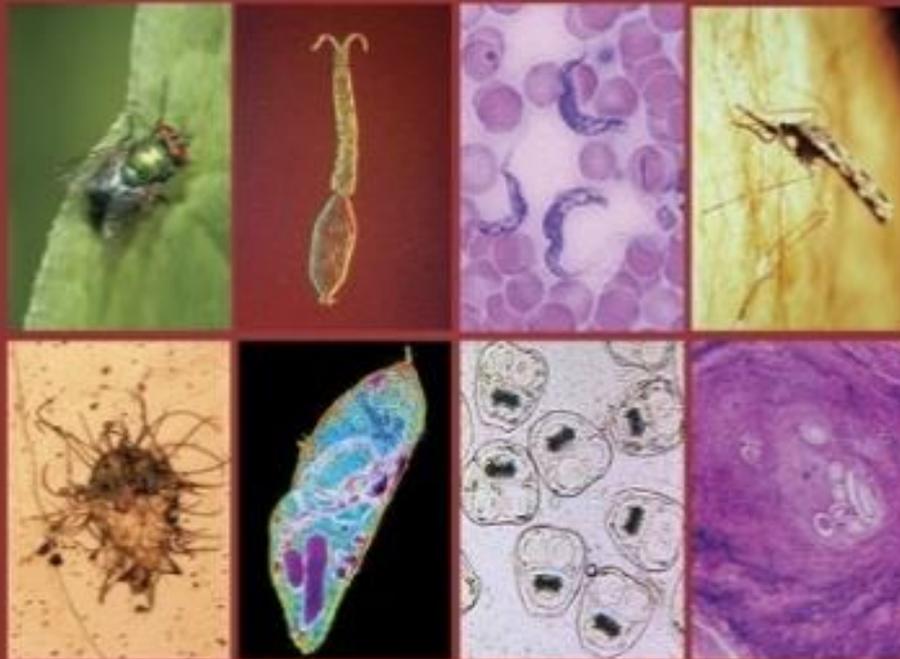




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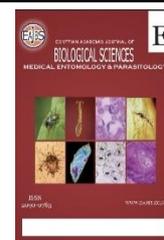
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## The Mechanisms of Lactobacillus Activities: Probiotic Importance of Lactobacillus Species

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### ABSTRACT

Lactobacillus is one of the largest genera included in the lactic acid bacteria. This genus contained a lot of species, with their differences in phenotypic and genotypic properties. They are gram-positive, aerotolerant non-spore-forming bacteria. They are commonly found in nature. Additionally, many lactobacillus species constitute a significant part of the human gastrointestinal microbial community and vaginal microbiota. They have a great role in the balance of intestinal microflora. The genus Lactobacillus is the most researched bacteria, which is widely used in probiotic and commercial products. Probiotics are supplements of food containing significant amounts of living microorganisms, and able to alter the composition of the gastrointestinal microbial community, and are used in the treatment of disturbed gastrointestinal microbiota that provides beneficial effects to human health. The mechanisms of probiotic lactobacillus activities include adhesion to intestinal mucosa to prevent the adherence and growth of pathogenic bacteria through competitive exclusion. Also, productions of antimicrobial substances, antiviral activities, antitumor activities, immunomodulatory activities, and maintenance of intestinal balance in certain GIT pathology are methods of promoting host conditions by certain species of this genus. In this article, many studies that report probiotic activities of specific Lactobacillus species are reviewed. So as to summarize the advantages and benefits that these bacteria promote in the host.

### INTRODUCTION

During birth, humans are exposed to a large microbial population when passed through the birth canal. So, microbial colonization in human guts begins immediately (Milani *et al.*, 2017). Gut microbiota is a variety of microorganisms residing in the mammalian gastrointestinal tract that provides protection for the hosts through the ability to overcome pathogenic microbes by competitive exclusions, including competition for attachment sites and nutritional sources. Also, they are able to secrete antimicrobial substances. The variation in the composition of gut microbiota depends on host specificity and is liable to exogenous and endogenous modifications throughout the lifetime (Sekirov *et al.*, 2010). Gut microbiota is important for normal nutritional metabolisms and has a role in the maturation of intestinal epithelium, lymphoid tissue and vasculature (Kho and Lal, 2018).

The strict anaerobic bacteria constitute a major portion of gut microbiota. The human intestinal tracts are predominantly colonized by the species of Bacteroidetes and Firmicutes phyla. Also, the species of other phyla are present in lesser amounts such as species of Proteobacteria, Actinobacteria, and Fusobacteria (Rinninella *et al.*, 2019).

Lactobacillus is one of the genera of Firmicutes phylum (Arumugam *et al.*, 2011). They are described as lactic acid-forming, gram-positive, aerotolerant, non-spore forming bacteria, which is composed of 237 species and 29 subspecies. Lactobacillus forms an important part of the microbiota that distributed in the gastrointestinal tract, female genitourinary tracts, and mouths (Rossi *et al.*, 2019). During glucose fermentation, lactobacilli produce lactic acid as a major end product of glucose metabolism through Embden-Meyerhof-Parnas pathway (Kononen, 2007). They use lactic acid and other metabolites such as hydrogen peroxide and bacteriocins to inhibit the growth of pathogenic microbes. Therefore, they were described as protective microorganisms (Reid and Burton, 2002).

