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Probiotics for better tomorrow: A review

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ABSTRACT:
Whether knowingly or unknowingly, humans have been consuming probiotic microorganisms through traditionally fermented foods for generations. This review provides a comprehensive overview of the current state of probiotic research, covering a wide range of topics, aimed to explore the impact of probiotics on therapeutic applications, including intestinal barrier, related immune function, inflammation, microbiota composition and its future directions. Advancements in multi-omics approaches and in nanotechnology, especially microencapsulation and electrospinning, will provide a deeper understanding of the mechanisms behind probiotic functionality, allowing for personalized and targeted probiotic therapies. This review aims to update the present knowledge, advanced research area and new technology used in to determine the potential role of probiotics for better tomorrow.

Keywords:
Probiotics, Therapeutic, Multi-omics, Nanotechnology, microencapsulation, electrospinning

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Introduction:

"Probiotics are foods and/or dietary supplements that contain non-pathogenic microbes such as bacteria and yeast that colonize the gut and may potentially provide various health benefits[1]." Including Balancing gut homeostasis by eliminating or inhibiting microbial flora[2,3], Strengthening of the immune system[4], Treatment for celiac Disease[5], and many more. In recent years, Consumers have become more concerned about health, diet and food safety for a better quality of life[6]. Traditional fermented foods including dairy products have been incorporating different strains of probiotics in them for a long time, Some of these products are yoghurt, cheese, ice cream and fermented milk[7–11], This is due to their distinctive physicochemical and nutritional capabilities which offer them the buffering capacity in the harsh acidic conditions of the stomach (pH 23) for a viable number of probiotic survival rates in the lower gut (6 to 8 log CFU/mL) assisting them to apply potential therapeutic effects[12–16].

Therapeutic Effects of Probiotics

The various benefits of probiotics for maintaining a healthy human life. It highlights the wide range of potential applications of probiotics throughout the body, showcasing their versatility in promoting well-being.

1. Gastrointestinal Tract Health: Probiotics are well-known for improving digestion, reducing cholesterol levels, and inhibiting the growth of harmful microorganisms in the gastrointestinal tract. Bifidobacterium, for instance, contributes to mucosal barrier defense[17-19].

2. Diarrhea Treatment: Probiotics have demonstrated effectiveness in managing different types of diarrhoea, including traveller's diarrhoea, antibiotic-induced diarrhoea, and rotavirus-related diarrhea in children[20, 21].

3. Inflammatory Bowel Disease: While the exact cause of conditions like ulcerative colitis and Crohn's disease is not fully understood, clinical studies have shown that probiotics can lead to remission in ulcerative colitis patients. However, their efficacy in Crohn's disease is less established[22].

4. Immune System Support: Probiotics can strengthen the immune system, particularly in allergic conditions, by impacting gut lymphoid cells and altering dietary protein immunomodulation[23].

5. Atopic Dermatitis: Probiotic consumption has been linked to a reduction in the intensity of eczema and various processes associated with the condition[24].

6. Urinary Tract Infections (UTIs): Probiotics like *Lactobacillus rhamnosus* GR-1 and L. fermentum B-54 with RC-14 have shown potential in preventing the recurrence of UTIs in women[25].

7. Cholesterol Reduction: Some evidence suggests that probiotics may contribute to lowering blood cholesterol levels, which could be beneficial in preventing obesity, diabetes, cardiovascular diseases, and stroke[26].

8. Dental Health: Probiotics hold promise in preventing dental caries by inhibiting the growth of cariogenic bacteria like Streptococcus sobrinus. They can also play a role in improving periodontal health and addressing halitosis[27].

9. Microbial Dysbiosis: Probiotics are used to counteract microbial dysbiosis caused by factors like antibiotic use, bacterial infections, and dietary changes. They can help restore the balance of beneficial bacteria and inhibit pathogenic growth[28].

10. Antibiotic-Associated Diarrhea (AAD): Probiotics are effective in preventing AAD, a common consequence of antibiotic use, and can also help address diarrhea caused by pathogenic bacteria like Clostridioides difficile[29].

11. Inflammatory Bowel Disease (IBD): Probiotics have been explored as potential treatments for IBD, including conditions like ulcerative colitis and Crohn's disease. However, their effectiveness varies between different forms of IBD[30].

