*fermentation*

*Editorial*

# New Insights into the Application of Lactic Acid Bacterial Strains in Fermentation

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In recent years, lactic acid bacteria, widely used in food fermentation, have been recognized as beneficial components of the human microbiome in which they play an important role. By studying the biochemical changes in lactic acid bacteria in the fermentation process and identifying the bacteria involved in food fermentation, more substances that are beneficial to human health can be discovered. The biological activity of next-generation probiotics in the gut has received increasing attention and is believed to interact with many metabolic diseases, diseases of aging, and rare diseases. Therefore, next-generation probiotics are the future of adjunctive biological therapy. This Special Issue aims to collect original research articles and reviews on fermentation applications and the characterization of the functional properties of these bacteria, as well as the analysis of their production of gamma-aminobutyric acid (GABA), short-chain fatty acids (SCFA), and other active compounds, as well as recent research on their ability to influence the gut microbiome. This Special Issue consists of 13 publications with different applications related to fermentation-related applications and the quality assessment of LAB, a method combining fermentation and self-buffering whole-cell catalysis for the efficient production of GABA using *Lactobacillus brevis* CD0817 [1]. Exopolysaccharides (EPS), produced by LAB and yeast, have many biological and technical properties that may be influenced by many factors that occur in fermentation [2]. In traditional hard Xinotyri cheese made from goat milk, changes in its microbial and biochemical properties include LAB during maturation and storage [3]. The effects of the fermentation processes include LAB under high pressure on ethyl carbamate (EC) (a probable human carcinogen) [4]. In addition, in terms of the active function and identification of new strains, lactic acid bacteria (LAB) NMCC-M2 isolated from buffalo milk (*Bubalus bubalis*) has the potential to target probiotics in cholesterol-lowering fermented foods [5]. The isolation and characterization of LAB *Pediococcus* sp. HLV from idli batter, a traditional Indian fermented product, reveal an economical and inexpensive raw material for the industrial production of lactic acid by the *Pediococcus* sp. strain, HLV1 [6]. Five types of lactic acid bacteria isolated from Amazonian cocoa fermentation may be used as starter agents that affect the quality of chocolate [7]. The value of acid-tolerant and potentially probiotic LAB isolated from kefir grains for the lactic acid fermentation of pomegranate juice has been evaluated [8]. The biomolecules produced by LAB have also been explicitly demonstrated in cells and animal treatment for their protection potential. *Lactobacillus crispatus* EVs regulate the physiological function of placental cells and can be used as an adjuvant therapy strategy for spontaneous preterm birth (sPTB) [9]. The lactic acid bacteria, *Lactiplantibacillus pentosus* SN001, is used in the preparation of fermented *Sargassum horneri* (*S. horneri*), which can be used as a dietary ingredient with enhanced antihypertensive effects [10]. The *Lactobacillus acidophilus* strain, AR1, has the potential to be added to dog food to avoid pathogenic and antibiotic complications [11]. On the other hand, plant lactic acid bacteria, which are promoted by pitaya flower buds (PFW), participate in PFW to improve the physiological damage caused by a high-fat diet [12]. Additionally, the effectiveness of the growth and decline of LAB bacteria in the production of low-salt (LS)/high-hydrostatic-pressure (HPP) soy sauce has been demonstrated in soy sauce production [13].

