

REVIEW ARTICLE

**ENCAPSULATION AND FORMULATION OF SINGLE CELL PROBIOTIC
USING *LACTOBACILLUS PLANTARUM*****Nitesh Singh* and Aastha Yadav**

Department of Biotechnology,

Pillai College of Arts, Commerce and Science (Autonomous), Panvel, Navi Mumbai

*Corresponding author E-mail: niteshsingh@mes.ac.in

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Abstract:

Lactobacillus plantarum, a type of lactic acid bacterium, has long been celebrated for its impressive probiotic properties. This beneficial microorganism is known for its remarkable ability to survive in a variety of environments, metabolize a wide range of substrates, and promote overall health by supporting gut microbiota and immune functions. Among its many applications, one of the most promising is its use in the production of probiotic tablets, which are gaining significant attention for their stability, convenience, and extended shelf life. In this chapter, we take a closer look at the journey of *L. plantarum* from the laboratory to the tablet form, with a special focus on the well-established *L. plantarum* strain WCFS1, known for its comprehensive characterization and broad utility. We explore the essential processes involved in its cultivation and fermentation, referred to as upstream and downstream processing, to ensure the bacteria remain viable and effective. We also dive into the formulation of these single-cell probiotic tablets, discussing their nutritional content, including beneficial vitamins, carbohydrates, and proteins that support health.

Keywords: *Lactobacillus plantarum*, WCFS1, Probiotic Tablets, Gut Microbiota, Fermentation Processes, Nutritional Benefits.

Introduction:

Probiotics, defined as live microorganisms that confer health benefits when consumed in adequate amounts, are pivotal in promoting gut health, enhancing immunity, and managing various metabolic disorders. Single-cell probiotics, primarily derived from bacterial genera such as *Lactobacillus*, *Bifidobacterium*, and yeast species like *Saccharomyces boulardii*, represent a critical advancement in probiotic technology. These organisms are metabolically versatile, capable of thriving in diverse environments, and are often more robust during storage and gastrointestinal transit than multi-strain formulations.

The unique properties of single-cell probiotics, such as their smaller size and faster replication rate, make them ideal candidates for producing tablets and other pharmaceutical-grade products. Among them, *Lactobacillus plantarum* has emerged as a model organism due to its ability to colonize

the gut effectively, produce bioactive compounds, and survive harsh conditions, such as acidic pH and bile salts.

Single-cell probiotics such as *Lactobacillus plantarum* and *Saccharomyces boulardii* are key examples. These organisms exhibit remarkable survivability, colonization ability, and health-promoting benefits. For instance, *Lactobacillus plantarum* produces lactic acid and other metabolites that create a hostile environment for pathogens, while *Saccharomyces boulardii* has been shown to combat intestinal infections and restore gut microbiota balance. The versatility of single-cell probiotics has propelled their use across various fields, including healthcare, functional foods, and pharmaceuticals. Their compact cellular structure, combined with their ability to thrive in diverse conditions, underscores their potential as powerful tools in probiotic research and development.

Unlike multicellular systems, single-cell probiotics have the advantage of being metabolically self-sufficient, which allows them to:

1. **Rapidly Adapt to Environments:** They can adjust their metabolic pathways to survive in harsh conditions, such as the acidic environment of the stomach or the bile-rich small intestine.
2. **Interact Directly with the Host:** Probiotic cells communicate with host cells via signaling molecules, leading to improved gut barrier function, immune modulation, and suppression of harmful microbes.
3. **Produce Bioactive Metabolites:** These include vitamins, enzymes, short-chain fatty acids (SCFAs), and antimicrobial peptides that contribute to gut health and overall well-being.
4. **Evolve and Diversify:** Their genetic plasticity allows them to evolve rapidly, adapting to new environments and challenges such as antibiotic exposure.