Probiotics are supplements of food that contain a sufficient number of living microorganisms to alter the composition of microbiota and they have many health benefits (Parker *et al.*, 2018). The term probiotic is a Greek word that refers to “for life”. Originally, probiotics were described

as microorganisms which secrete substances, which enhance and control intestinal flora (Fuller, 1999). But recently, probiotics are being described as live microorganisms that are in the form of food supplements (Hill *et al.*, 2014). According to clinical studies, probiotics are beneficial for reducing inflammatory conditions and gastrointestinal infections, preventing antibiotic-associated diarrhea, and some other conditions (Kho and Lal, 2018). Probiotics also have roles in the enhancement of the host immune system, reduction of cholesterol levels and blood pressure, and use of nutrients from food. The strains of microorganisms that are used as probiotic and exert beneficial effects should display certain preferable properties such as acid and bile tolerance to be orally administered. Also, the adherence capacity to intestinal mucosa and epithelia, in order to prevent the adhesion and colonization of pathogens. The values of these parameters are still under discussion because differences between action in vivo and in vitro may occur.

Generally, using the lactobacillus is safe. However, some health issues were reported such as bacteremia and abscess in severe ulcerative colitis and immunocompromised patients. Another issue is risk of disseminating antibiotic resistance. Hence, convenience and proper identification of the strains and genetic profiles are required (Castro-González *et al.*, 2019).

**Table 1:** Basonyms (old names) and new names of certain Lactobacillus strains.

Basonyms	New names	Meaning of the genus name	Genbank 16S rRNA gene accession number	Genbank genome accession number	Reference
<i>Lactobacillus casei</i>	<i>Lacticaseibacillus casei</i>	Lactobacilli related to cheese	AF469172	BALS000000000	(Zheng <i>et al.</i> , 2020)
<i>Lactobacillus paracasei subsp. Paracasei</i>	<i>Lacticaseibacillus paracasei subsp. Paracasei</i>	Lactobacilli related to cheese	D79212	AZGH000000000	(Zheng <i>et al.</i> , 2020)
<i>Lactobacillus rhamnosus</i>	<i>Lacticaseibacillus rhamnosus</i>	Lactobacilli related to cheese	<u>D16552</u>	<u>AZCQ000000000</u>	(Zheng <i>et al.</i> , 2020)
<i>Lactobacillus salivarius</i>	<i>Ligilactobacillus salivarius</i>	Uniting (host adapted) lactobacilli	AF089108	AYYT000000000	(Zheng <i>et al.</i> , 2020)
<i>Lactobacillus plantarum</i>	<i>Lactiplantibacillus plantarum</i>	Lactobacilli related to plants	AJ965482	AZEJ000000000	(Zheng <i>et al.</i> , 2020)
<i>Lactobacillus pentosus</i>	<i>Lactiplantibacillus pentosus</i>	Lactobacilli related to plants	D79211	AZCU000000000	(Zheng <i>et al.</i> , 2020)
<i>Lactobacillus fermentum</i>	<i>Limosilactobacillus fermentum</i>	biofilm-forming lactobacilli	JN175331	JQAU000000000	(Zheng <i>et al.</i> , 2020)
<i>Lactobacillus brevis</i>	<i>Levilactobacillus brevis</i>	Dough-leavening lactobacilli	<u>M58810</u>	AZCP000000000	(Zheng <i>et al.</i> , 2020)
<i>Lactobacillus rami</i>	<i>Lentilactobacillus rami</i>	Slow growing lactobacilli	<u>AB366389</u>	<u>AZEI000000000</u>	(Zheng <i>et al.</i> , 2020)
<i>Lactobacillus amylophilus</i>	<i>Amylolactobacillus amylophilus</i>	Starch degrading lactobacilli	<u>M58806</u>	<u>AYYS000000000</u>	(Zheng <i>et al.</i> , 2020)
<i>Lactobacillus acidophilus</i>	Unchanged	Rod-shaped bacillus from milk	AY773947	AZCS000000000	(Zheng <i>et al.</i> , 2020)
<i>Lactobacillus crispatus</i>	Unchanged	Rod-shaped bacillus from milk	<u>AF257097</u>	<u>AZCW000000000</u>	(Zheng <i>et al.</i> , 2020)
<i>Lactobacillus gasseri</i>	Unchanged	Rod-shaped bacillus from milk	<u>AF519171</u>	<u>NC_008530</u>	(Zheng <i>et al.</i> , 2020)
<i>Lactobacillus johnsonii</i>	Unchanged	Rod-shaped bacillus from milk	<u>AJ002515</u>	<u>AZCY000000000</u>	(Zheng <i>et al.</i> , 2020)

## Lactobacilli Activities in Gastrointestinal Tract:

### 1. Adhesion to Intestinal Mucosa:

The intestinal microbiome has a significant role in the combination and homeostasis of the gastrointestinal tract and maintenance of the host energy metabolism (Pflughoeft and Versalovic, 2012). Alteration in the intestinal microbial composition is known as dysbiosis. This may result from the disruption of microbiome-host symbiosis interaction. This alteration leads to change in the functions of GIT because it favours the invasion and growth of pathogenic bacterial species (Frank *et al.*, 2011). Intestinal dysbiosis has been associated with infection in GIT, and is a hallmark of inflammatory bowel disease, including Crohn's disease and ulcerative colitis (Wlodarska, Kostic

and Xavier, 2015). Dysbiosis can trigger disease in any age of life, from first weeks of life, as detected in necrotizing enterocolitis (Neu & Walker, 2011), and during adulthood in upgrade colorectal cancer (Schwabe and Jobin, 2013). One of the desirable traits of probiotic lactobacilli is a good adherence capacity. Generally, adhesion to epithelial cell lines, extracellular matrix molecules, and immobilized intestinal mucus were studied in vitro in short-term assay (Vélez *et al.*, 2007). However, some studies have already investigated the adhesion and colonization of probiotic lactobacilli in vivo in both humans (Garcia-Gonzalez *et al.*, 2018), and animals (Šikić Pogačar *et al.*, 2020). The adherence of lactobacilli in the intestine is a multi-factorial process. There are many factors that mediate the adhesion of