Changes in extracellular biopolymers contribute to fermented products' organoleptic properties or bioactivity. Exopolysaccharides (EPS) produced by LAB have many biological and technical properties that can be influenced by the numerous factors that occur in fermentation. A review of these microorganisms has resulted in the produced beverages being a good source of benefits for human health; some EPS-producing strains in particular have biological effects such as antioxidant, prebiotic, and immunomodulatory activities. Additionally, in technical applications using EPS-producing LABs as starting bacteria, they can function as thickeners, stabilizers, and emulsifiers in the food industry and can also help maintain the nutritional properties of products [2]. The further exploration of the biochemical metabolites of bioactive lactic acid bacteria is a topic of great economic benefit and technical application. In the trend of using lactic acid bacteria to produce gamma-aminobutyric acid (GABA), the use of *Lactobacillus brevis* CD0817 effectively combines fermentation and self-buffered whole-cell catalysis to produce GABA. After the completion of GABA fermentation, cells were recovered for whole-cell catalysis to catalyze the decarboxylation of the substrate L-glutamic acid to GABA. The results showed that cells cultured in a fermentation medium supplemented with 20.0 g/L glucose for 16 h were the most suitable for the whole-cell catalytic production of GABA. At 16 h, the content of fermented GABA reached 204.2 g/L. Under the optimized whole-cell catalytic conditions (temperature 45.0 °C, time 12.0 h, wet cell weight 25.0 g/L, L-glutamic acid 120.0 g/L), GABA 85.1 g/L was obtained. The purity of the GABA products reached 97.1%, and the recovery rate was 87.0%. These data suggest that *Levilactobacillus brevis* (*Lactobacillus brevis*) CD0817 effectively combines fermentation and self-buffering whole-cell catalysis with potential applications in the production of GABA [1]. In addition, the fermentation of lactic acid bacteria *Lactiplantibacillus pentosus* SN001 can enhance the antihypertensive effect of *S. horneri*. In vivo studies in mice and spontaneously hypertensive rats (SHR) suggest that fermented *S. horneri* has antihypertensive effects. The ACE inhibitory activity of *S. horneri* varied from 3.6% to a maximum of 63.3% after fermentation. In addition, glycerol was identified due to ACE inhibitory components in the fermentation product. Therefore, Keratococcus fermented with Lactobacillus pentosus SN001 is expected to be used as a functional material with antihypertensive activity. This achievement is likely to lead to the effective utilization of *S. horneri*, and the identification of glycerol is expected to provide essential knowledge for the field of medicine [10].

Three *Lactobacilli* strains isolated from clinically healthy dogs showed considerable survival potential under simulated gastrointestinal environment conditions, low pH, and high bile salt concentrations, as well as good adhesion properties to MODE-K cells. *Lactobacillus* significantly inhibited the growth of pathogenic bacteria and their adhesion to MODE-K cells. Real-time PCR analysis further demonstrated that *L. acidophilus* strains, AR1 and AR3, inhibited the *Salmonella*-induced production of pro-inflammatory cytokines (IL-6, IL-8, and IL-1) and enhanced the expression of tight junction proteins (occludin). The results of this in vitro study suggest that the *L. acidophilus* strain, AR1, has the potential to be added to dog food in the future to promote health and prevent or treat intestinal infectious diseases [11].

The results of studies targeting the microbial stage in the vaginal environment to modulate the physiological activity of host cells suggest that *L. crispatus*-derived extracellular vesicles (EV) (1010 particles/mL) can reduce oxidative stress in 3A-sub-E placental cells due to oxidative stress. They resulted in cell death. *L. crispatus*-derived EVs (1010 particles/mL) entered 3A-sub-E placental cells after 12 h of treatment and significantly promoted $H_2O_2$-induced Akt phosphorylation in 3A-sub-E placental cells after 24 h of treatment. This indicated that *L. crispatus*-derived EVs improved Akt phosphorylation and attenuated cellular senescence and the death of placental cells induced by oxidative stress. Furthermore, *L. crispatus*-derived EVs enhanced resistance to $H_2O_2$ induction mediated by increased mitochondrial fusion. Overall, *Lactobacillus crispatus* in the vagina can regulate the physiological functions of placental cells by delivering *Lactobacillus crispatus*-EVs and reducing cell senescence. Since cellular senescence is associated with spontaneous preterm birth (sPTB), the maintenance of *L. crispatus* populations could be used as an adjunct therapeutic strategy to avoid sPTB [9].