12. Crohn's Disease (CD): Probiotics, while showing promise in some studies as adjuvant therapy for CD, have yielded mixed results, and further research is needed to establish their role.

In summary, the text highlights the potential benefits of probiotics in various aspects of human health, from gastrointestinal and immune health to dental care and addressing specific conditions. However, the efficacy of probiotics can vary based on factors such as the specific strains used, dosage, and individual responses, necessitating further research to fully understand their effects and applications[31].

Recent advances in the field of probiotics

Probiotics is at the peak of the moment. Many new advances have been seen over the period of time. Life-threatening infections such as Clostridioides difficile infection (CDI), which is caused by a gram positive bacteria intestinal pathogen C. The use of probiotics is investigated to balance the gastrointestinal (GI) tract microflora[32]. Probiotics have also gained huge amount of attention in recent years due to its advantageous advances in biomedical applications such as antimicrobial agents, aiding in tissue repair, and treating diseases. To ensure the good bacteria performs well it is essential to have minimum of 10^7 CFU/mL or gram of the product being used[33].

In biomedical field, biopolymers have won hearts because of their excellent biocompatibility and regularity. Biopolymers are a class of biological materials which can be used in various domains like cell tissue engineering, drug delivery and biosensors[34]. Chitosan (CS) which is a cationic polysaccharide consisting of D-glucosamine and N-acetyl-glucosamine residues linked by β-(1 → 4) bonds[35]. Studies have shown that using CS as a coating material in alginate beads can ameliorate the survival of encapsulated bacteria in harsh conditions like the gastrointestinal tract and high temperatures[36].

Microencapsulation:

Current microencapsulation technologies include layer-by-layer techniques, spray drying, emulsification, extrusion, and electrospraying[37]. Microencapsulation has put forward fruitful means of protecting probiotics from degradation. Initially, it's possible to formulate them to establish a tangible barricade that safeguards the probiotics from detrimental components in their surroundings, like gastric acid, bile salts, or digestive enzymes[38] Next, they might be crafted with supplements that generate a conducive environment for probiotics resistant to acidity, manage pH levels, and support their growth[39]. Ultimately, microparticles can be tailored to trap particular compounds that boost probiotic viability and are subsequently released by the probiotics[40].

Electro-spinning:

Electro spinning stands as a versatile technique employed for the continuous production of nanofibers spanning from nanometres to micrometres in diameter[41]. Initially pioneered by Formhals in 1934, electro spun fibres have found extensive applications in tissue engineering, energy conversion and storage, drug delivery and release, food packaging, sensors, catalysis, filtration, and virtually every realm of research[42]. In the realm of the food industry, electro spun nanofibers primarily find utility in the encapsulation (antioxidants, antimicrobial agents, enzymes, and probiotics) and packaging domains[43].

Nano-technology:

Recent years have seen a promising growth in the field of research into the use of nanostructured systems for the treatment of infectious diseases that are resistant to traditional therapies like antibacterial medicines. Patient's quality of life and life expectancy have increased as a result of these disorders[44]. The ability of Nano emulsions to merge with the outer membranes of bacteria is thought to be the basis for the antimicrobial action and mechanism of nanoemulsions, according to molecular studies. They exhibit broad-spectrum activity due to the rupture of membrane bilayers and cellular permeability caused by electrostatic interactions between the cationic charge of nanoparticles and the anionic charge of microorganisms[45].

New discovery and research methodologies:

Recent advancements in technologies and methodologies are driving significant progress in the field of prebiotics and probiotics, and these developments will continue to be crucial in the future. There is a growing emphasis on ensuring the reproducibility of microbiome results and eliminating biases introduced by various protocols. Challenges in this regard include interpretative variations at different stages of research, from sample collection and sequencing to data analysis.

The use of 'omics' technologies has led to notable breakthroughs, transitioning from standalone data generation to integrated systems biology approaches[46]. These technologies are being refined with better bioinformatics tools and the integration of machine learning and artificial intelligence for more holistic predictions.