Lactobacilli to the different parts of intestinal mucosa (Vélez *et al.*, 2007). One of the factors of adhesion is mucus binding proteins. Mucus is a protective layer that covers the epithelial cells of the intestine. The main components of mucus are mucin, which is a complex mixture of highly glycosylated proteins, and glycolipids. The amount of mucin is balanced between its production by goblet cells, and its degradation by proteases and physical erosion through transit function in the gut (Deplancke and Gaskins, 2001). Mucus may provide a natural environment for commensal bacteria including 1 lactobacilli (Paone and Cani, 2020). The mucus-binding protein of *Lactobacillus reuteri* 1063 (Roos and Jonsson, 2002), the lectin-like mannose-specific adhesin of *Lactobacillus plantarum* WCFS1 (Pretzer *et al.*, 2005), and the mucus-binding protein of *Lactobacillus acidophilus* NCFM (Buck *et al.*, 2005) all are identified as lactobacilli mucus adhesins.

Sortase-dependent proteins are another mediator for adhesion, that have been detected in lactobacilli. Sortase-dependent proteins are a group of surface proteins in gram-positive bacteria. This molecule contains C-terminal motif LPxTG that cleaves by the sortase enzyme. After the cleavage, the surface protein is connected with the peptidoglycan, then incorporated into the envelope. Finally, it is displayed on the microbial surface (Marraffini, DeDent and Schneewind, 2006). These cell surface proteins contained repeated unit homologous to mucus binding domains (MUD). MUD recognize terminal sialic acid, N-acetylgalactosamine and N-acetylglucosamine residues in mucin chains and result in the adhesion to the intestinal mucus (Muscarello *et al.*, 2020).

The four sortase-dependent proteins have been well identified and functionally characterized of their lactobacillus species, which are *Lactobacillus reuteri* 1063 Mub (Roos and Jonsson, 2002), *Lactobacillus Plantarum* WCFS1 Msa (Pretzer *et al.*, 2005) *Lactobacillus acidophilus* NCFM

Mub (Buck *et al.*, 2005), and *Lactobacillus salivarius* UCC118 LspA (Claesson *et al.*, 2006; Van Pijkeren *et al.*, 2006).

Some of the lactobacilli surface layer proteins work as an adhesin. Surface layer protein encoding genes of many lactobacillus species have been cloned, sequenced and analysed. However, all the biological functions of these proteins aren't confirmed (Hynönen and Palva, 2013). The four identified surface layer proteins, which are able to adhere to intestinal cell, extracellular matrix, and lipoteichoic acid of other bacteria are CbsA of *Lactobacillus crispatus* JCM 5810 (Sillanpaa *et al.*, 2000), surface layer protein of *Lactobacillus helveticus* R0052 (Johnson-henry *et al.*, 2007), surface layer protein of *Lactobacillus brevis* ATCC 8287 (Åvall-Jääskeläinen *et al.*, 2002) and surface layer protein of *Lactobacillus acidophilus* NCFM (Buck *et al.*, 2005). Also, some nonprotein molecules on the surface of lactobacilli have been reported to have a role in the adherence of lactobacilli with GIT, such as LTA. Also, other lactobacilli species display adherence to the GIT mucosa such as *Lactobacillus gasseri* SBT2055SR (Fujiwara *et al.*, 2001), *Lactobacillus reuteri* DSM 12246, *Lactobacillus rhamnosus* 19070–2, *Lactobacillus rhamnosus* LGG (Jacobsen *et al.*, 1999), *Lactobacillus acidophilus* 821–3 (Hütt *et al.*, 2011), *Lactobacillus rhamnosus* 19070–2, and *Lactobacillus reuteri* DSM 12246 (Rosenfeldt *et al.*, 2003).

## 2. Activities in Inflammatory Bowel Disease:

Inflammatory bowel disease (IBD) is a general name for chronic inflammatory conditions of the gastrointestinal tract. The most prevalent forms of IBD are Crohn's disease (CD) and ulcerative colitis (UC) (Seyedian, Nokhostin and Malamir, 2019). These are both chronic and relapsing diseases of intestinal mucosa. Affected site and type of ulcers are different. CD is a discontinuous epithelial cell inflammation with deep ulcer and the whole

gastrointestinal tract can be affected. However, UC is a continuous mucosal inflammation of the colon and rectal area with superficial ulcers. Some studies were performed to evaluate the microbial diversity in IBD patients. Generally, the results show a decreased microbial diversity and stability of intestinal microbiota in IBD patients. So, it is suggested that the composition of intestinal microbiota has an important role in the aetiology and pathogenesis of IBD (Jacoline Gerritsen, *et al.*, 2011). Therapeutic roles of probiotic strains for IBD treatment was outlined from clinical observations because they can restore microbiota-host symbiosis (Scalaferrri *et al.*, 2013).