In a fermentation application study of the new strains, forty-six LAB strains were isolated from buffalo milk (*Bubalus bubalis*), of which five strains were identified as *Weissella confusa* (NMCC-M2), *Leuconostoc pseudo-mesenteroides* (NMCC- M4), *Lactococcus lactis Subsp. hordniae* (NMCCM5), *Enterococcus faecium* NMCC-M6, and *Enterococcus lactis* NMCC-M7. NMCC-M2 exhibited the best probiotic properties, including survival at pH 3 and 0.5% (*w*/*v*) bile salts, complete sensitivity to the tested antibiotics, high enzymatic potential, and in vitro cholesterol reduction. Therefore, the isolated strain NMCC-M2 can be a potential targeted probiotic in cholesterol-lowering fermented foods [5].

In addition, fifty lactic acid-producing bacteria were isolated from idli batter, a traditional fermented product in India, and six strains were selected. The phenotypes were Gram-positive, cocci, and catalase-negative. Finally, one strain was selected to grow at 50 °C. Suitable strains were further characterized. Molecular characterization by 16S rRNA gene analysis and BLAST analysis showed 99% similarity to *Pediococcus pentosaceus* Ni1142. The isolated culture was named *Pediococcus* sp. HLV1, and the sequences were submitted to the NCBI database with accession number MH921241. *Pediococcus* sp. HLV1 showed the optimal growth and production of lactate at pH 7.0 and 40 °C with glucose as the carbon source (10%) and yeast extract as the nitrogen source (0.3%). *Pediococcus* spp. also showed antibacterial activity against Gram-positive and Gram-negative bacteria. Using the above optimal conditions, lactic acid in fresh mango peel extract was investigated, yielding 5.2% (*v*/*v*) lactic acid at the end of fermentation. In other words, the isolated LAB *Pediococcus* sp. strain, HLV1, can grow at high temperatures (45 °C), produce lactic acid, and survive at 50 °C. The *Pediococcus* sp. strain, HLV1, has the potential to produce lactic acid by fermenting mango peel, thereby allowing mango peel to become an economic raw material source for industries related to *Pediococcus* application [6].

The isolation and identification of LAB from cocoa bean fermentation feedstocks in the Brazilian Amazon using MRS agar has been studied. By sequencing the 16S region of LAB isolated from MRS agar, five LAB species registered in the GenBank database could be identified, including the most prevalent *Pediococcus acidilactici* followed by *Lactobacillus farraginis, L. parafarraginis, L. zeae*, and *L. casei*. These are likely to be used as leavening agents to improve the produced chocolate's quality and economic value [7].

In fruit and vegetable processing, fruit and pomegranate juices demand food substrates for microorganisms' survival and fermentation. The acid-tolerant and potentially probiotic *Lactobacillus paracasei* SP3 strain, recently isolated from kefir grains, has potential applications in producing novel functional beverages based on pomegranate juice fermentation. The study results showed that the *L. paracasei* strain, SP3, was effective for lactic fermentation during the fermentation of pomegranate juice at 30 °C for 24 h and storage at 4 °C for 4 weeks. The ethanol concentrations were kept low (0.3–0.4 % *v*/*v*), and low levels of acetic acid (0.6 g/L) were detected. The *L. paracasei* SP3 cell viability remained high (>7 log cfu/mL), even by week 4. Compared with unfermented fruit juice, fermented pomegranate juice recorded higher levels of total phenolic content (123.7–201.1 mg GAE/100 mL) and antioxidant activity (124.5–148.5 mgTE/100 mL) in all the periods studied. The *L. paracasei* strain, SP3, resulted in a significant reduction in the level of hydrolyzable tannins (42%) in the juice, thereby reducing its astringency and improving the product's organoleptic characteristics. It showed great potential when it was applied as a free culture without applying the microencapsulation method commonly used in these fermentations, resulting in a product with potential functional properties and high nutritional value [8].

