Review on the Commercial and Therapeutic Implementation of Lactobacillus Species

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Authors’ contributions

This work was carried out in collaboration among all authors. Author PC conceived of the presented idea followed and guided by authors UP, SB and PC developed the theory and authors SB and UP performed the framework of the article. The verification of the methods and strategies of the article is done by authors PC, SB and UP drafted the entire manuscript and edited the gathered contributions. All authors read and approved the final manuscript.

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ABSTRACT

Lactic acid (LA) is one of the primary metabolites that is considered to be a natural product derived from microorganisms such as Lactic Acid Bacteria (LAB). The most prominent LA-producing LAB is the genus of Lactobacillus spp. and is found in almost all dairy products along with non-dairy food products. The application of LA can be found in various sectors including food, fermentation, pharmaceuticals, and chemical industries. LAB have lots of perspective in the production of organic acids as well as complex compounds that find potential application in food products such as yoghurt, cheese, buttermilk in addition to therapeutics uses such as probiotics that help in preventing gastrointestinal infections; improve pathogen protection; and alter host immunological responses. Moreover, fermentation with LAB also facilitates efficient nutrient quality and provides an effective method of food preservation. The present study provides an overview of the potential application of LA produced by Lactobacillus spp. in different sectors.

Keywords: Lactic acid; LAB; probiotics; LAB fermentation; LA pharmaceuticals; LAB dairy products; primary metabolites.
1. INTRODUCTION

Lactic acid (LA) is a significant compound considering the fact that it is applicable in various industries including food, pharmaceutical, and chemical industries. It also acts as flavouring agent, acidulants and to an extent displays antibacterial activity [1]. LA is predominantly produced by Lactic Acid Bacteria (LAB). The LAB are a broad group of microorganisms found in plants, meat, and dairy products. They are further classified into eight genera including Lactobacillus, Leuconostoc, Carnobacterium, Bifidobacterium, Streptococcus, Lactococcus, Enterococcus, and Sporolactobacillus [2].

Conventionally, LAB have been exploited for the production of several natural products ranging from simple organic acids, esters, and biological solvents to complex compounds that find potential application in food along with biodegradable polymers like Poly Lactic Acid (PLA) which are attributed to the presence of hydroxyl and carboxyl groups in its structure [3] [4]. The majority of LAB species are food-grade organisms that are given Generally Recognized As Safe (GRAS) status by the US Food and Drug Administration (FDA). Besides, they are capable of synthesizing potential probiotics, prebiotics, LA, etc.

LA possesses antimicrobial activity due to its bioactive nature (protonated form) at low pH and has a synergistic effect. Microbial fermentation of LA has become the most preferred method for enhancing cereal quality, texture, flavour, pH regulation, and safety due to its correlation with physicochemical properties [5]. LA is often considered as ‘Milk Acid’ even though it is also derived from the fermentation of non-dairy products including beer, fruits, vegetables, and sourdough. LAB have been utilized as starter cultures for the fermentation process including dairy products such as milk, curd, cheese, buttermilk, and non-dairy products such as alcoholic beverages, sourdough, pickles, and sauerkraut. Apart from several advantages, LA is also known to have some disadvantages such as serving as an etiological agent for diseases like endocarditis, bacteremia, and septicemia, and may act as a contaminant producing off-flavours [6].

Fermentation with LAB seems to be a cost-effective and efficient method of preserving food as it enhances the nutritional quality of the products. The strain selection is a crucial step, especially in terms of LA purity and manufacturing capacity. Depending upon the fermentative end-products, the LAB are classified as homofermenters or heterofermenters. Homofermentative LAB (Streptococcus, Lactococcus, Lactobacillus, and Pediococcus) yield LA from glucose fermentation whereas, Heterofermentative LAB (Leuconostoc and Betabacteria) are utilized as flavour-enhancing agents especially in dairy industries. The type as well as the quantity of LA produced during fermentation, are important in food production and play a significant role in the taxonomic classification of LAB. Distinguished by their fermentative capability, LAB enhance nutrients, improve organoleptic properties, improve food safety, and offer health advantages [7]. Lactobacillus spp. have been found to flourish in various fermentation processes especially in winemaking conditions and manifest several beneficial biological characteristics, thus making them better starter cultures for Malolactic fermentation (MLF) [8]. The starter culture of LA, substrates, and operational modes all enhance the performance and efficacy of LA fermentation process [9]. Probiotics can be defined as live microorganisms that are beneficial to human health [10].