The decreasing cost of molecular methodologies, driven by microfluidics and nanofluidics, is generating a wealth of data. This includes techniques like droplet quantitative PCR reactions, allowing for absolute quantification of molecules with minimal sample volumes[47]. Advances in sequencing quality, such as single-molecule real-time sequencing, enable finer characterization of microorganisms at species and even strain levels.

Novel cultivation methods, like microfluidic encapsulation and multiple culture conditions, are revealing new microbial species with potential benefits[48,49]. These isolates can be comprehensively characterized through sequencing and analysis techniques.

Bioinformatic platforms and parallel-computing workflows are enhancing the conversion of sequence data into usable information, facilitating the creation of probiotic databases and large-scale strain analyses[50,51]. Improved software interfaces are also simplifying analysis procedures.

However, it's important to note that in vitro information from these technologies might not always translate directly to in vivo effects. The challenge lies in bridging this gap and ensuring that in vitro mechanisms align with clinical outcomes. To address this, researchers are developing humanistic models that simulate interactions more accurately, such as humanized animal models, organoids, coculture experiments, and 'organ on a chip' models. While these models aid in discovery and prediction, in vivo assessments remain essential to confirm the efficacy of probiotics and prebiotics

Future of Probiotics research:

The opportunities in the fields of probiotics and prebiotics are rooted in understanding their effects on the microbiota and their interactions with the host. This perspective is informed by early career researchers who participated in the 2019 meeting of the International Scientific Association for Probiotics and Prebiotics - Student and Fellows Association (ISAPP-SFA). Probiotic and prebiotic research is driven by genetic characterization and modification of strains, advanced in vitro, in vivo, and in silico techniques, and metabolomics tools to uncover their effects on the host. These tools provide unparalleled insights into how probiotics and prebiotics function within the host ecosystem.[52] Young scientists need to acquire diverse skill sets or collaborate in interdisciplinary teams to conduct comprehensive experiments and analyze data systematically. This is crucial for understanding microbial structures and interactions across body sites and for determining how administered probiotic strains and prebiotic substances impact the host. Implementing such strategies will help bridge the gap between research findings and real-world health outcomes.

Considerations for Probiotics Research

Genetic Characterization and Modification of Probiotics

In the realm of probiotics research, the genetic characterization and modification of probiotic strains have gained significance, aided by bioinformatics and in silico techniques. Whole genome sequencing (WGS) has become more accessible and should be a standard practice before introducing new probiotic strains to the market[53]. Genomes should be rigorously annotated and deposited in central databases for transparency and safety assessment. WGS helps identify genetic elements and predict their functions, shedding light on safety concerns such as virulence factors and antibiotic resistance.

Genetic manipulation is a valuable tool for studying probiotic mechanisms[54] and potentially enhancing strains[55]. However, limited genetic tools, legal frameworks, and ethical considerations hinder progress.

Encouraging the sharing of genetic constructs through repositories would expedite research, especially for creating safe vectors and tailored probiotics[56,57]. Strengthening genetic and bioinformatic skills is crucial in advancing probiotics research.

In vitro models are increasingly relevant, reducing dependence on animal models for preclinical assessment. While humanized animal models are used, in vitro human cell and tissue models, like organoids and organs-on-chips, offer promising alternatives[58,59]. These models allow researchers to explore probiotic effects on human systems with better relevance and precision. The shift away from mammal studies by regulatory bodies opens avenues for the development and implementation of innovative in vitro models.

In summary, probiotics research is embracing genetic characterization, modification, and advanced in vitro models, driven by bioinformatics and interdisciplinary expertise. The focus is on enhancing safety, understanding mechanisms, and developing more relevant testing methods that align with changing regulatory perspectives.

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Conflict of Interest:

The authors declare that they have no conflict of interest.

References

1. Bodke, H. & Jogdand, S. Role of Probiotics in Human Health. *Cureus* (2022) doi:10.7759/cureus.31313.

2. Cordeiro, M. A. *et al.* Fermented whey dairy beverage offers protection against Salmonella enterica ssp. enterica serovar Typhimurium infection in mice. *J Dairy Sci* **102**, 6756–6765 (2019).

3. Mousavi Khaneghah, A. *et al.* Interactions between probiotics and pathogenic microorganisms in hosts and foods: A review. *Trends Food Sci Technol* **95**, 205–218 (2020).

