

Review Article

Functional Potential of Different Strains of Probiotic Bacteria in Probiotic Beverages: A Focus on Plant-Based Substrates

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ABSTRACT

The growth of the global awareness on health and wellness has spurred increased interest in functional foods, particularly on probiotic beverages. Among the various probiotic strains, *Lactobacillus plantarum* stands out due to its adaptability, survivability under diverse conditions, and production of health-promoting metabolites such as lactic acid, bacteriocins, and exopolysaccharides. Recent advancements have shifted focus towards non-dairy, plant-based alternatives like soy milk, which aligns with consumer preferences for vegan and lactose-free options while providing nutritional and prebiotic advantages. Studies on the viability and stability of probiotics in various food matrices have demonstrated effective survival strategies, including prebiotic supplementation. Moreover, kinetic modeling and optimization techniques, such as response surface methodology (RSM) and one-factor-at-a-time (OFAT), are crucial in maximizing biomass yield and metabolite production under controlled fermentation conditions. This paper highlights the significance of optimizing fermentation environments to enhance the growth, viability, and functional potential of probiotic strains, with a focus on their applicability in the food, nutraceutical, and biotechnology industries.

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1. Introduction

Previous studies have observed a discernible increase in consumers' interest in their health and well-being. This tendency has been fueled by several causes, including increasing awareness of the importance of nutrition, increased worry about chronic diseases, and a desire for a higher standard of living. As a result, the production and consumption of functional foods—such as probiotic beverages—have increased. Due to

its potential health advantages, which include improved digestion, immune system function, and overall gut health, drinks containing live beneficial bacteria, or probiotics, are becoming increasingly well-liked (Zommiti *et al.*, 2020). Consumers are increasingly searching for items that offer these practical benefits in addition to basic sustenance. Furthermore, due to advancements in food science and technology, manufacturers can now more readily produce probiotic beverages with improved flavour, texture, and shelf stability, thereby boosting their availability and appealing to a larger market (Gupta *et al.*, 2023). The food and beverage industries are focusing their research on probiotic drinks with appealing consumer attributes, health benefits, and good nutrition because consumers strongly prefer functional foods with nutrients and bioactives that can lower the risk of cancer, cardiovascular, and cerebrovascular diseases, as well as other chronic illnesses.

Drinks containing probiotics have increased in popularity because of their probable advantages for gut health and general well-being. It is assumed that the live bacteria in these drinks settle in the gut, where they interact with the pre-existing microbiota and give various positive benefits. One of the main benefits of probiotic beverages is the preservation and restoration of a balanced and healthy gut flora population. The human gut is home to billions of bacteria known as the gut microbiota, which are crucial for proper digestion, immune system function, nutritional absorption, and even mental health. By replenishing and enhancing the diversity of the gut microbiota, probiotic beverages contribute to a healthy microbial ecology. Probiotic drinks contain live beneficial bacteria, usually *Bifidobacterium*, *Lactobacillus*, or other probiotic strains (Koirala & Anal, 2021). Additionally, probiotic drinks have been associated with several distinct health benefits, including improved immune system function, enhanced nutritional absorption and digestion, relief from gastrointestinal disorders such as irritable bowel syndrome (IBS), and even support for mental health through the gut-brain axis (Reynoso-Garcia *et al.*, 2022). According to some studies, probiotic drinks may benefit skin, weight management, and metabolic health in addition to gastrointestinal health. For instance, according to research done by Dhama *et al.*, (2016), where several strains and diseases have been compiled, it is found that diverse types of probiotic strains can cure inflammatory diseases such as atopic eczema and dermatitis.