Antimicrobial metabolites produced by diverse strains of LAB from various sources are an active research area in order to optimize antimicrobials and their use in bacteriostatic and bactericidal applications. Studies have found that LAB imposes beneficial effects on skin health, including skin condition improvement and disease prevention [11]. It has been observed that taking L. delbrueckii and other LAB orally can help prevent the formation of atopic diseases [12]. Many beneficial health effects have been demonstrated by L. helveticus either through direct or indirect methods including pathogenic inhibition, modification of gut microbiota, modulation of the host immunity, generation of bioactive compounds from food products, or improvement of the food quality [13]. This study highlights the potential use of LA produced by the LAB in the food, fermentation, and therapeutic domain.

2. IMPLEMENTATION OF LAB IN FOOD SECTOR

Notable applications of LA in the food sector include confectionery, beverages, fermented meat, dairy products, salads, dressings, and
ready-to-eat foods. According to Codex Alimentarius Commission (1995), LA is considered as an acidity regulator for certain types of foods such as creams, whey protein cheese, frozen vegetables, nuts and seeds, salt substitutes, and infant foods and therefore added as food additives. Use as additives are important for food preservation because of its antimicrobial activity along with regulating the microflora in the food microenvironment thus enhancing the shelf-life of the food products [14]. LA can also be employed in low amounts in the preparation of other foods including jellies and fruit syrups [14]. Based on vegetables, LA can be utilized in both raw and freshly picked-up vegetables as well as frozen vegetables, seaweeds, nuts, and seeds.

2.1 Application of Lactobacillus spp. in Dairy Products

The rapid growth of technology and awareness have made people know about food and food-related health. They choose to eat foods that could help them avoid various health problems. Therefore, the use of probiotics in food is widely accepted to boost their potential and health benefits. Lactobacillus spp. is considered to be one of the microorganisms categorized under LAB and are present in various types of environments ranging from food (dairy and non-dairy products) to the human Gastrointestinal (GI) tract. These are gram-positive, rod-shaped, non-spore forming bacteria that generate LA as a by-product of their fermentative metabolism. Microorganisms such as L. acidophilus are known to possess such properties including maintaining cholesterol levels, reducing the chances of carcinogenicity, and reducing lactose intolerance, diarrhoea, and constipation. Probiotics are living bacteria cultures that are available in dehydrated or liquid form and are utilized in a range of dietary supplements. Freeze-dried powders, granules, capsules, and suppositories are all common forms of probiotics that are available commercially. Currently, the focus of microencapsulation research has been on probiotic sustainability in food products, with little testing on probiotic viability after distribution into the body. In this particular method, microcapsules are used to isolate the probiotic from the adverse surrounding environment while also allowing it to proliferate [15].