The possible mechanisms behind the therapeutic effects of probiotic lactobacilli on IBD include preventing the growth of pathogenic microbes that may result from decreased intestinal microflora count in IBD patients. Lactobacilli compete with pathogenic microbes to bind epithelial cells of intestines and other components such as mucin. Therefore, they prevent epithelium attachments and invasions by enteropathogenic bacteria. A study reports the incubation of *Lactobacillus plantarum* 299v with HT-29 intestinal epithelial cells, shows increased expression levels of MUC2 and MUC3 gene and prevented adherence of pathogenic *E. coli* to HT-29 intestinal epithelial cells (Bai and Ouyang, 2006).

Another mechanism is regulating immune response of intestinal mucosa and mucosal immune cells. Normal intestines tolerate enteric microflora, but IBD patients response to luminal antigens include the resident microflora, bacterial products, and food antigens abnormally and cause of inflammation. This inflammation is triggered by excessive release of inflammatory products, such as cytokines, chemokines and active oxides by mucosal immune cells.

Overproduction of proinflammatory cytokines alter the biological function of epithelial cells, for

example, TNF $\alpha$  triggers epithelial cells to secrete IL8, and express membrane TLR4 excessively. TLR4 leads to hyper-reaction and response to lipopolysaccharide (LPS) in bacterial cell wall. IL8 attracts and stimulates other leukocytes. Also, the pathogens enhance secretion of IL8 and TNF $\alpha$ . Probiotic strains interact with intestinal epithelia and decrease synthesis of inflammatory products elicited by diverse proinflammatory stimuli.

The mechanism of this immunosuppressive process involves prevention of the inhibitor nuclear factor  $\kappa$ B pathway by blocking of I $\kappa$ B- $\alpha$  degradations, which inhibit nuclear translocation of active NF- $\kappa$ B dimer and later relevant gene expression. That indicates probiotics can regulate immune response and tolerance of the intestinal mucosa (Bai and Ouyang, 2006). Ayyana *et. al.* report anti-inflammatory activity of *Lactobacillus mucosae* AN1 and *Lactobacillus fermentum* SNR1 in rats. The upregulation of anti-inflammatory cytokine genes and down-regulation pro-inflammatory cytokine genes in probiotic-treated rat tissues were studied by RT-PCR (Ayyanna, Ankaiah and Arul, 2018).

### 3.Diarrhea:

The treatment for diarrhea is supportive treatments that used to control symptoms such as rehydration and recuperating. Probiotic bacteria can be beneficially used to control diarrhea in children, traveller's diarrhea, antibiotics associated diarrhea caused by *clostridium difficile* infections through different mechanisms (Hitzeman and Romo, 2011).

Antibiotic-associated diarrhea caused by *Clostridium difficile*, which is a spore-forming, gram-positive, anaerobe bacteria that can be treated with the use of *L. rhamnosus* L34 and *L. casei* L39, two vancomycin-resistant lactobacilli probiotics that have effectiveness by inhibiting the production of IL-8 through suppressing the activation of phospho-nuclear factor- $\kappa$ B during infection with *Clostridium difficile*

(Boonma *et al.*, 2014). Also, the effects of *L. acidophilus* GP1B on the expression of virulence genes in *C. difficile* were investigated. This study explained the quorum sensing between both species. Quorum sensing (QS) is a mechanism of cell-to-cell communication through the production and secretion of extracellular signalling molecules called autoinducers. When these autoinducers accumulate in the environment and reach the threshold concentration, they change level of genes expression inside the cells. *C. difficile* produces an extracellular signal product known as LuxS or autoinducer-2 (AI-2). AI-2 has been involved in the controlling of virulence in pathogenic bacteria. The cell extract (CE) of *L. acidophilus* GP1B interferes with QS in *C. difficile* by decreasing AI-2 production. CE of *L. acidophilus* GP1B downregulates expression of virulence genes in *C. difficile* at the level of mRNA. Those virulence genes including luxS (autoinducer), tcdA (toxin A), tcdB (toxin B), and txeR (sigma factor). Moreover, the growth of *C. difficile* in the mouse model is inhibited by *Lactobacillus acidophilus* GP1B, as a result of reduction in pH due to production of organic acids produced by the probiotic bacterium (Yun, Oh and Griffiths, 2014).

#### 4. *Helicobacter pylori* Infection:

*Helicobacter pylori* is a microaerophilic, gram-negative pathogenic bacteria that lives in the stomach of human and can cause gastric cancer, chronic gastritis, and peptic ulcer disease (Lehours and Yilmaz, 2007). Antibiotic treatment for *H. pylori* infection has disadvantages, such as increased risk for antibiotic resistance. As a result, probiotic treatment is highly recommended (Chen *et al.*, 2012). In vitro study of probiotics for treatment of *H. pylori* showed that they work either through immunological or nonimmunological modulation (Haller *et al.*, 2000). Immunomodulatory effects of probiotics were studied on animals include decrease of inflammation and gastric activity through