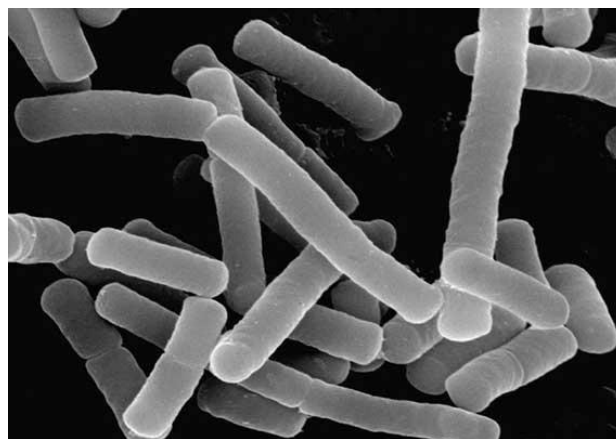


Figure 1: *Lactobacillus plantarum*

Upstream Processing of Single Cell Probiotics:

The upstream process in the production of single-cell probiotics encompasses the preparation and optimization of all elements required for efficient microbial growth. It is critical to ensure a high yield of viable cells while maintaining cost-effectiveness and scalability.

1. Strain Selection and Development:

Strain selection and development are critical to ensuring the probiotic's efficacy, stability, and scalability in industrial production. *Lactobacillus plantarum*, a lactic acid bacterium, is widely recognized for its probiotic potential. This bacterium is known for its ability to

survive in diverse environments, metabolize a variety of substrates, and confer health benefits to the host. One of the prominent applications of *L. plantarum* is its use in the production of probiotic tablets, which are gaining popularity due to their stability, ease of consumption, and extended shelf life.

- *Lactobacillus plantarum* 299v is known for its role in alleviating Irritable Bowel Syndrome (IBS) symptoms.
- *L. plantarum* WCFS1 exhibits robust metabolic versatility and produces antimicrobial peptides. These attributes make *L. plantarum* a preferred candidate for both therapeutic and industrial applications.

Criteria for Selection:

- **Survivability:** Ability to withstand low pH, bile salts, and oxidative stress in the gastrointestinal tract.
- **Functional Traits:** Production of beneficial metabolites like lactic acid, hydrogen peroxide, and bacteriocins.
- **Safety:** Must be non-pathogenic, non-toxic, and Generally Recognized as Safe (GRAS).
- **Stability:** High viability during storage and processing.

2. Media Optimization

- **Objective:** To develop a cost-effective growth medium that supports robust cell proliferation without compromising cell viability. The growth medium for *L. plantarum* WCFS1 is designed to optimize cell growth and metabolite production. Key components include:
- **Carbon Source:** Glucose or sucrose is used as the primary energy source, ensuring rapid bacterial proliferation and metabolic activity.
- **Nitrogen Source:** Yeast extract or peptone provides essential amino acids, peptides, and growth factors required for robust cell development.
- **Salts:**
 - Sodium acetate: Serves as a buffering agent and carbon source under specific conditions.
 - Magnesium sulfate: Supports enzyme activation and cell membrane stability.
 - Manganese sulfate: Essential for enzymatic functions and metabolic regulation.
- **Trace Elements:** Additional micronutrients such as zinc, cobalt, and iron can be included to further enhance metabolic efficiency.
- **pH Adjustment:** The initial pH is adjusted to 6.2–6.5 using NaOH or HCl, creating an optimal environment for *L. plantarum*'s growth.

3. Fermentation Process

Fermentation is a critical step in the production of single-cell probiotics, as it directly affects the viability and functionality of the bacterial cells. The process involves several detailed steps to ensure optimal growth and metabolite production of *Lactobacillus plantarum* WCFS1:

- Inoculum Preparation:** A seed culture is prepared by inoculating *L. plantarum* WCFS1 into a small volume of sterile growth medium. The inoculum is incubated under controlled conditions (37°C, pH 6.2) until it reaches the exponential growth phase, ensuring high cell viability.