4. Maldonado Galdeano, C. *et al.* Beneficial Effects of Probiotic Consumption on the Immune System. *Review Article Ann Nutr Metab* **74**, 115–124 (2019).

5. Wagh, S. K., Lammers, K. M., Padul, M. V., Rodriguez-Herrera, A. & Dodero, V. I. Celiac Disease and Possible Dietary Interventions: From Enzymes and Probiotics to Postbiotics and Viruses. *Int J Mol Sci* **23**, (2022).

6. Koirala, S. & Anal, A. K. Probiotics-based foods and beverages as future foods and their overall safety and regulatory claims. *Future Foods* **3**, 100013 (2021).

7. Papadopoulou, O. S., Doulgeraki, A., Panagou, E. & Argyri, A. A. Editorial: Recent advances and future perspective in probiotics isolated from fermented foods: From quality assessment to novel products. *Front Microbiol* **14**, (2023).

8. Zucko, J. *et al.* Probiotic – friend or foe? *Curr Opin Food Sci* **32**, 45–49 (2020).

9. Roobab, U. *et al.* Sources, formulations, advanced delivery and health benefits of probiotics. *Curr Opin Food Sci* **32**, 17–28 (2020).

10. Guimarães, J. T. *et al.* Impact of probiotics and prebiotics on food texture. *Curr Opin Food Sci* **33**, 38–44 (2020).

11. Anal, A. K. Quality Ingredients and Safety Concerns for Traditional Fermented Foods and Beverages from Asia: A Review. *Fermentation 2019, Vol. 5, Page 8* **5**, 8 (2019).

12. Tomar, O. The effects of probiotic cultures on the organic acid content, texture profile and sensory attributes of Tulum cheese. *Int J Dairy Technol* **72**, 218–228 (2019).

13. Reid, G. The growth potential for dairy probiotics. (2015) doi:10.1016/j.idairyj.2015.04.004.

14. Linares, D. M. *et al.* Lactic Acid Bacteria and Bifidobacteria with Potential to Design Natural Biofunctional Health-Promoting Dairy Foods. *Front Microbiol* **8**, 248410 (2017).

15. Kalicka, D., Znamirowska, A., Pawlos, M., Buniowska, M. & Szajnar, K. Physical and sensory characteristics and probiotic survival in ice cream sweetened with various polyols. *Int J Dairy Technol* **72**, 456–465 (2019).

16. Araújo, E. A. *et al.* Probiotics in Dairy Fermented Products. *Probiotics* (2012) doi:10.5772/51939.

17. Trapp, C. L., Chang, C., Halpern, G. M., Keen, C. L. & Gershwin, M. E. The influence of chronic yogurt consumption on populations of young and elderly adults. *International Journal of Immunotherapy* **9**, 53–64 (1993).

18. Yasui, H., Nagaoka, N., Mike, A., Hayakawa, K. & Ohwaki, M. Detection of Bifidobacterium Strains that Induce Large Quantities of IgA. *https://doi.org/10.3109/08910609209141310* **5**, 155–162 (2009).

19. SÜTAS, Y., HURME, M. & ISOLAURI, E. Down-Regulation of Anti-CD3 Antibody-Induced IL-4 Production by Bovine Caseins Hydrolysed with Lactobacillus GG-Derived Enzymes. *Scand J Immunol* **43**, 687–689 (1996).

20. Saavedra, J. M. *et al.* Feeding of Bifidobacterium bifidum and Streptococcus thermophilus to infants in hospital for prevention of diarrhoea and shedding of rotavirus.

21. Black, F. T. *et al.* Prophylactic Efficacy of Lactobacilli on Traveler's Diarrhea. in *Travel Medicine* 333–335 (Springer Berlin Heidelberg, 1989). doi:10.1007/978-3-642-73772-5_70.

22. Bjarnason, I., Sission, G. & Hayee, B. A randomised, double-blind, placebo-controlled trial of a multi-strain probiotic in patients with asymptomatic ulcerative colitis and Crohn's disease. *Inflammopharmacology* **27**, 465–473 (2019).

23. Ouwehand, A. C., Tölkkö, S., