikumar et al. (2014)
The extra cellular products of <i>L. plantarum</i> revealed anticancer effects by increased trans-epithelial electrical resistance of H4 cells and decreased the secretion of pro-inflammatory cytokines IL-6 and IL-8	Dimitrovski et al. (2014)
Oral supplementation of <i>L. plantarum</i> stimulated the expression of IL-12 and IFN- $\gamma$ in splenocytes and activates MHC class II markers, CD80 and CD 86 in dendritic cells. This study confirmed that the probiotic strain has immune-modulatory effects	Ku et al. (2014)
This study showed that the <i>L. plantarum</i> ameliorates colitis by inhibiting the TLR-4-linked NF- $\kappa$ B and MAPK signaling pathways	Jang et al. (2014)
Administration of <i>L. plantarum</i> , regulates lipid metabolism in adipose tissues by lowering cholesterol level	Li et al. (2014)
<i>Lactobacillus plantarum</i> enhances the antiproliferative activity in the vascular smooth muscle cell through the suppression of cell cycle progression and expression of cell cycle-related proteins	Lee et al. (2014)
This study states that the oral administration of <i>L. plantarum</i> expressing the hemagglutinin (HA) gene of H9N2 AIV significantly induces the mucosal, humoral and cellular immune responses. Therefore, this vaccine could be used to prevent the spreading of H9N2 avian influenza virus and also transmission of AIV	Shi et al. (2014)
The reported data confirmed that the administration of <i>L. plantarum</i> significantly reduced levels of the total cholesterol, $\gamma$ -glutamyl transpeptidase, low-density lipoprotein, glucose, homocysteine and interleukin-6 in postmenopausal women. These symptoms in postmenopausal women are an important risk factor for cardiovascular morbidity, especially stroke and coronary heart disease	Barreto et al. (2014)
The study demonstrates for the first time the protective role of <i>L. plantarum</i> on symptoms of irritable bowel syndrome	Stevenson et al. (2014)
This study confirmed that the oral administration of <i>L. plantarum</i> stimulates high levels of pro-inflammatory cytokine IL-12 and low levels of anti-inflammatory cytokine IL-10, whereas in hepatic and renal cells it induces the levels of alanine amino transferase, gamma glutamyl transferase, plasmatic triglycerides, total cholesterol, creatinine and urea concentrations	Salah et al. (2013)
Lipoteichoic acid obtained from <i>L. plantarum</i> inhibited the production of pro-inflammatory cytokines such as NF- $\kappa$ B and suppresses the atherosclerotic plaque inflammation	Kim et al. (2013)
This study confirmed that the administration of heat killed or live <i>L. plantarum</i> attenuates the symptom of Crohn's disease and ulcerative colitis	Chiu et al. (2013)
<i>L. plantarum</i> recovered from Korean traditional pickle exhibited significant antioxidant activity. This study concluded that the administration of this strain has various oxidative effects	Arasu et al. (2014)
<i>Lactobacillus plantarum</i> derived from the intestine induces the enhanced production of cytokine	Salah et al. (2013)
Regular intake of the diet with <i>Lactobacillus</i> strains reduces the body weight and white cell size of the adipose tissue	Grover et al. (2012)
This study depicts that the supplementation of <i>L. plantarum</i> prevents dermatitis by increment of type 1 helper T cell activation and regulatory T cell activation	Won et al. (2012)
<i>Lactobacillus plantarum</i> exerts anti-cancer effects in 1,2-dimethyl hydrazine (DMH)-induced colorectal cancer	Kumar et al. (2012)
Oral administration of <i>L. plantarum</i> K68 prevents the spreading of ulcer and exhibited comparatively better anti-inflammatory and immune modulatory activities by inhibiting the synthesis of factor- $\alpha$ and prostaglandin E(2) in macrophage	Liu et al. (2011)
This study documented that the plantaricin A produced by <i>L. plantarum</i> stimulates in vitro proliferation and migration of human keratinocytes	Pinto et al. (2011)
This study concluded that the <i>L. plantarum</i> strains are mainly involved in the T-cell differentiation, thereby improving the immune responses toward antigens	Vissers et al. (2010)
Tight junction formation in the intestine is stimulated by in-vivo administration of <i>L. plantarum</i>	Anderson et al. (2010)
Bacterial infection causes a serious problem in curing wounds especially in the ulcer stage. In this report, external application of <i>L. plantarum</i> on the ulcer patient cures the wounds of the diabetic patient. The investigations concluded that levels of polymorphonuclear, apoptotic and necrotic cells were completely decreased	Pera et al. (2010)
Oral administrations of <i>L. plantarum</i> cure obstructive jaundice and protect the liver from different barriers	Zhang et al. (2010)
<i>Lactobacillus plantarum</i> coated with proteins and polysaccharides exhibited interesting hypocholesterolemic effects. This combination speeds up the degradation of hepatic cholesterol into bile acids	Jeun et al. (2010)
<i>Lactobacillus plantarum</i> may improve human colon cancer. This is the first report about the pharmacological application of probiotic bacteria in the treatment of colon cancer and the mechanism of intestinal epithelial cells in immune responses	Paolillo et al. (2009)

(Kim et al., 2013) and induces the enhanced production of cytokines in the human intestine (Salah et al., 2012). On the other hand, *L. plantarum* strains are mainly involved in the T-cell differentiation thereby improving immune responses toward antigens (Visser et al., 2010) and preventing dermatitis by increment of type 1 helper T cell activation and regulatory T cell activation (Won et al., 2012).

#### 4. Conclusion

In conclusion, among the lactic acid bacteria, *L. plantarum* plays an important role in medical applications. The summarized recent report related to *L. plantarum* would be useful to the pharmaceutical industry for the preparation of medical formulations without side effects.

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