The production of probiotic drinks in recent times focuses on the demands of consumers, including the alternative for lactose-free products that still provide the same function as dairy milk-based probiotic drinks. The production of probiotic beverages with soy milk instead of dairy milk is a noteworthy development in functional foods that corresponds with current dietary and health trends. This strategy supports sustainability and innovation in the food and beverage industry in addition to meeting the demands of vegan and lactose-intolerant consumers. Although soy milk may not display the same benefits as dairy milk does, considering the nutritional content, some potentials of soy milk shall not be overlooked. Proteins, vitamins (particularly B vitamins), minerals, and isoflavones—which may have anti-inflammatory and antioxidant effects on the body—are abundant in soy milk. With the exception of methionine, soybeans contain nearly all of the essential amino acids. Studies have shown that certain soybean peptides have antibacterial, immunomodulatory, antioxidant, and insulin-modulating properties. As phytochemicals, soy isoflavones have been related to health effects, which differ based on how bioavailable they are in the host organism due to their molecular makeup (Cai *et al.*, 2021). It has been shown that pectinases, which are produced and secreted during microbial fermentation, raise the protein content of milk made from grain. Oligosaccharides are present in soy milk and function as prebiotics, promoting the development and viability of probiotics. These prebiotics may aid the growth of beneficial bacteria in the stomach. In this situation, probiotic fermentation is a desirable alternative since it adds a substantial number of active probiotics to the finished product while also enhancing its nutritional value, flavor and aroma, texture and stability, and microbiological safety (Zhu *et al.*, 2020).

Lactobacillus plantarum is a common, useful species of lactic acid bacteria (LAB) that may be found in fermented foods and the human gastrointestinal tract (Behera *et al.*, 2018). Its numerous essential properties make it perfect for use in probiotic applications. To begin with, *L. plantarum* may grow in a variety of environmental conditions, including those with different pH levels, temperatures, and stress levels. Due to its adaptability, it can survive and colonise in the gut since it can thrive in a range of conditions, including the acidic environment of the stomach (Parlindungan *et al.*, 2021). Second, *L.*

plantarum has potent probiotic properties, including the ability to adhere to intestinal epithelial cells and compete with harmful bacteria for nutrients and binding sites. This competitive exclusion process contributes to the preservation of gastrointestinal health by preventing harmful germs from colonising the stomach. Furthermore, lactic acid, bacteriocins, and short-chain fatty acids are among the metabolites produced by *Lactobacillus plantarum* that contribute to its probiotic qualities (Seddik *et al.*, 2017). These metabolites help regulate immune responses, strengthen the intestinal barrier, and regulate the gut microbiota, all of which contribute to improved gut health and overall well-being.

2. Probiotics

The FAO/WHO definition of probiotics, which is still applicable after 19 years, may be reduced to four straightforward and useful criteria that help determine if a certain strain of microbe is suitable for use in food and nutritional supplements. Probiotic strains need to be adequately described, safe for the intended use, backed by a minimum of one successful human clinical trial carried out following generally accepted scientific standards, or, if applicable, in accordance with the guidelines and regulations of local or national authorities, and alive in the product at an effective dose for the duration of its shelf life. Probiotics are currently defined by the International Scientific Association for Probiotics and Prebiotics (ISAPP) as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host," which is a slight modification of the definition originally provided by the World Health Organisation (WHO) in 2001 (Zucko *et al.*, 2020). The definition of probiotics indicates that they should be given in levels sufficient to produce a health benefit for the host, without mentioning a specific dosage. Given that probiotics are live microorganisms with the ability to replicate inside their hosts, it is thus plausible that, over time, a small number of probiotic cells may be sufficient to provide a positive impact, provided they develop sufficiently within the host. This is undoubtedly the case with disease-causing pathogenic microorganisms, which, due to their virulence and ability to multiply within the host, may have harmful effects on human health at incredibly low levels. Probiotics have a great safety record, and because they haven't been the subject of many MTD or dose-ranging studies, the majority of studies only select a daily dose of 10^8 – 10^{11} colony-forming units (CFU), which corresponds to effective levels from previous research. Because of the gut-brain axis, probiotics are being ingested more often for their benefits to immune system function, digestive health, and maybe even mental health. Additionally, the COVID-19 pandemic increased consumer interest in immunity-boosting goods. The demand for functional beverages has increased significantly as consumers shift away from sugar-filled drinks in symbiosis of healthier options such as probiotic beverages, including kefir, kombucha, and yogurt-based drinks. Fermented milk and yogurt products alone are currently valued at €46 billion, with 77% of the market concentrated in Europe, North America, and Asia (Bintsis & Papademas, 2022). The increasing popularity of plant-based probiotic drinks can also be attributed to the growing trend of veganism and flexitarian diets. Probiotics are now widely available and in great demand worldwide, thanks to a substantial growth in the industry. The probiotic market was estimated to be worth USD 35 billion in 2015 and was expected to grow to USD 74 billion by 2024, according to Vera-Santander *et al.*, (2023). The term "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" refers to gram-positive bacteria, which are the basis for probiotics. Probiotics help to maintain healthy intestinal function in the immune system, controlling the defence mechanism, lowering serum cholesterol, lowering plasma LDL levels, and restoring glucose homeostasis. They also help to prevent and treat food allergies and diarrhoea, relieve lactose intolerance, and restore normal intestinal transit.

2.1 Viability of Probiotic Bacteria in Different Food Products

Researchers have been investigating the use of microencapsulation to enhance the ability of probiotics to withstand extreme temperatures, pH fluctuations, and storage. Microencapsulation in alginate or chitosan-coated capsules significantly improved the survival of *Lactobacillus acidophilus* and *Bifidobacterium lactis*

in yoghurt during cold storage, maintaining viable counts above the minimum therapeutic threshold of 10^6 CFU/mL, according to studies by Rutella *et al.*, (2019). During this time, the creation of symbiotic products—which mix probiotics and prebiotics to increase the latter's survival—has become increasingly popular. In a research done by Canbulat & Ozcan (2015) stated that adding inulin as a prebiotic to yoghurt enhanced *Lactobacillus rhamnosus* growth and survival, retaining viable numbers even after six weeks of storage. The use of *Bifidobacterium longum* in yoghurt compositions was documented by Patterson *et al.*, (2020). Similar to a study by Nualkaekul *et al.*, (2011), the vitality of *B. longum* remained high up to 10^7 for up to six weeks, according to this study, which concentrated on the strain's survival at refrigerated temperatures. To preserve viability, the study highlighted the significance of limited oxygen exposure and ideal pH regulation.

Ngamsomchat *et al.*, (2022), with a special emphasis on *L. plantarum*, investigated probiotic viability in goat cheese in different concentrations of salt. Cheese is a great probiotic carrier because of its fat content and low acidity. They found that probiotics remained viable for up to 12 weeks when refrigerated. An investigation of *Lactobacillus rhamnosus* in probiotic cream cheese was conducted by Shahraki *et al.*, (2023). According to their research, probiotics' ability to survive during the manufacturing and storage of cheese was greatly increased by encasing them in calcium alginate. After 45 days of storage, probiotics were still alive above 10^6 CFU/g, demonstrating that cream cheese might be a useful probiotic carrier. Research by González-Orozco *et al.*, (2023) examined the survivability of *Lactobacillus kefiranofaciens* in several kefir formulations using kefir, a naturally fermented milk product. According to this study, the probiotics' high survivability was made possible by the special matrix of kefir, and their symbiotic association with yeast further improved the probiotics' fermentation efficiency. According to Hasgucmen & Sengun (2020), *Lactobacillus rhamnosus* demonstrated strong cold resistance, maintaining viability above 6 log CFU/g after 120 days of frozen storage at -20°C , and even after 5 days at 4°C ; notably, a higher survival rate was observed at -20°C than at 4°C , suggesting that freezing conditions may better preserve probiotic viability over time.

The market for plant-based probiotic products has expanded in parallel with the increasing consumer interest in plant-based and dairy-free diets. Plant-derived substrates—including cereals (e.g., oats, rice, barley), legumes (e.g., soybeans, chickpeas), fruits, and vegetables—are rich in essential nutrients such as dietary fibers, polyphenols, and oligosaccharides, which promote the growth, metabolic activity, and viability of probiotic strains (Marco *et al.*, 2021). These materials are not only abundant and sustainable but also meet the rising demand for vegan-friendly functional foods. Moreover, recent studies have demonstrated that plant-based matrices can improve probiotic survival during storage and gastrointestinal passage, while offering additional health benefits through their bioactive compounds (Vinderola *et al.*, 2023; Žvirdauskienė *et al.*, 2025). The key characteristics and benefits of common plant-based substrates used for probiotics are summarized in Table 1.

Table 1: Overview of Plant-Based Matrices Supporting Probiotic Growth and Viability

Substrate Type	Key Features & Benefits	Supporting Citations
Fruits & Vegetables	Support growth of probiotic strains (e.g., <i>Lactobacillus</i> , <i>Bifidobacterium</i>); rich in prebiotic fibers; suitable for juices and beverages; maintain probiotic viability during storage	Dahiya & Nigam (2022)
Cereals & Grains	Good fermentation matrix; support probiotic survival and flavor development; used in beverages and snacks	Kirmizigul & Sengun, (2023)
Legumes & Soy	Provide protein and prebiotic fibers; suitable for vegan/vegetarian products	Kumar <i>et al.</i> , (2022)
Plant-Based Milks	Used as dairy alternatives; support probiotic viability; appeal to lactose-intolerant and vegan consumers	Aspri <i>et al.</i> , (2020)

Consumers are increasingly looking for non-dairy probiotic choices because of dietary preferences, allergies, and sustainability concerns, according to a study by Fidelis & Granato (2021). Consumers are prepared to spend more for plant-based meals that provide extra health advantages, such as probiotics for immune and digestive support, according to surveys done in North America and Europe. Maintaining desired sensory qualities, such as flavour and texture, is one of the challenges in creating plant-based probiotic meals. Consumers are increasingly looking for non-dairy probiotic choices because of dietary preferences, allergies, and sustainability concerns, stated by Fidelis & Granato (2021) consumers are prepared to spend more for plant-based meals that provide extra health advantages, such as probiotics for immune and digestive support, according to surveys done in North America and Europe. Maintaining desired sensory qualities, such as flavour and texture, is one of the challenges in creating plant-based probiotic meals. Probiotic fortification enhanced the product's health appeal, but if it is not correctly balanced, it may change the texture or flavour, which looked at consumer acceptability of oat-based probiotic drinks (DeBruyne & Hekmat, 2024). This demonstrates the necessity of meticulous formulation to guarantee customer delight while providing probiotic advantages. When paired with prebiotic fortification, soy milk's greater protein content also promoted improved development of *B. longum* and *L. plantarum* (Xu *et al.*, 2022). In addition, He *et al.*, (2022) investigated the addition of *Lactobacillus acidophilus* to oat milk, emphasizing the beneficial relationship between probiotics and the beta-glucan in oats. Both the probiotics' survivability and the oat milk's nutritional profile were improved by this combination.

2.2 Microbial Growth Kinetics and Optimization

Research on the kinetics of microbial growth offers a chance to develop biotechnology. To improve microbiological analysis, it is essential to estimate the growth kinetics and yield variables. Kinetic models are highly helpful in the design and management of biotechnological processes because they offer sophisticated information on the behaviour of microbial development through precise, reproducible, in-depth experiments and mathematical models (Sriraman *et al.*, 2024). Understanding microbial development and ecosystem dynamics is largely dependent on bacterial biomass, which is an essential measure of microbial activity. It describes the entire mass of bacteria, including both living and dead cells, that are present in a certain ecosystem. Bacterial biomass is considered a general index of microbial activity and is used to calculate growth rates. Increased microbial growth, nutrient cycling, and metabolic processes are often indicated by higher biomass. Monitoring bacterial activity in response to environmental changes, including temperature, pH, and nutrient availability, is a common practice in ecological research. Bacterial population growth rates may be computed by analysing bacterial biomass over time (Haralambiev *et al.*, 2020). A crucial metric in microbiology, growth rate indicates how rapidly a bacterial population grows in a certain environment. This is very helpful in industrial settings like fermentation, where maximising growth rates may boost production efficiency (Parekh *et al.*, 2000).

Cell growth refers to a gain in mass and size that is regulated by the chemical, biological, and physical surroundings. Increases in the macromolecular and chemical components of the cell are used to measure microbial development, and each bacterium has a distinct growth pattern (Sakthiselvan *et al.*, 2019). Microbial growth kinetics describes the link between a microbe's particular growth rate and substrate concentration. Laboratory culture conditions greatly influence microbial growth kinetics. In batch culture, the composition and condition of microbial cells vary with time, affecting the rate of biomass increase. Alternatively, in continuous culture, the substrate concentration is at a balance, and the culture develops in a stable physiological condition, yielding more exact and reproducible results. However, continuously growing conditions provide an artificial environment that does not fully describe microbial kinetic processes.

For instance, a study by Yeboah (2023) recorded various substrates to enhance LAB growth. In this study, the use of plant-based hydrolysates that serve as an effective carbon source has been highlighted. It showed that the growth of *Lactobacillus delbrückii* spp. *Bulgarius* in soy milk hydrolysate supplemented with a nitrogen source was enhanced, with a doubling time reduced. A kinetic analysis for *Lactobacillus casei* was carried out to determine biomass production, product inhibition effects, and substrate consumption rates. It was recorded that the fed-batch cultures at high biomass concentration resulted in

higher productivity (0.45 g/L h⁻¹), complete lactose conversions (<1.0 g of lactose/L at the end of each fed-batch cycle), and higher viable cell counts (2×10^{10} cell/g of freeze-dried product) (Aguirre-Ezkauriata *et al.*, 2010). According to Nancib *et al.*, (2015), the maximum growth rate of *L. casei* subsp. *Rhamnosus* was 0.1 h⁻¹, where the specific growth rate decreased with the increase in lactate concentration in the MRS medium, and the specific growth rate reduced to 22% at 84 g/L lactic acid concentration. A 0.66 h⁻¹ of maximum specific growth rate was acquired in a study by Zacharof *et al.*, (2013) about the cell growth dynamics of *Lactococcus lactis*. The achievement of the maximum specific growth rate of *Lactococcus lactis* assists in obtaining the optimum condition for the volumetric cell productivity. A study by Ricciardi *et al.*, (2019) recorded the maximum growth rate of *L. casei* in whey permeate medium ranges from 0.29-0.65 h⁻¹ cultivated at 37°C in sterile optimized medium supplemented with 2.5 g/l yeast extract, 2.5 g/l tryptone, 0.1 g/l MgSO₄·7H₂O, 0.02 g/l MnSO₄·H₂O, 0.5 ml/l Tween 80.

Optimization method using response surface (RSM) is still less than other methods, such as ONE-FACTOR-AT-A-TIME (OFAT). RSM has three typical experiment designs: Plackett-Burmann Design (PBD), Box-Behnken Design (BBD), and Central Composite Design (CCD). The PBD was primarily utilised to assess the major effects of the components and to filter important factors affecting biomass output of *L. plantarum* (Choi *et al.*, 2021). It was reported that different types of media produced different biomass production. The highest biomass production was in yeast extract supplemented with a nitrogen source, with 1.722 g/L. In the work of Mathiyalagan *et al.*, (2021), culture conditions such as pH, temperature, and incubation period were tested using PBD before BBD. The temperature and pH were shown to be major determinants, with 41.8°C and pH 7.02. Portilha-Cunha *et al.*, (2020) evaluated a total of six parameters including temperature, pH, glucose concentration, aeration degree, NaCl concentration, and agitation rate), but only temperature was considered a significant factor because the maximum growth rate was recorded under anaerobic conditions, 37°C, and in the absence of NaCl. In the study by Hemalatha & Devi (2022), temperature and pH were regarded as significant parameters and then examined using CCD; the optimal temperature for the development of *L. plantarum* was 40°C, and the optimum pH was pH 6.0. The most common method of optimization from recent research is OFAT.

According to Sliżewska & Chlebicz-Wójcik's (2020) study, the one-factor-at-a-time (OFAT) method was used to determine the optimum temperature and initial pH of the medium for a few *Lactobacillus* strains (*L. paracasei*, *L. pentosus*, *L. plantarum*, *L. reuteri*, and *L. rhamnosus*). *L. plantarum* produced the most exopolysaccharide when fermented at 27°C and 100 rpm for 36 hours (Goveas *et al.*, 2021). In terms of biomass production, Choi *et al.*, (2021) discovered that the optimal growing conditions for *L. plantarum* were 30°C, pH 6.5, and an agitation speed of 200 rpm in modified medium. The optimization and kinetic growth of different Lab strains are summarized in Table 2.

3. Metabolites of Probiotic Bacteria

The byproduct that was continually created during the fermentation and development process was the LAB metabolites. Table 3 summarises the probiotic strains, their potential beneficial metabolites, and the conditions required for metabolite production. Raw milk is the starting point for the dairy industry, and LAB is essential to the transformation of raw milk into dairy products, including cheese, yoghurt, and fermented milk. Lactose was transformed to lactic acid by LAB during fermentation; this biochemical conversion was advantageous since it served many purposes and produced a desired end product (de Souza *et al.*, 2023). For example, eliminating harmful or anti-nutritional elements to avoid lactose intolerance, increasing of the calcium bioavailability, improving the product's texture, and reducing its syneresis (Sharma *et al.*, 2021).