- ii. Bioreactor Setup:** The sterilized bioreactor is filled with the prepared growth medium and connected to monitoring systems for temperature, pH, and dissolved oxygen. The bioreactor is preconditioned to 37°C and microaerophilic conditions to create an optimal environment for the strain.
- iii. Inoculation:** The prepared inoculum (2–5% v/v) is aseptically transferred to the bioreactor. The initial optical density (OD₆₀₀) of the culture is monitored to ensure a consistent starting point for fermentation.
- iv. Fermentation Parameters:**
 - **Temperature:** Maintained at 37°C to support optimal enzyme activity and bacterial metabolism.
 - **pH Control:** Automated addition of NaOH or HCl keeps the pH constant at 6.2, preventing growth inhibition due to acid accumulation.
 - **Agitation:** Stirring at 150–200 rpm ensures uniform mixing and nutrient distribution.
 - **Aeration:** Adjusted to maintain microaerophilic conditions, as excess oxygen can inhibit lactic acid bacteria.
- v. Monitoring and Sampling:** Regular sampling is performed to measure optical density (OD₆₀₀), pH, and metabolite production (e.g., lactic acid concentration). Cell growth is monitored to determine the transition from the exponential to stationary phase, which indicates the optimal harvest time.
- vi. Termination:** Fermentation is stopped after 16–24 hours, once the culture reaches its peak viable cell density. The bioreactor is cooled to prevent further metabolic activity and degradation of metabolites.

4. Harvesting

The culture is harvested at the late exponential or early stationary phase to maximize viable cell count and maintain probiotic efficacy. The harvesting process involves the following steps:

- i. Determination of Harvest Point:** The growth phase of the culture is closely monitored using optical density (OD₆₀₀) and dry cell weight measurements. Harvesting is initiated once the culture reaches peak viability, typically at OD₆₀₀ values of 2.5–3.0.
- ii. Separation of Biomass:** Centrifugation is performed at 5,000–10,000 g for 10–15 minutes to separate the bacterial cells from the spent medium. Large-scale industrial setups may use continuous flow centrifuges for efficiency.
- iii. Supernatant Removal:** The supernatant containing residual metabolites and spent medium is discarded or used for downstream byproduct recovery (e.g., lactic acid).
- iv. Recovery of Biomass:** The bacterial pellet is carefully collected and immediately subjected to washing to remove any remaining medium components or impurities.
- v. Temperature Control:** The entire harvesting process is carried out at low temperatures (4°C) to prevent thermal stress and maintain cell viability.

Downstream Processing:

Downstream processing refers to the series of steps involved in purifying, stabilizing, and packaging the product after it has been produced during upstream processes, such as fermentation. It is a critical phase that ensures the probiotic product is of high quality, free of contaminants, and ready for long-term storage or consumption.

- i. **Cell Washing:** The harvested cells are carefully washed to ensure purity and remove impurities. This step involves: Preparation of Washing Solution: A sterile phosphate-buffered saline (PBS) solution is prepared with pH adjusted to 7.2–7.4. The solution is sterilized to avoid introducing contaminants. Washing Procedure: The bacterial pellet from the centrifugation step is resuspended in the PBS solution at a ratio of 1:3 (pellet to solution). Gentle mixing is performed to avoid cell damage while ensuring thorough washing. Centrifugation: The suspension is centrifuged again at 5,000–8,000 g for 10 minutes to re-pellet the washed cells. This process is repeated 2–3 times to ensure complete removal of medium residues and impurities.
- ii. **Concentration:** Concentration of washed cells is vital to prepare a high-density biomass for further processing. The steps include: Ultrafiltration: The washed cell suspension is passed through an ultrafiltration membrane with a molecular weight cutoff suitable for retaining cells while removing excess liquid. This process helps in achieving a thick, concentrated cell slurry. Alternative Centrifugation: In cases where ultrafiltration is unavailable, high-speed centrifugation (10,000–12,000 g) is used to concentrate the cells. Yield Assessment: The concentrated biomass is measured for viable cell count (e.g., CFU/mL) to ensure it meets the required density (10^9 – 10^{11} CFU/g).
- iii. **Stabilization and Preservation:** Maintaining the viability of concentrated cells is critical for their efficacy in the final tablet form. This involves: Cryoprotectant Addition: A cryoprotectant solution is prepared using components like skim milk powder (10%), trehalose (5%), or glycerol (5%). The solution is mixed with the concentrated biomass to create a uniform suspension. Pre-Freezing: The suspension is divided into aliquots and frozen rapidly at -80°C to preserve cellular integrity and minimize ice crystal formation. Lyophilization (Freeze-Drying): The frozen samples are placed in a freeze-dryer. Primary Drying: Water is sublimated under vacuum conditions at -40°C . Secondary Drying: Residual moisture is removed by gradually raising the temperature to 20 – 25°C while maintaining vacuum. Moisture Content Check: The final product is analyzed to ensure moisture levels are below 4%, ensuring long-term storage stability.
- iv. **Sterilization and Packaging:** The sterilization and packaging process is crucial for maintaining the sterility, shelf life, and functionality of the probiotic tablets:
 - **Sterilization:** The packaging materials are sterilized using methods like autoclaving, gamma irradiation, or ethylene oxide treatment. Sterile working conditions are maintained during packaging to prevent contamination.