regulation of immune system by control of chemokines and cytokines. A study demonstrates the ability of the probiotic strain *Lactobacillus fermentum* UCO-979C to improve the gastric innate immune response against *H. pylori* infection in mice. The observations reveal that the exopolysaccharide expressed by *L. fermentum* UCO-979C is partially responsible for its immunomodulatory effect, and its anti-inflammatory activity. *Lactobacillus fermentum* UCO-979C modulates the immune response through changing the cytokines such as TNF- $\alpha$ , IL-8, and MCP-1. The levels of these cytokines are significantly reduced, and the level of IL-10 increased in the gastric mucosa of *H. pylori*-infected mice. Several studies have explained the ability of probiotic lactobacilli to regulate the expression of cytokines in gastric epithelial cells by the inhibition of nuclear factor- $\kappa$ B activation. In addition, the ability of *L. fermentum* UCO-979C to change the expression of negative regulators of TLR4 signaling in intestinal epithelial cells were described. *L. fermentum* UCO-979C reduces the expression of MKP-1 and Tollip in intestinal epithelial cells after the activations of TLR4 which lead to down-regulate of the inflammatory factors such as IL-8 and C3. The detailed molecular mechanism of this hypothesis requires more research and investigation to explain the process (Garcia-Castillo *et al.*, 2020). While nonimmunological mechanisms are including secretions of antibacterial substances, competition for inhabitation, and enhancing mucous barriers (Ji and Yang, 2020).

#### Antimicrobial Activities:

Lactobacilli are known to produce a wide range of compounds that exhibit direct antimicrobial activities against viruses and bacteria. These compounds include organic acids, hydrogen peroxide and bacteriocins (Yusuf, 2013).

Acidity is one of the important environmental stressors, that produced as a

result of food fermentation and production of lactic acid by the lactic acid bacteria. The undissociated form of lactic acid crosses the plasma membrane of pathogenic bacteria either through diffusion or carrier on the plasma membrane. The lactic acid dissociates in the cytoplasm by releasing protons into the cytosol. The influx of protons lead to acidification of the cytoplasm, disappearing the membrane potential and reducing the activity of enzymes that sensitive to acidic pH. Consequently, proteins and DNA become damaged, energy supply processes and macromolecule synthesis are impeded (Tachedjian, Hanlon and Ravel, 2018).

Bacteriocins are peptides, produced by bacteria. Bacteriocins have antimicrobial actions and act as local antibiotic against pathogenic bacteria, which is work either as bactericidal or bacteriostatic (Mokoena, 2017). Bacteriocins generally had a narrow spectrum activity, because more active against closely related species. However, a lot of bacteriocins produced by lactobacilli have broad spectrum activity against several groups of bacteria, such as species of anaerobes bacteria (e.g., *Clostridium* species), gram-positive bacteria (e.g., *Staphylococcus spp.*, *Streptococcus spp.*), and gram-negative bacteria (e.g., *Salmonella* species, and *Campylobacter* species) (Mcfarland et al. 2018; Zhou et al. 2020; Kang et al. 2017; Wasfi et al. 2018). The amounts of productions and activities of these peptides affected by some physical and chemical factors, such as acidity, source of nutrients and chemical compositions of the environment. Most bacteriocins act through formation of pores in the membranes of the target cells (Negash and Tsehai, 2020). Bacteriocin is a general term, it is specifically named depend on the genus or species producer, for example, plantaricin produce by *L. plantarum* (Yusuf, 2013).

The antibacterial activity of lactobacilli species was studied, such as the ability of *Lactobacillus plantarum* GK81,

*Lactobacillus acidophilus* GK20 and *Lactobacillus plantarum* JSA22 to inhibit salmonella infection in the epithelial cells of intestines (Eom, Song and Choi, 2015). In addition, the cell-free supernatant of lactobacillus prevents the growth of *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. Also, cell-free supernatant inhibits the formation of biofilms by both species (El-Mokhtar et al., 2020).

About the antiviral activity of lactobacilli, various modes of lactobacilli action against different viruses were reported. It may be direct interaction with the viruses or indirect activity, which resulted in the reduction of viral titre. Direct lactobacillus-virus interaction occurs as trapping or adsorptive mechanism. In a study, this type of mechanism was reported, which showed direct interaction of *Lactobacillus paracasei* A14, *Lactobacillus paracasei* F19, *Lactobacillus paracasei/rhamnosus* Q8, *Lactobacillus plantarum* M1.1, and *Lactobacillus reuteri* DSM12246 with the virus vesicular stomatitis virus (VSV) and trapped it (Botić et al., 2007).

Indirect modes of action such as the immunomodulatory activity of many lactobacillus species include *Lactobacillus plantarum* strain YU, in mouse showed high interleukin 12-inducing activity in the peritoneal macrophages and dendritic cells. The increased production of IL-12 by CD11c+ dendritic cells is due to the activation of toll-like receptors by the probiotic strains. So, this process is TLR2 and TLR4-dependent manner. Also, natural killer cell activity in the spleen is enhanced, because IL-12 increases the NK cells' activity.

Production of IgA from Peyer's patch cells is upregulated, which may be due to improvements of gut microflora and stimulations of antigen-presenting cells (APC) in the gut (Kawashima et al., 2011). Also, the *Lactobacillus rhamnosus* GG (LGG) prevents loss of early influenza virus control through immunomodulatory activity by up-

regulation of type I IFN pathway (Kumova *et al.*, 2019).

### 1. Antitumor Activity:

Probiotic lactic acid bacteria produce exopolysaccharides (EPS) and surface carbohydrate polymers with various biological functions. EPS exhibits anti-proliferative effects on many types of tumour cells such as intestine, liver, and breast. The mechanism of anti-tumour activity includes promoting apoptosis, inducing cell cycle arrest, anti-mutagenic, anti-oxidative, and anti-angiogenesis effects (Wu *et al.*, 2021).

The tumour cells treated with EPS, display morphological changes associated with apoptosis such as vacuolation, cytoplasm condensation, nuclear disintegration, chromatin condensation, swollen mitochondria, and apoptotic bodies around the nucleus. Cysteine aspartyl-specific proteases (caspases) proteins play an important role in the mechanism of programmed cell death. Caspase is activated by two pathways, extrinsic or death receptor pathway and intrinsic or mitochondrial pathway which lead to apoptosis. Initiator caspases are including caspase-2, -8, -9, -10 and caspase-3, -6, -7 are belong to executioner caspases. The mechanism of the extrinsic pathway begins by the combination of cell death signals such as FAS ligand (FASL), tumour necrosis factor (TNF) with death receptors such as FAS and TNF receptors. Then death-inducing signalling complex formed that activates the initial caspase-8, further executioner caspase-3 is activated and causes apoptosis subsequently (Wu *et al.*, 2021). EPS of *L. plantarum* NCU116 (EPS116) increases the expression of pro-apoptotic genes, including FAS and FAS ligand (FASL) in mouse intestinal epithelial cancer cells. This report suggests the suppressive capacity of *Lactobacillus plantarum* NCU116 on the proliferation of CT26 cells may be mediated through toll-like receptor-2 (TLR-2) and the activation of c-Jun dependent FAS/FASL-mediated

apoptotic pathway (Zhou *et al.*, 2017). Lactobacilli species exert antioxidant activity through different mechanisms. For example, *Lactobacillus casei* KCTC 3260 and *Lactobacillus helveticus* CD6 were found to possess a strong antioxidant capacity through the chelation of ions. *Lactobacillus casei* KCTC 3260 is able to chelate Fe<sup>2+</sup> or Cu<sup>2+</sup>, and *Lactobacillus helveticus* CD6 has higher chelation of Fe<sup>2+</sup> ion. The exact chelation mechanism is not well understood, but one of the opinions said this chelation ability of the probiotic strains may be due to the physiological chelators that present in the intracellular cell-free extract of probiotic strains. Also, probiotic bacteria are able to produce metabolites with antioxidant activity such as glutathione (Wang *et al.*, 2017). Zhang *et al.* revealed high doses of EPS (50 mg/kg per day) produced by *L. plantarum* YW11 functionally relieved the oxidative stress in mice and serum level of glutathione peroxidase (GSH-Px) is increased (Zhang *et al.*, 2017).

### Immunomodulatory Activity:

Immune system is a complex system, composes of various cells and chemicals such as cytokines of innate and adaptive immune responses. The interaction between probiotics and immune system is through direct bacterial contact with intestinal epithelial cells, or through internalization by M cells, following initiating immune responses mediated by macrophages, T-lymphocytes and B-lymphocytes. The two major mechanisms behind the immunomodulation in the hosts by probiotics are regulations of the genes expression and signalling pathways (Yan and Polk, 2011, Zhang *et al.* 2019).

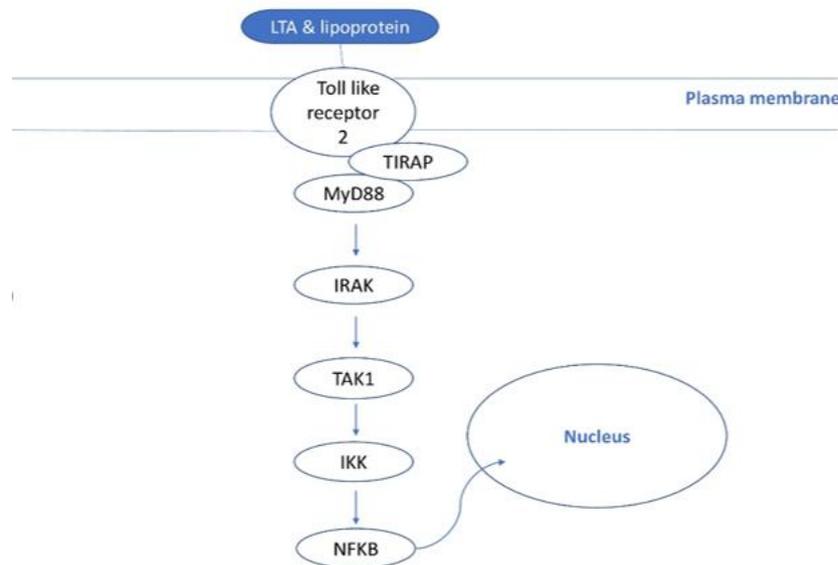
The interactions of lactobacilli with mucosal immune cells or epithelial cells are crucial to modulate specific function of the mucosal immune system. These interactions are mediated by pattern recognition receptors (PRR) such as Toll-like receptors, nucleotide oligomerization domain-like receptors and C-type lectin.

Activations of these PRR signalling pathways have crucial effects on both innate and adaptive immune response such as regulations of antigen-presenting cells, native and regulatory T cells. The studies were observed the ability of various lactobacillus species to activate toll-like receptors. Onishi *et al.* revealed that total RNA and genomic DNA of phagocytosed *L. gasseri* OLL2809 activates TLRs 7 and 9, and upregulates IL-12 productions in J774.1 cells (Onishi *et al.*, 2020). *Lactobacillus acidophilus* enhances intestinal epithelial tight junction barriers and reduces intestinal inflammations through toll-like receptor 2 dependent manner (Al-Sadi *et al.*, 2021). The proteins extracted from *Lactobacillus crispatus* KT-11 promote interleukin 12p40 productions by toll-like receptor 2 in J774.1 cell cultures (Keisuke *et al.*, 2021).