Despite a continuous increase in demand, lactic acid production has not increased (Bahry *et al.*, 2019). It has been demonstrated that lactic acid directly improves human health in addition to increasing the nutritional content of food. Both homolactic fermentation and h5 heterolactic fermentation were implicated in the metabolism of LAB (Wang *et al.*, 2023). A strain of *Lactobacillus* sp. was identified by its genetic composition, which dictated the sort of lactic acid fermentation it performed. Because of its high

output and optical purity of lactic acid, homofermentative LAB was chosen for commercial lactic acid production (Abedi & Hashemi, 2020). However, because heterofermentative LAB produced less lactic acid and more carbon dioxide during fermentation, it was less appropriate for commercial production (Zhang *et al.*, 2022). Heterofermentative LAB, for instance, was not frequently utilised as a starting culture in the dairy sector because the carbon dioxide it generated might result in issues such as fractures in dairy products and inflated packaging (Abedi & Hashemi, 2020). Fermentation techniques to increase lactic acid output and purity are now the most widely used methods in the business. The generation of carbon dioxide is one of the main disadvantages of heterofermentative LAB in industrial settings. In fermentation-based food products, this gas may result in foaming and unintended textural changes, making process control more difficult and necessitating extra measures to limit gas release. Furthermore, the taste, acidity, and general quality of the finished product may be changed by the presence of other metabolic byproducts, including acetic acid, which may not be preferred in sectors that produce only lactic acid (Zhang *et al.*, 2022). The generation of carbon dioxide is one of the main disadvantages of heterofermentative LAB in industrial settings. In fermentation-based food products, this gas may result in foaming and unintended textural changes, making process control more difficult and necessitating extra measures to limit gas release. Furthermore, the taste, acidity, and general quality of the finished product may be changed by the presence of other metabolic byproducts, including acetic acid, which may not be preferred in sectors that produce only lactic acid.

Table 2: Summary of studies on the optimization and kinetic growth of various lactic acid bacteria strains using different plant-based substrates

LAB strain	Plant Substrate	Optimal condition	Key findings	Reference
<i>Lactobacillus delbreuckii</i> spp. <i>Bulgarius</i>	Soy milk hydrolysates	42°C for 12 hours, 100 rpm	Reduction of exponential rate time from 24 hours to 12 hours compared to incubation in MRS medium	Yeboah (2023)
<i>Lactobacillus casei</i>	Goat milk whey	Batch, fed-batch and continuoos experiments in bioreactor (37°C, pH 5.5, 300 rpm)	$\mu = 1.22 \text{ h}^{-1}$ at 1.0 g/L lactic acid concentration; $\mu = 0.02 \text{ h}^{-1}$ at 5 g/L lactic acid concentration	Aguirre-Ezkauriatza <i>et al.</i> , (2010)
<i>Lactobacillus casei</i>	Date juice glucose	Batch fermentation, pH 6.0, 38°C, 200 rpm	$\mu = 0.1 \text{ h}^{-1}$; specific growth rate reduced to 22% at 84 g/L lactic acid concentration	Nancib <i>et al.</i> , (2015)
	Cheese whey protein permeate medium	Batch cultivation, 37°C, pH 6.5	$\mu = 0.29-0.65 \text{ h}^{-1}$	Ricciardi <i>et al.</i> , (2019)
<i>Lactobacillus plantarum</i>	Yeast extract, maltose and soy tone	Batch cultivation, pH 6.5, 30°C, 200 rpm	Highest biomass production in yeast extract (1.722 gL ⁻¹)	Choi <i>et al.</i> , (2021)
<i>Lactococcus lactis</i>	Glucose, yeast extract, peptone	Batch fermentation, pH 6.5, 30°C, 350 rpm	$\mu = 0.66 \text{ h}^{-1}$	Zacharof & Lovitt (2013)

Note: μ = specific growth rate (h^{-1}); LAB = lactic acid bacteria.