- **Packaging Materials:** Primary Packaging: Aluminum blister packs or moisture-resistant plastic containers are used to protect the lyophilized tablets from light, air, and moisture. Secondary Packaging: Cardboard boxes with desiccant packs are used to provide additional protection and absorb residual moisture.
- **Sealing:** Each container or blister pack is sealed under sterile, nitrogen-flushed conditions to minimize oxygen exposure.
- v. **Quality Control:** Quality control checks ensure that the downstream processes result in a product that meets industry standards for probiotic count, purity, and stability.
Tests:
 - **Viable Cell Count:** Ensures the tablets contain the specified CFU (colony-forming units).
 - **Moisture Content:** Confirms that moisture levels are below 4% to prevent microbial growth.
 - **Stability Tests:** Evaluate the product's shelf life under various storage conditions.
 - **Packaging Integrity:** Ensures the packaging is tamper-proof and resistant to environmental factors.
 - **Quality Assurance:** Final product batches undergo testing for sterility, moisture content, and packaging integrity to ensure compliance with industry standards.
- vi. **Final Labeling and Storage:** The packaged product is labeled with details such as the strain used (*Lactobacillus plantarum* WCFS1), CFU count, manufacturing and expiration dates, and storage instructions. The final product is stored under recommended conditions, typically at low temperatures, to maintain probiotic viability.

Formulation:

- i. **Encapsulation-Based Ingredients:** The probiotic tablet formulation starts with the active ingredient, *Lactobacillus plantarum* WCFS1, in its lyophilized and encapsulated form. Encapsulation materials include biocompatible agents like alginate or chitosan, which protect the probiotics from harsh gastric conditions. Prebiotics such as fructooligosaccharides (FOS) or inulin are added to provide synbiotic effects, enhancing the growth and efficacy of the probiotics. Excipients play a critical role in ensuring the tablet's stability and functionality, including fillers (e.g., microcrystalline cellulose or lactose), binders (e.g., povidone or starch), disintegrants (e.g., croscarmellose sodium), and lubricants (e.g., magnesium stearate).
- ii. **Tablet Formulation Steps:** The process begins with mixing the lyophilized *L. plantarum* WCFS1 powder with excipients in a hygienic blender to ensure uniform distribution. Mixing is carried out at a speed of 20–30 rpm for 10–15 minutes using equipment such as a double-cone or V-blender. Granulation follows, employing either wet or dry granulation depending on the moisture sensitivity of the formulation. For wet granulation, a binder solution, such as hydroxypropyl methylcellulose (HPMC), is added. The granules are then sieved to achieve a uniform particle size. Drying of the granules is conducted using a fluidized bed dryer at a controlled temperature of 40–50°C until the moisture content is reduced to below 5%. These

dried granules are compressed into tablets using a tablet press machine. Compression parameters include a force of 5–10 kN, with each tablet weighing approximately 500 mg and shaped as either round or oval for standard dosing.