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## References

1. Li, H.; Sun, T.; Jia, M.; Wang, L.; Wei, C.; Pei, J.; Lin, Z.; Wang, S. Production of Gamma-Aminobutyric Acid by Levilactobacillus brevis CD0817 by Coupling Fermentation with Self-Buffered Whole-Cell Catalysis. *Fermentation* **2022**, *8*, 321. [CrossRef]
2. Cázares-Vásquez, M.L.; Rodríguez-Herrera, R.; Aguilar-González, C.N.; Sáenz-Galindo, A.; Solanilla-Duque, J.F.; Contreras-Esquivel, J.C.; Flores-Gallegos, A.C. Microbial exopolysaccharides in traditional mexican fermented beverages. *Fermentation* **2021**, *7*, 249. [CrossRef]
3. Pappa, E.C.; Bontinis, T.G.; Samelis, J.; Sotirakoglou, K. Assessment of the Microbiological Quality and Biochemical Parameters of Traditional Hard Xinotyri Cheese Made from Raw or Pasteurized Goat Milk. *Fermentation* **2022**, *8*, 20. [CrossRef]
4. Lai, C.-Y.; Hou, C.-Y.; Chuang, P.-T.; Hsu, W.-H.; Wu, S.-C. Microbiota and Mycobiota of Soy Sauce-Supplied Lactic Acid Bacteria Treated with High Pressure. *Fermentation* **2022**, *8*, 338. [CrossRef]
5. Hameed, A.; Condò, C.; Tauseef, I.; Idrees, M.; Ghazanfar, S.; Farid, A.; Muzammal, M.; Mohaini, M.A.; Alsalman, A.J.; Hawaj, M.A.A.; et al. Isolation and Characterization of a Cholesterol-Lowering Bacteria from Bubalus bubalis Raw Milk. *Fermentation* **2022**, *8*, 163. [CrossRef]
6. Katepogu, H.; Wee, Y.J.; Almaary, K.S.; Elbadawi, Y.B.; Gobinath, R.; Chinni, S.V.; Lebaka, V.R. Isolation and Characterization of Pediococcus sp. HLV1 from Fermented Idly Batter. *Fermentation* **2022**, *8*, 61. [CrossRef]
7. Chagas Junior, G.C.A.; Ferreira, N.R.; Gloria, M.B.A.; Gobira, R.M.; Maia, F.d.A.; Lopes, A.S. Identification of Lactic Acid Bacteria on Raw Material for Cocoa Bean Fermentation in the Brazilian Amazon. *Fermentation* **2022**, *8*, 199. [CrossRef]
8. Mantzourani, I.; Terpou, A.; Bekatorou, A.; Plessas, S. Valorization of Lactic Acid Fermentation of Pomegranate Juice by an Acid Tolerant and Potentially Probiotic LAB Isolated from Kefir Grains. *Fermentation* **2022**, *8*, 142. [CrossRef]
9. Wang, L.M.; Lee, B.H.; Hou, C.Y.; Hsu, W.H.; Tai, C.J. Probiotics-Derived Extracellular Vesicles Protect Oxidative Stress against H2 O2 Induction in Placental Cells. *Fermentation* **2022**, *8*, 74. [CrossRef]
10. Tamura, M.; Shimizu, S.; Nagai, H.; Yoshinari, O.; Hamada-Sato, N. Enhancement of the Antihypertensive Effect of Fermented Sargassum horneri with Lactiplantibacillus pentosus SN001. *Fermentation* **2022**, *8*, 330. [CrossRef]
11. Zhang, G.; Raheem, A.; Gao, X.; Zhang, J.; Shi, L.; Wang, M.; Li, M.; Yin, Y.; Li, S.; Cui, X.; et al. Cytoprotective Effects of Lactobacilli on Mouse Epithelial Cells during Salmonella Infection. *Fermentation* **2022**, *8*, 101. [CrossRef]
12. Lee, B.H.; Hsu, K.T.; Chen, Y.Z.; Tain, Y.L.; Hou, C.Y.; Lin, Y.C.; Hsu, W.H. Polysaccharide Extracts Derived from Defloration Waste of Fruit Pitaya Regulates Gut Microbiota in a Mice Model. *Fermentation* **2022**, *8*, 108. [CrossRef]
13. Shi, Y.C.; Lai, C.Y.; Lee, B.H.; Wu, S.C. The Bacterial and Fungi Microbiota of Soy Sauce-Supplied Lactic Acid Bacteria Treated with High-Pressure Process. *Fermentation* **2022**, *8*, 97. [CrossRef]