Table 3: Probiotics strain, Possible Beneficial Metabolites, and the Condition of Metabolites Production

Strain	Metabolites	Remarks	Reference
<i>Lactobacillus spp., Streptococcus thermophilus</i> zlw TM11, <i>Lactococcus lactis</i> , <i>Lactobacillus delbrueckii</i> subsp. <i>bulgaricus</i> 34.5 etc	Viscous exopolysaccharides	Strain cultivated at a temperature of 35.6°C, initial pH of 7.4 and 6.4% of inoculation size applied.	Han <i>et al.</i> , (2015)
<i>Lactobacillus plantarum</i> , <i>Leuconostoc lactis</i>	Bacteriocins; leucosin	36°C at a pH of 6.5 using 1% inoculum size.	Wang <i>et al.</i> , (2023)
	Riboflavin	The strain that produced the most riboflavin was named RYG-YYG-9049. After 20 hours of fermentation at 37 °C, the RYG-YYG-9049-M10 strain was able to enhance the amount of riboflavin in fermented soy milk by ten times.	Yuan <i>et al.</i> , (2023)
<i>Lactobacillus</i> & <i>Limosilactobacillus reuteri</i>	3-hydroxypropionic acid	Produced through the glycerol metabolism pathway.	Kumar <i>et al.</i> , (2022)
<i>Lactobacillus plantarum</i> , <i>Pediococcus acidilactici</i> , <i>Streptococcus spp</i>	Succinic acid	Undertaken in wet and spray-dried fish-based raw material for 3 weeks at room temperature (25°C).	Kuley <i>et al.</i> , (2020)
<i>Levilactobacillus brevis</i> , <i>Limosilactobacillus fermentum</i> , <i>Lactiplantibacillus plantarum</i>	Phenolic acid	Produced through decarboxylase and reductase	Filannino <i>et al.</i> , (2015)

4. Metabolites of Probiotic Bacteria

The byproduct that was continually created during the fermentation and development process was the LAB metabolites. Table 3 summarises the probiotic strains, their potential beneficial metabolites, and the conditions required for metabolite production. The dairy, cosmetics, pharmaceutical, and chemical industries all made use of lactic acid, one of the key metabolites. Raw milk is the starting point for the dairy industry, and LAB is essential to the transformation of raw milk into dairy products, including cheese, yoghurt, and fermented milk. Lactose was transformed to lactic acid by LAB during fermentation; this biochemical conversion was advantageous since it served many purposes and produced a desired end product (de Souza *et al.*, 2023). For example, eliminating harmful or anti-nutritional elements to avoid lactose intolerance, increasing the minerals' (calcium's) bioavailability, improving the product's texture, and reducing its syneresis (Sharma *et al.*, 2021).

Despite a continuous increase in demand, lactic acid production has not increased (Bahry *et al.*, 2019). It has been demonstrated that lactic acid directly improves human health in addition to increasing the nutritional content of food. Both homolactic fermentation and h5 heterolactic fermentation were implicated in the metabolism of LAB (Wang *et al.*, 2023). A strain of *Lactobacillus* sp. was identified by its genetic composition, which dictated the sort of lactic acid fermentation it performed. Because of its high output and optical purity of lactic acid, homofermentative LAB was chosen for commercial lactic acid production (Abedi & Hashemi, 2020). However, because heterofermentative LAB produced less lactic acid and more carbon dioxide during fermentation, it was less appropriate for commercial production (Zhang *et al.*, 2022). Heterofermentative LAB, for instance, was not frequently utilised as a starting culture in the dairy sector because the carbon dioxide it generated might result in issues such as fractures in dairy products and inflated packaging (Abedi & Hashemi, 2020). Fermentation techniques to increase lactic acid output and purity are now the most widely used methods in the business. The generation of carbon dioxide is one of the main disadvantages of heterofermentative LAB in industrial settings. In fermentation-based food products, this gas may result in foaming and unintended textural changes, making process control more difficult and necessitating extra measures to limit gas release. Furthermore, the taste, acidity, and general quality of the finished product may be changed by the presence of other metabolic byproducts, including acetic acid, which may not be preferred in sectors that produce only lactic acid (Zhang *et al.*, 2022). The generation of carbon dioxide is one of the main disadvantages of heterofermentative LAB in industrial settings. In fermentation-based food products, this gas may result in foaming and unintended textural changes, making process control more difficult and necessitating extra measures to limit gas release. Furthermore, the taste, acidity, and general quality of the finished product may be changed by the presence of other metabolic byproducts, including acetic acid, which may not be preferred in sectors that produce only lactic acid.