Application of L. acidophilus in fermentation has been widely exploited especially in the preparation of yoghurt or curds, sweet acidophilus milk (prepared by adding L. acidophilus in milk yielding low lactose-containing buttermilk). Basically, when LA along with rennin is added to milk, it causes coagulation and precipitation of a particular milk protein known as Casein, which is segregated with appropriate methodologies and is then used to make curds or yoghurts. Curds are a good source of vitamins especially Vitamin C, minerals, carbohydrates particularly lactose, fats, and other milk proteins such as Whey proteins. Sweet acidophilus milk is prescribed for people suffering from lactose malabsorption or lactose intolerance. Other dairy products where this microorganism is utilized include tempeh and miso. Lactobacillus spp. is the most common microorganism used in dairy products. One of the strains of L. acidophilus has been widely used as probiotics in traditional food and nutritional supplements because of its safety, activity in humans, and industrial modification, and therefore has been marketed as fermented dietary products in the US mid-1970s. Different countries incorporate fermented milk products in their routine nutritious diets. Implementing L. acidophilus in goat milk had increased LA content along with hydrogen peroxide and other by-products making the surroundings unfavourable for unwanted microorganisms, thus help maintain the equilibrium of intestinal microbial flora, beneficial against antibiotic-induced diarrhoea, vaginal and urinary tract diseases. Moreover, acidophilus-containing goat milk aids in the synthesis of niacin, folic acid, and Vitamin B6 in the body. Studies on the potential application of L. acidophilus on immunity-boosting, decrease in vaginal and urinary tract infections, and reduction in cholesterol levels find extensive future research areas that need to be explored [16]. Some of the fermented milk-product include yoghurt (L. bulgaricus and S. thermophilus) and Kefir (L. caucasicus) [17]. Whole, low-fat, or skimmed milk can be used to make yoghurt. LA enhances the consistency of yoghurt, providing its unique texture and sour flavour. Kefir has a mild alcoholic flavour and a thin yoghurt-like texture.

Apart from its use in the above-mentioned dairy products, L. acidophilus along with other LAB such as L. casei, L. paracasei, Bifidobacterium also finds its application in various types of cheese production like cheddar cheese [18], minas fresh cheese [19], and probiotic white cheese [20]. Curd is the main ingredient of a variety of cheeses [21]. During the ‘Ripening'
stage, excess lactose that remains after casein coagulation is fermented by the activity of the microorganisms. Ripening causes the cheese to get flavour and texture. Some cheese such as cottage, cream cheese, and ricotta cheese can be produced without the need for the ripening stage.

2.2 Application of Lactobacillus spp. in Non-Dairy Products

Vegetarian often do not prefer dairy products so the utilization of non-dairy probiotics food products become important. Generally, the dairy sector accounts for about 33% of the global share of probiotics usage in food products where 78% of probiotics are distributed in the form of yoghurt [22]. Manipulating the structural characteristics of food components, probiotics can be administered into fruit juices and cereals [23]. Non-dairy products are generally seen as fermented products from rice, fruits, soybean, and vegetables like potatoes. Lactobacillus spp. are frequently employed in fruit-based and vegetable-based products such as carrot, beet, celery, garlic, spices, onions, and peas [16]. Non-dairy products are generally seen as fermented products from rice, fruits, soybean, and vegetables like potatoes. The introduction of LAB starter culture to the malolactic fermentation aims to convert the naturally occurring malic acid into LA, resulting in lower wine acidity and more desirable flavours [24]. Administration of probiotics in food for their growth, stability, and development inside the food environment as well as maintain a synergistic relationship with other strains requires strain selection and/or improvement techniques [25]. LA has been used as an acidifying agent by introducing it directly to vegetables and fruits to lower the pH and thus, inhibit the growth and proliferation of microorganisms. Apart from LA, LAB can also be used as pH regulators when added to vegetables. Fermentation of the vegetables occurs when a suitable concentration of salts is externally added (so that equilibrium is attained) and LAB is used as inoculums. Some examples of food products that are obtained from the fermentation of vegetables include Pickles (Lactobacillus sp.) and Sauerkraut (Leuconostoc mesenteroides, L. cucumeris, and L. pentaceticus) [26].

The manufacturing of sourdough requires LA as an acidulant. L. sanfrancisco along with non-baker’s Yeast (Saccharomyces exiguus) are used in the preparation process [21]. Rye bread is predominantly composed of sourdough with a high LA content and heterofermentative microorganisms, whereas mixed wheat bread requires a sourdough with greater acetic acid content. LA acts as a preservative in meat. Bacterial fermentation is permitted during the production of fermented sausages when an appropriate starter culture is used. When the pH is adjusted to 5, LA is formed that results in preservation as well as impart secondary characteristics such as the unique flavour of fermented sausages [26].

3. ADAPTATION IN FERMENTATION SECTOR

Malolactic fermentation (MLF) is a type of secondary fermentation in wine that occurs during or after the alcoholic fermentation process. It is carried out by one or more LAB. MLF can be carried out by Lactobacillus plantarum (especially under high pH conditions and in co-inoculation with yeasts), and several strains are commercially available as MLF starting cultures [27]. In this study, it has been observed that L. plantarum strains can be employed to acidify grape must before it becomes alcoholic. Saccharomyces cerevisiae and L. plantarum co-inoculation in grape must increases bacterial tolerance to wine’s harsh environment and reduces total fermentation time. The study also suggested that secondary metabolic reactions important for wine aroma and flavour during the fermentation process can be performed including various metabolisms such as citrate metabolism, amino acid metabolism, polysaccharide metabolism, etc. Another study governed by Palombo et al. (2015) revealed that L. plantarum was able to degrade nondigestible alpha-galactoside sugars and demonstrated beneficial technological qualities including hydrolase activities [28]. Culture-dependent and independent methods were obtained to evaluate the use of L. plantarum collected from spontaneous caper berry fermentation as a starter culture to improve the microbiological quality of the process and produce a consistent homogeneous product with desirable and controllable qualities, and thus preventing spoilage [28].

Another study demonstrated that Amylolytic lactic acid bacteria (ALAB) fermentation can improve the nutritional quality of a rice-based foodstuff by increasing the availability of lysine
and improving the digestibility of starch in young children [29]. The use of ALAB to ferment the amylaceous matrix provides a technological advantage by removing the need for exogenous amylases for starch liquefaction. Amylolysis activity has also been discovered in several Lactobacillus and Bifidobacterium probiotic strains. L. helveticus is a probiotic which has a significant anti-hypertensive and angiotensin-converting enzyme inhibitory (ACEI) activity. The study showed that it can be employed as a starter culture in the production of semi-hard cheeses and fermented milk products [30]. As fermentation starters or food supplements, several probiotic L. helveticus strains are employed. These bacteria produce unique bioactive peptides during milk fermentation because they have significant extracellular proteinase activity. The addition of L. helveticus to fermented milk increased acidity and proteolysis. The treatment raised the ACEI activity of the fermented milk substantially. It has been reported that the addition of L. helveticus in milk fermentation resulted in a shorter fermentation period and a higher concentration of volatile flavour compounds, particularly benzaldehyde and acetoin, was fermented simply by commercial starter bacteria [30].

LAB such as L. sakei and their derived antibacterial substances have long been employed as significant compounds of hurdle technology to improve food safety, shelf life, taste development, control of viable microorganisms, and preservation of fermented sausages [31]. The species L. sakei undoubtedly possesses many fitness traits that ensure its optimal growth and the capacity to survive under the conditions encountered during meat storage and fermentation [32]. L. casei has been employed in the production of numerous fermented food products, such as acid-generating starter cultures (for milk fermentation) and ‘adjunct cultures’ to enhance flavour in a variety of cheeses because of its ability to influence sourdough acidification, gluten protein degradation, amino acid and aroma compound production, thus improving sensorial and nutritional properties of bakery foods [33]. Coelho et al., (2018) conducted a study to show the application of the probiotic L. paracasei and the prebiotic lactulose in dry-fermented sausages and its effect on technological, microbiological, and sensory qualities of the final product [34]. Furthermore, this study also reported a possibility of symbiotic activity between prebiotic and probiotic while producing dry-fermented sausages.