- iii. **Encapsulation and Coating Process:** The encapsulation process begins by suspending probiotic cells in a sterile sodium alginate solution (2–3% w/v). This suspension is extruded dropwise into a calcium chloride solution (0.1–0.5 M), where calcium alginate beads are formed. To further enhance stability and provide additional protection against gastric acidity, these beads are coated with a layer of chitosan. The encapsulated beads are then freeze-dried to reduce moisture content to below 4%, ensuring their long-term stability. The final tablets undergo an enteric coating process to shield the probiotics from gastric acid, allowing them to reach the intestine intact. The coating solution is prepared using cellulose acetate phthalate dissolved in a mixture of ethanol and water. The coating process involves spray coating in a fluidized bed or pan coater, with inlet and outlet temperatures maintained at 50–60°C and 40–45°C, respectively. The coating weight gain is carefully controlled to be 5–10% of the total tablet weight.

Nutritional Value:

Probiotic tablets formulated with *Lactobacillus plantarum* WCFS1 offer significant nutritional and health benefits, making them a valuable addition to dietary supplements.

- i. **Rich in Bioactive Compounds:** *L. plantarum* produces bioactive metabolites such as short-chain fatty acids (SCFAs), bacteriocins, and exopolysaccharides, which enhance gut health and support the immune system. The SCFAs act as an energy source for colonocytes and maintains intestinal homeostasis.
- ii. **Nutrient Synthesis and Absorption:** Probiotic *L. plantarum* enhances the bioavailability of essential nutrients like vitamins (B-complex, folate) and minerals (calcium, iron, magnesium). It aids in breaking down indigestible carbohydrates, enabling the release of nutrients for absorption.
- iii. **Protein and Enzyme Production:** Contains enzymes such as proteases and lactase that aid in digestion, especially for individuals with lactose intolerance. Contributes to amino acid metabolism and synthesis of essential peptides.
- iv. **Calorie-Free Probiotic Support:** As a non-caloric supplement, it provides functional health benefits without contributing to caloric intake, making it suitable for individuals managing weight.
- v. **Gut Microbiota Regulation:** Regular consumption supports the balance of beneficial gut microbiota, which is linked to improved metabolism, mental health, and immunity.

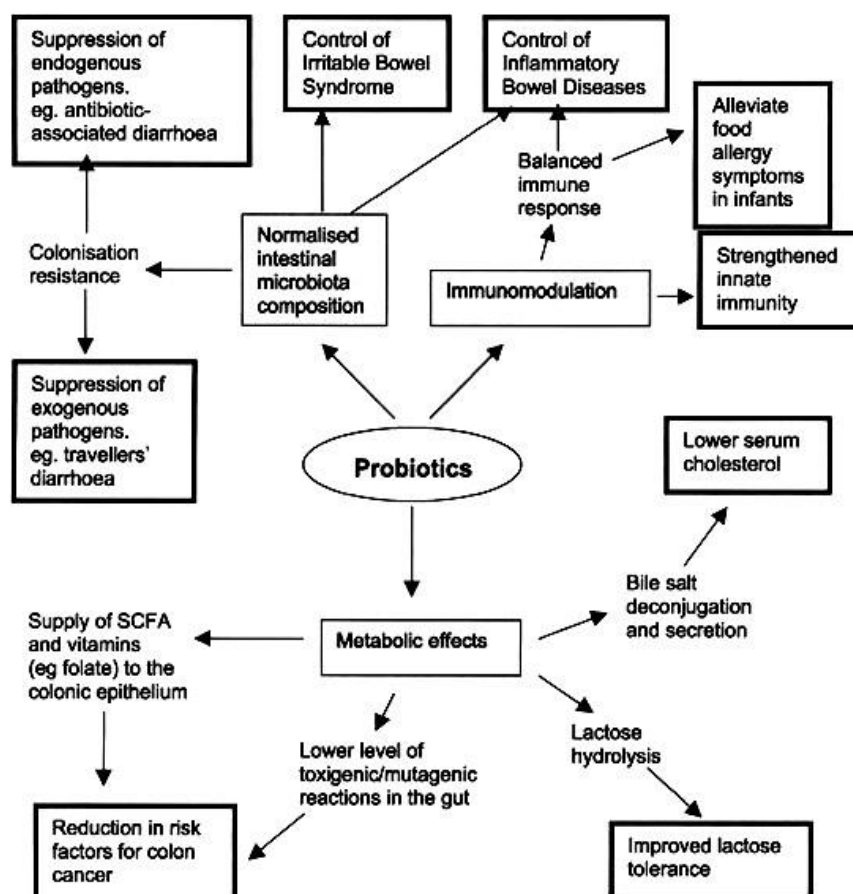


Figure 2: Benefits of probiotics

Comparison with Other Available Probiotics:

Feature	<i>L. plantarum</i> WCFS1	Other Common Probiotics (e.g., <i>L. acidophilus</i> , <i>B. bifidum</i>)
Acid and Bile Tolerance	High; survives gastrointestinal tract	Moderate; often requires protective encapsulation
Vitamin Production	Produces B1, B2, B6, B9, B12, and K	Limited or absent
Antimicrobial Compounds	Produces bacteriocins and exopolysaccharides	Limited production
Shelf Stability	Long shelf life with encapsulation	Moderate; prone to viability loss during storage
Prebiotic Utilization	Efficiently metabolizes FOS and GOS	Limited substrate metabolism
Target Applications	Gut health, immunity, mental health, nutrition	Primarily gut health
Genome Mapping	Fully sequenced; precision-engineered	Limited genetic characterization
Adaptability	Survives wide temperature and pH ranges	Often limited to specific conditions
Antioxidant Activity	High, with production of antioxidant metabolites	Moderate to low

Applications:

Single-cell probiotic tablets using *L. plantarum* WCFS1 have a wide range of applications across various domains:

- i. **Dietary Supplements:** Widely used as over-the-counter probiotics for promoting digestive health, immune function, and nutrient absorption.
- ii. **Functional Foods:** Incorporated into fortified foods and beverages such as yogurts, smoothies, and energy bars for added probiotic benefits.
- iii. **Clinical Nutrition:** Used in medical nutrition therapy for patients with gastrointestinal disorders, antibiotic-associated diarrhea, or inflammatory bowel disease (IBD).
- iv. **Animal Feed:** *L. plantarum*-based probiotics are included in livestock feed to improve digestion, immunity, and growth performance in animals.
- v. **Fermented Products:** Plays a vital role in the production of fermented vegetables (e.g., sauerkraut, kimchi), enhancing taste, preservation, and probiotic content.
- vi. **Cosmetics and Skincare:** Emerging applications involve probiotic-infused skincare products that promote a balanced skin microbiome, reduce inflammation, and combat acne.
- vii. **Pharmaceuticals:** Development of probiotic-based treatments for gut dysbiosis, allergies, and even cancer therapy support through immune modulation.
- viii. **Public Health Programs:** Probiotic tablets can be integrated into government health initiatives to combat malnutrition, improve gut health, and enhance immunity, particularly in low-resource settings.
- ix. **Sports Nutrition:** Probiotic supplements are increasingly marketed to athletes for improving nutrient assimilation, reducing inflammation, and enhancing recovery.
- x. **Veterinary Medicine:** Probiotic tablets are finding applications in pet care, supporting digestive health and immunity in cats, dogs, and other animals.

Future Prospects:

The future of single-cell probiotic tablet production using *L. plantarum* WCFS1 holds immense potential in addressing global health and nutritional challenges:

1. Targeted Therapy:

The potential of *L. plantarum* WCFS1 in targeted therapeutic applications is immense. Unlike generic probiotics, this strain can be engineered or selected for specific functionalities, such as: Inflammatory Bowel Diseases (IBD): By modulating gut microbiota and reducing inflammation markers like TNF-alpha, it can play a crucial role in alleviating symptoms of Crohn's disease and ulcerative colitis. Metabolic Disorders: Its ability to regulate lipid metabolism and glucose levels opens doors to treating obesity, Type 2 diabetes, and hyperlipidemia. Mental Health: Through the gut-brain axis, *L. plantarum* WCFS1 contributes to neurotransmitter production (e.g., serotonin) and may aid in managing anxiety, depression, and stress-related disorders.