Heterofermentative LAB, for instance, was not frequently utilised as a starting culture in the dairy sector because the carbon dioxide it generated might result in issues such as fractures in dairy products and inflated packaging (Abedi & Hashemi, 2020). Fermentation techniques to increase lactic acid output and purity are now the most widely used methods in the business. Growth medium played a crucial role in the production of lactic acid. It has been demonstrated that milk is a dependable growth medium for the production of lactic acid by *L. plantarum*. After 12 hours of fermentation in skim milk, the *L. plantarum* reached a final pH of 4.32 and a titratable acidity of 0.74% (Wang *et al.*, 2019). These drawbacks make homofermentative LAB the ideal method for large-scale production, as it produces lactic acid from almost all carbohydrates with little byproducts. These strains are the best option for use in food preservation, dairy fermentation, and the manufacturing of bioplastics because they provide more efficiency, better yield, and simpler downstream processing. Nonetheless, in certain situations where their distinct byproducts—such as ethanol or acetic acid—contribute to the intended sensory or preservation qualities of fermented foods, heterofermentative LAB continue to be significant.

According to de Souza *et al.*, (2023), LAB may generate metabolites such as bacteriocins, viscous exopolysaccharides (EPS), and aromatic compounds. These metabolites have traits that affect fermented

foods' nutritional value and sensory qualities (texture, colour, flavour, and scent) (Moradi *et al.*, 2021). The bacterial strain, medium conditions, incubation length, temperature, and starting pH were all important determinants during the formation of these metabolites (Daba *et al.*, 2021). The EPS produced by LAB has drawn a lot of interest from businesses and researchers in addition to lactic acid and bacteriocin. Naturally occurring LAB-produced EPS has been shown to provide health benefits in addition to improving the rheology of dairy products (Oleksy-Sobczak & Klewicka, 2019). Bacteriocins, LAB's metabolites, were responsible for its well-known antibacterial qualities. According to Zangeneh *et al.*, (2020), bacteriocins are well-known antibacterial proteins that are produced by bacterial ribosomes and either kill or stop the development of infections. It provided a number of advantages, including high stability, nontoxicity, no residue, and nonresistance. Because of this, the usage of LAB bacteriocins in food has increased dramatically, particularly in fermented goods. It may eventually take the place of chemical preservatives in food items to increase their safety and shelf life (Ibrahim *et al.*, 2019).

Bacteriocins are well-known low-molecular peptides that have minimal oral toxicity in humans and have demonstrated encouraging potential for use as bio-preservatives in the food sector (Pei *et al.*, 2020). Numerous studies have demonstrated the protective benefits of bacteriocins against pathogens in a variety of food categories, including vegetables, fermented dairy products, and bakery goods (Zangeneh *et al.*, 2020; Ibrahim *et al.*, 2019; Goel *et al.*, 2020). Both bactericidal and bacteriostatic effects of bacteriocin produced cell death by either preventing the formation of cell walls or rupturing the membrane by creating holes (Sharma *et al.*, 2021). Bacteriocins' wide pH tolerance, heat stability, and enzyme resistance were the main factors in their widespread application in the food sector (Sharma *et al.*, 2021). Additionally, it was shown that the bacteriocin generated by *L. plantarum* was resistant to pepsin, trypsin, and proteinase-K and could tolerate a broad pH range (2–10) and high heat processes (60–121°C). When compared to the control (fresh bacteriocin), there was no discernible variation in the bacteriocin's activity. Class I (lantibiotics), Class II, and Class III are the three groups into which LAB-produced bacteriocins may be divided according to their structure and characteristics (Pérez-Ramos *et al.*, 2021). Nisin and pediocin were the only commercially available bacteriocin kinds utilised in the dairy business, despite the fact that there were other varieties as well (Mora-Villalobos *et al.*, 2020). Since the lipids in meat products may alter nisin's effectiveness, nisin was particularly effective in dairy products but less effective in meat products (Todorov *et al.*, 2022). Environmental conditions also have a significant role in influencing bacteriocins' effectiveness and production.

5. Conclusion

This study highlights the growing relevance of *L. plantarum* in the development of functional, plant-based probiotic beverages. Its robust adaptability, metabolic versatility, and ability to produce health-promoting metabolites position it as a key candidate in probiotic applications. Incorporating non-dairy substrates such as soy milk addresses dietary and sustainability concerns while supporting probiotic viability and functionality. Moreover, optimizing fermentation conditions through kinetic modeling and experimental designs like OFAT and RSM is essential for maximizing biomass yield and product quality. These findings underscore the potential of *L. plantarum* in advancing probiotic food innovation and improving public health outcomes.

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