When TLRs signaling activated, TLR in the enterocyte membrane or subcellular compartments binds to the myeloid

differentiation primary response (MyD88) adapter protein to induce signaling. MyD88 recruits tumour necrosis factor receptor-associated factor (TRAF6) and members of the interleukin-1 receptor-associated kinase family (IRAKs), which lead to activations of the transforming growth factor beta-activated kinase 1 (TAK1) complex. Then the inhibitor of nuclear factor- $\kappa$ B kinase (IKK) complex is activated by the activated TAK complex. The activated IKK phosphorylates the inhibitor of nuclear factor- $\kappa$ B (NF- $\kappa$ B) and cause its degradation. Then NF- $\kappa$ B translocates into the nucleus and activates gene expression of cytokines and chemokines.

The activated TAK1 complex simultaneously activates the mitogen-activated protein kinase (MAPK) pathway. Finally resulting in phosphorylation of interferon regulatory factor 3 (IRF3). Then, IRF3 translocates into the nucleus and activates transcription of type 1 interferons, particularly IFN- $\beta$  (Wells, 2011).



**Fig. 1:** Simplified scheme of NF- $\kappa$ B activation via toll-like receptor 2 signalling pathway. This diagram is a modified scheme of Figure 1 published by Wells, 2011.

## Role Of Lactobacilli in The Treatment or Reducing the Effect of Metabolic Disorders:

### 1.Cholesterol-Lowering Activity:

Cholesterol is an important component of body tissue. However, high level of serum cholesterol is a major risk factor for heart diseases (Aloğlu and Öner, 2006). Pharmacological treatments for

lowering cholesterol are available but they have many side effects and costly. The lactobacilli are able to interact with metabolism of bile salt. Lactobacilli reduce cholesterol by deconjugation of bile acids because deconjugated bile acid is less reabsorbed from intestinal lumen due to less solubility. So, it leads to increase excretions of free bile acid in faeces. Hence, cholesterol is a precursor of bile acid, increased requirement of cholesterol to replace bile acid that removed in faeces, resulting in decreased serum cholesterol level (Staley *et al.*, 2018; Khare and Gaur, 2020). Wang *et al.* shows lactobacillus strain overexpression of bile salt hydrolase has a significant role in hypercholesterolemia by decreasing absorption of cholesterol and rising cholesterol catabolism. Also, indicates effective ability of *Lactobacillus plantarum* AR113 and *Lactobacillus casei* pWQH01 to inhibit the cholesterol absorptions and accelerations of cholesterol transportations (Wang *et al.*, 2019).

## 2.Diabetes:

Diabetes is a chronic metabolic disorder, characterized by high blood glucose level, resulted from insufficient insulin secretion by beta cells of pancreas (type 1 diabetes) or resistance to insulin actions (type 2 diabetes) (Alberti and Zimmet, 1998). Previously, elevation of proinflammatory biomarkers and oxidative stress have been observed in diabetic patients (Reis *et al.*, 2010). And inflammatory immune response plays an important role in the progression of diabetes type 1 and 2 (Donath and Shoelson, 2011). Generally, type 2 diabetes is treated with intestinal alpha-glucosidase inhibitors. The inhibitory activity of lactobacilli against intestinal maltase, sucrase, lactase and amylase, which are enzymes involved in hydrolysis of carbohydrates, was evaluated in faeces of healthy breast-fed infants <9 months in age. *Lactobacillus Plantarum*, *Lactobacillus fermentum* and *Lactobacillus acidophilus* were isolated. Then, heat-killed

sonicated extracts (SE) from these bacteria were prepared for investigation of their glucosidase inhibitory potential in the presence of different sugars and rat intestinal acetone powder. The study demonstrate that lactobacillus strains present in the human gut have alpha- and beta-glucosidase inhibitory activities and ability to reduce blood glucose responses in vivo (Panwar *et al.*, 2014). The postponement in the progression of glucose intolerance, ameliorated effect on oxidative stress and beta-cell functions by the action of *Lactobacillus paracasei* NL41 have been detected in rats (Zeng *et al.*, 2019).

## Renal Disease:

The end stage of renal disease is characterized by very low glomerular filtration rate (<15 ml/ min/1.73 m<sup>2</sup>), which causes increased plasma concentration of uremic waste such as creatinine, urea and uric acid (Di Cerbo *et al.*, 2013). The accumulation of these wastes leads to inhibit several physiological and biochemical functions in the body. Such as decreased serum level of calcium and activated vitamin D with the increased phosphorus and parathyroid hormone (Kestenbaum and Belozeroff, 2007). Chronic renal failure has been also characterized by overgrowth of small intestinal bacteria, which is a pathological condition described as a competition between bacteria and the intestinal lumen for nutrients introduced in the body through feeding (Rana and Bhardwaj, 2008; Strid *et al.*, 2003). In this condition, bacteria may also use of intraluminal proteins in the host small intestine, which may cause to deficiency of protein and overproduction of ammonia by bacteria (Bures *et al.*, 2010). Several studies support using probiotics as an alternative method of therapy and treatment of end stage renal disease (Ranganathan *et al.*, 2010). Several clinical studies have been performed to assess the ability of natural and genetically modified bacteria on uremic pathology, which often develops in patients with end-stage renal disease (Mangione and Dal Canton, 2011).