2. Personalized Nutrition:

Advances in gut microbiome research allow for a more tailored approach to health and nutrition.

L. plantarum WCFS1 could be matched to individual needs by: Microbiome Profiling: Identifying specific deficiencies or imbalances in an individual's gut microbiota and addressing them with a targeted probiotic supplement. Diet Compatibility: Customizing formulations for people with lactose intolerance, gluten sensitivity, or specific dietary preferences, such as vegan or ketogenic diets. Chronic Conditions: Offering precise nutritional solutions for patients with conditions like irritable bowel syndrome (IBS) or chronic fatigue syndrome (CFS), improving quality of life through gut microbiota modulation.

3. Functional Foods:

Beyond tablets, *L. plantarum* WCFS1 can revolutionize the food and beverage industry: Dairy Products: Fortified yogurts, kefir, and cheeses enriched with this strain offer an easy, daily dose of probiotics with enhanced health benefits. Non-Dairy Alternatives: Probiotic-infused almond milk, oat milk, or coconut-based products cater to plant-based diets. Snack Innovations: Functional snacks like probiotic granola bars or cookies infused with *L. plantarum* provide health benefits on the go. Probiotic Beverages: Innovative drinks such as kombucha, herbal teas, or sports drinks enriched with *L. plantarum* WCFS1 can cater to a growing market of health-conscious consumers.

4. Pediatrics and Geriatrics:

Special formulations of *L. plantarum* WCFS1 tailored for vulnerable populations: Pediatric Health: For children, it can boost immunity, improve digestion, and prevent common gastrointestinal infections like rotavirus. Its role in reducing allergies and promoting healthy growth makes it invaluable for pediatric nutrition. Geriatric Health: In the elderly, it can counteract age-related gut dysbiosis, enhance nutrient absorption, and strengthen immunity, reducing the risk of chronic illnesses and infections. It also addresses constipation and malabsorption issues commonly faced by seniors.

5. Biopharmaceuticals:

The application of *L. plantarum* WCFS1 in biopharmaceuticals is a promising area for future exploration: Capsules and Sachets: Pharmaceutical-grade probiotic capsules and sachets containing *L. plantarum* could be used as adjunct therapy for gastrointestinal and metabolic disorders. Combination Therapies: When combined with prebiotics (synbiotics) or other probiotic strains, these formulations can enhance overall efficacy. Therapeutic Development: Research into its immunomodulatory properties may lead to its use in vaccine adjuvants, allergy prevention, and even autoimmune disease management.

6. Sustainability:

The production of *L. plantarum* WCFS1 aligns with global sustainability goals, making it accessible and eco-friendly: Low-Cost Substrates: The strain can be cultivated using agro-industrial by-products such as whey, molasses, or fruit peels, reducing production costs and waste. Energy Efficiency: Modern fermentation technologies, such as continuous bioreactors, optimize yield with minimal energy consumption. Global Accessibility: Cost-effective production ensures affordability, making this probiotic available even in low-income regions where malnutrition and gastrointestinal disorders are prevalent. Environmental Impact: As a plant-based and biodegradable product, *L.*

plantarum WCFS1 contributes to a reduced carbon footprint compared to synthetic or animal-derived supplements.

Conclusion:

The development of *Lactobacillus plantarum* WCFS1 probiotic tablets represents a significant advancement in the field of functional foods and therapeutic probiotics. This strain offers a comprehensive range of health benefits, including enhanced gut health, immune modulation, nutrient synthesis, and potential mental health improvements. Its resilience to harsh gastrointestinal and industrial processing conditions, combined with its ability to produce bioactive compounds, positions it as a superior alternative to many commercially available probiotics.

The novelty of this product lies in its precision-engineered formulation, high viability, and multifunctional applications. With its versatility in addressing diverse health concerns and potential for integration into personalized nutrition, pediatrics, geriatrics, and biopharmaceuticals, *L. plantarum* WCFS1 tablets pave the way for innovative, sustainable, and impactful solutions in global health. This probiotic tablet sets a benchmark for next-generation probiotics, promising to bridge the gap between health, nutrition, and therapeutic advancements.