4. THERAPEUTIC EFFICACY OF LAB

Lactobacillus helveticus is a widely used type thermophilic starter in the fermentation of milk for the production of a variety of cheeses. A substantial amount of scientific data suggests that strains of the L. helveticus species have health-promoting characteristics (as shown in Table 1), in addition to their technical significance. Multiple in vitro investigations revealed that L. helveticus has a number of typical probiotic characteristics, including the capacity to survive gastrointestinal transit, attach to epithelial cells, and prevent disease. In vivo investigations in mice revealed that L. helveticus can prevent gastrointestinal infections, improve pathogen protection, alter host immunological responses, and change the makeup of the intestinal microbiota. L. helveticus has also been shown to have a number of health-promoting characteristics in interventional investigations and clinical trials. In 2002, the infectious disorder was the second biggest cause of mortality, behind cardiovascular disorders, accounting for around 26% of all deaths globally. Antimicrobial resistance acquired by infections and the consequent spread of resistant bacteria is a serious public health problem. Antibiotic resistance is mostly caused by the inappropriate use of antibiotics, which are frequently administered needlessly. Lactobacillus helveticus has also been shown to work in tandem with other bacteria strains to combat infections. For instance, L. helveticus strain was combined with L. rhamnosus to treat mice infected with Citrobacter rodentium, a murine pathogen that resembles enterohemorrhagic E. coli infections in people (EHEC) [35]. The scientists found a decrease in weight loss, colonic epithelial cell hyperplasia, and mucosal barrier dysfunctions when mice were pre-treated with a combination of the two lactobacilli and subsequently infected with C. rodentium, resulting in enhanced newborn mouse survival. Growing evidence supports the concept that one of the primary methods by which beneficial microorganisms might positively impact the host’s health is through their capacity to interact with the host’s immune system, triggering reactions at both the local and systemic levels. So many strains of L. helveticus can have a favourable impact on the host’s physiology as well as prevent or treat pathologic diseases. In vitro studies have
suggested a number of modes of action, including direct pathogen suppression, macrophage activation, and host gene expression regulation. In addition, in vivo clinical and interventional investigations have shown *L. helveticus* probiotic effectiveness [13]. The probiotic characteristics of strains belonging to the *L. helveticus* species are comparable to those of bacteria traditionally regarded to be probiotics, such as *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, and *Bifidobacterium animalis* subsp. *lactis*. Additionally, characteristics specific to *L. helveticus* strains, such as the synthesis of ACE inhibitory peptides or cinnamoyl esterase activity, promote the use of this bacteria above other probiotic species. Due to its capacity to tolerate different environmental stressors such as high temperatures, *L. helveticus* may adapt to industrial fermentation settings more easily than other intestinal probiotic *Lactobacilli* and *Bifidobacteria*. Due to its ability to survive various environmental stresses such as high temperatures or low pH, osmotic pressure, and oxygen, *L. helveticus* can adapt to industrial fermentation conditions more easily than most intestinal probiotic lactobacilli and *Bifidobacteria*, making it easier to incorporate into new probiotic formulas.

5. INDUSTRIAL GROWTH INVOLVING HEALTH MANAGEMENT

Probiotics are active microorganisms and, when given in sufficient numbers, provide health advantages. Probiotic-containing products have long been researched and praised for their beneficial effects on GI health. However, their positive effect, which is backed up by a growing body of clinical data, extends beyond the gastrointestinal system [41]. Probiotic microorganisms have been claimed to have anti-cariogenic action and an anti-malodor impact via inhibiting streptococci. In the literature, probiotics have been used to treat gingivitis and periodontal disease [42], as well as to reduce the risk of candidal mucosal infections in patients with denture-associated stomatitis. One of the strains of *L. casei* is known as Shirota (LcS), found in the probiotic-fermented milk drink Yakult, has a 75-year track record of safe consumption and proven health benefits, as