Some strains of lactobacilli are oxalate degrading microorganism such as *Lactobacillus acidophilus* and *Lactobacillus gasseri* (Chamberlain *et al.*, 2019). An experimental study was performed on stone-forming rats. *Lactobacillus casei* HY2743 and *Lactobacillus casei* HY7201 were used for the treatment. As the result, treated group shows decreased urine oxalate excretion and less abundant crystals in kidneys in compare to control group, because both strains are able to degrade oxalate (Kwak *et al.*, 2006). *Lactobacillus paracasei* and *Lactobacillus plantarum* together effectively improve renal functions by decreasing kidney injury, oxidative stress and proinflammatory reactions in the kidneys (Hsiaowen Huang, *et al.*, 2021).

#### **Vaginal Colonization:**

The female lower genital tract is a dynamic ecosystem, where more than 200 bacterial species, aerobes and anaerobes coexist together. This microbiome is influenced by genes, ethnic background, environmental and behavioural factors. *Lactobacillus* species is a dominant part of vaginal microbial compositions that play a crucial role vaginal health and wellness (Auriemma *et al.*, 2021). Bacterial vaginosis is switching of vaginal microbiota from a lactobacillus-dominated to a more diverse microbiota, with the presence of clinical symptoms. Bacterial species of genera Gardnerella, Atopobium, Prevotella, Fusobacterium, and Dialister are particularly associated with bacterial vaginosis (Onderdonk, Delaney and Fichorova, 2016). Lactobacilli protect the vagina through several mechanisms such as preventing colonization of pathogenic bacteria through direct killing of pathogens by secreting of compounds such as lactic acid, hydrogen peroxide and bacteriocins. Lactic acid acidifies the vagina pH 3.0-4.5, and other bacteria unable to growth in this acidic environment. The promotion of autophagy is another characteristic of lactic acid. Lactic acid upregulates autophagy by inhibiting the productions of cyclic

adenosine monophosphate (cAMP) in the cells. Defective and old organelles such as mitochondria, defective proteins and intracellular bacteria, viruses and protozoa separated by enclosing in a double membrane structure called an autophagosome. The autophagosome combines with a lysosome and the enclosed products are broken down by lysosomal enzymes. Thus, lactic acid promotes the clearance of vaginal epithelial cells from intracellular pathogens and prevent the cells from the consequences of oxidative stress (Zhou *et al.*, 2015). Also, lactic acid enhances survival of vaginal epithelial cells through modulations of repairing damaged DNA by preventions of histone deacetylase activity that provoke acetylation of histones on the surface of DNA (Wagner *et al.*, 2015)

Vaginal yeast infection is another common type of vaginitis. Commonly occurred due to the colonization of fungus *Candida albicans* in the vagina (Marrazzo, 2002). Lactobacilli protect the vagina against *Candida* infections. The mechanisms of this protection are through the adherence of lactobacilli to the vaginal mucosa and compete for adhesion sites. In vitro model revealed the protective ability of *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14, by using VK2 E6/E7 vaginal epithelial cells (Wagner, 2012, Superti, 2020).

#### **Possibility of Reducing the Spread of Antibiotic Resistance by Other Pathogens:**

The probiotic lactobacilli may reduce the dissemination of antibiotic resistance genes by other pathogens. One of the possible mechanisms by which probiotics achieve this effect is by stabilization of the GIT microbiota. Because overuse of antibiotics is a significant cause to change normal GIT microbiota and increases the number of pathogenic bacteria, which harbour more antibiotic resistant genes (Elvers *et al.*, 2020). Another mechanism is improving the

functions of antibiotics through some ways such as disruption of biofilms, decreasing the pathogenic bacterial populations at mucosal sites, and enhancing mucosal immunity. These factors help antibiotics to kill or stop the growth of microbes earlier before they become resistant. Probiotic lactobacilli reduce the risk of some infectious disease. Hence, reduction in need for antibiotics and selective pressure (is the factors that change the environmental conditions. In order to survive in this environment, the microorganisms acquired new characteristics) that result in decreasing mutations in the microbes. Because overuse of antibiotics is a major cause of disseminating antibiotic resistance genes especially in heavy antibiotic consumers, which harbour more antibiotic resistance genes (Alaa *et al.*, 2020). Some species of lactobacilli harbour resistance genes but they are more given 'generally regarded as safe (GRAS) status in compare to other species of lactic acid bacteria (Ouweland *et al.*, 2016). A study investigates the antibiotic susceptibility profile and acquired resistance genes in lactic acid bacteria isolated from human vagina. In the PCR results, no acquired genes were identified in the genome of any of the lactobacillus species (Sirichoat *et al.*, 2020). More studies and investigations are required to find out that probiotic lactobacilli can directly reduce the dissemination of antibiotic resistance.

#### CONCLUSION:

Lactobacilli are extremely studied because of their ability to promote health benefits to the hosts including humans. The beneficial effects of certain species of lactobacilli such as *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *Lactobacillus reuteri* and some other species have been observed in clinical studies. Major health benefitting mechanisms of lactobacilli include enhancements of intestinal epithelial barrier functions, alterations of gut microbiota, prevention of growth of pathogenic microbes in the intestines and vaginal

mucosa, production of antimicrobial substances such as bacteriocin, immunomodulatory effects and anti-proliferative effects. Due to these beneficial effects of lactobacilli, they have been essential in biotherapeutic and prophylactic for preventions and treatment of diseases. Bacteremia and abscess in severe ulcerative colitis and immunocompromised patient were reported caused by lactobacilli. So proper identification of probiotic strains, genotypic and phenotypic characteristics are demanded.

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