References:

1. Abdelazez, A., Abdelmotaal, H., Zhu, Z.-T., Jia, F.-F., Rokayya, S., Zhang, L.-J., Al Tawaha, A. R., & Meng, X.-C. (2018). Potential benefits of *Lactobacillus plantarum* as probiotic and its advantages in human health and industrial applications: A review. *Advances in Environmental Biology*. <https://doi.org/10.22587/aeb.2018.12.1.4>
2. Jankovic, I., Sybesma, W., Phothisath, P., Ananta, E., & Mercenier, A. (2010). Application of probiotics in food products—Challenges and new approaches. *Current Opinion in Biotechnology*, 21(2), 175–181. <https://doi.org/10.1016/j.copbio.2010.03.009>
3. Al-Yami, A. M., Al-Mousa, A. T., Al-Otaibi, S. A., & Khalifa, A. Y. (2022). *Lactobacillus* species as probiotics: Isolation sources and health benefits. *Journal of Pure and Applied Microbiology*, 16(4), 2270–2291. <https://doi.org/10.22207/JPAM.16.4.19>
4. Hutkins, R. W. (2006). *Microbiology and technology of fermented foods*. Blackwell Publishing.
5. Li, Y., Tomita, H., Lv, Y., Liu, J., Xue, F., Zheng, B., & Ike, Y. (2011). Molecular characterization of Erm(B)- and Mef(E)-mediated erythromycin-resistant *Streptococcus pneumoniae* in China and complete DNA sequence of Tn2010: Erythromycin-resistant *S. pneumoniae* in China. *Journal of Applied Microbiology*, 110(1), 254–265. <https://doi.org/10.1111/j.1365-2672.2010.04875.x>
6. Potential benefits of *Lactobacillus plantarum* as probiotic and its advantages in human health and industrial applications: A review. (2018). *Advances in Environmental Biology*, 12(1). <https://doi.org/10.22587/aeb.2018.12.1.4>
7. Strain-specific probiotics: Linking probiotic strains with health and disease. (2024). *International Journal of Veterinary Science*, (Pre and Pro), 234–246. <https://doi.org/10.47278/book.CAM/2024.415>

8. Zikou, E., Dovrolis, N., Dimosthenopoulos, C., Gazouli, M., & Makrilakis, K. (2023). The effect of probiotic supplements on metabolic parameters of people with type 2 diabetes in Greece—A randomized, double-blind, placebo-controlled study. *Nutrients*, 15(21), 4663. <https://doi.org/10.3390/nu15214663>
9. Aljohani, A., Rashwan, N., Vasani, S., Alkhawashki, A., Wu, T. T., Lu, X., Castillo, D. A., & Xiao, J. (2024). The health benefits of probiotic *Lactiplantibacillus plantarum*: A systematic review and meta-analysis. *Probiotics and Antimicrobial Proteins*. <https://doi.org/10.1007/s12602-024-10287-3>
10. Tarapatzi, G., Filidou, E., Kandilogiannakis, L., Spathakis, M., Gaitanidou, M., Arvanitidis, K., Drygiannakis, I., et al. (2022). The probiotic strains *Bifidobacterium lactis*, *Lactobacillus acidophilus*, *Lactiplantibacillus plantarum* and *Saccharomyces boulardii* regulate wound healing and chemokine responses in human intestinal subepithelial myofibroblasts. *Pharmaceuticals*, 15(10), 1293. <https://doi.org/10.3390/ph15101293>
11. Fenster, K., Freeburg, B., Hollard, C., Wong, C., Laursen, R. R., & Ouwehand, A. C. (2019). The production and delivery of probiotics: A review of a practical approach. *Microorganisms*, 7(3), 83. <https://doi.org/10.3390/microorganisms7030083>
12. Jankovic, I., Sybesma, W., Phothisirath, P., Ananta, E., & Mercenier, A. (2010). Application of probiotics in food products—Challenges and new approaches. *Current Opinion in Biotechnology*, 21(2), 175–181. <https://doi.org/10.1016/j.copbio.2010.03.009>