| Strain number | Therapeutic part | Functionality | Experimental method | References |
|---------------|------------------|---------------|---------------------|------------|
| R0052         | *Lactobacillus rhamnosus* in combination with bacterial cells | Improvement of mucosal barrier function and hyperplasia of colonic epithelial cells in mice infected with *Citrobacter rodentium* | *In vivo* | [35] |
| M92           | S-layer protein, the isolated part | Bacterial auto-aggregation and co-aggregation with *Salmonella Typhimurium* are facilitated. | *In vivo study in mice* | [36] |
| HY7801        | Bacterial cells | *Candida albicans*-induced vaginitis is inhibited, and myeloperoxidase activity in vaginal tissue is reduced. | *In vivo study in mice* | [37] |
| M92           | Bacterial cells | Total enterobacterial and *Salmonella* spp. faecal counts have decreased. | *In vivo study in mice* | [36] |
| Bar13         | Bacterial cells | In faecal samples, an increase in butyrate and a reduction in pyridine. | *In vitro study in human* | [38] |
| MIMLh5        | Hydrolyzates of Casein | In human epithelial Caco-2 cells, NF-κB activation is reduced. | *In vitro* | [39] |
| R0052         | Bacterial cells in a *B. longum* probiotic formulation | Post-myocardial ischemia anxiety and depression in mice are reduced. | *In vivo* | [40] |
evidenced by extensive scientific research focusing on its reduction of functional and infectious gut diseases, as well as its immune-modulating effect. Oral probiotics’ mechanisms of action vary by species and might be complex, but the major ideas are mechanical pathogen displacement and the synthesis of organic acids and antibacterial chemicals. Yakult’s use for a positive impact on the oral cavity has gotten very little consideration. LcS has been shown to have inhibitory effects against peri pathogenic and malodorous bacteria like Porphyromonas gingivalis and Fusobacterium nucleatum in vitro experiments [43]. Lactobacillus spp., and other probiotic bacteria have been characterized as attempting to improve a healthy digestive tract and modulating immunity. Some probiotic species may have a positive impact on dental health, according to the research. LcS is a well-researched probiotic strain that has been linked to a number of health benefits [44]. Future research might emphasize patients at higher risk of oral infection, where an inadequately identified flora, such as an increased presence of periopathogens or clinically confirmed halitosis, could be greatly influenced by probiotic intake.

In LA bacteria, antioxidant enzymes including SOD, NADH-oxidase, and NADH-peroxide, as well as heterologous non-haem catalase, are key enzymatic defense mechanisms against oxidative stress (LAB). Some LAB strains’ antioxidant activity might be linked to their synthesis of cell-surface chemicals, such as Lactococcus lactis subsp. extracellular polysaccharides [45]. Lactobacillus is a diverse LAB genus with major significance in food and feeds fermentation. Lactobacilli are now employed as probiotics, silage inoculants, and fermented food starters. However, lactobacilli have a wide variety of uses in food biotechnology, and a number of significant strains must be found and described. In this regard, access to and interchange biological material inside and across strain collections will be critical for broadening the spectrum of lactobacilli biotechnological uses.

6. CONCLUSION AND FUTURE PERSPECTIVE

In the food industry, LAB has a broad array of applications. It has been obtained from a range of food products, including meat, milk, fruits, vegetables, and cereal commodities, and has also been used as a starter culture in various fermentation processes. Furthermore, the probiotic activities of a few LAB strains were extensively studied and have been found to show antibacterial activity. The use of inoculants containing different strains of Lactobacillus alone or in combination with other strains and applied to farm silos to create successful products in increasing the aerobic stability of silage has been found as a result of the study. These inoculants aid in the transition of silage fermentation to a more heterolactic type, reducing the quantity of yeasts and therefore extending the duration that the silage remains stable after exposure to air. Further research is needed, however, to determine the effects of good on-farm management practices and silage inoculation with Lactobacillus alone or in combination with homolactic acid bacteria on improving the aerobic stability of bunker silages during consumption. Lactobacilli may function as a factory of the cell for the de novo production of bioactivities from a variety of dietary protein sources, contributing to their metabolic activity. Immunomodulating, antimicrobial, antihypertensive, and opioid-like characteristics are among the biological actions linked with such peptides. Milk proteins are known to be the main source of bioactive peptides, which can be encoded within the amino acid sequence of dairy proteins and must be released and activated by proteolysis. These findings have sparked interest in producing value-added fermented foods that are specifically enhanced with chemicals that have been shown to provide consumers with certain health benefits. Thus, it has been clearly observed that different strains of Lactobacillus have various functions in industrial as well as therapeutic purposes. Several studies and further researches are being done to find better and improved strains of lactobacillus which can give an enhanced amount of lactic acid.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.
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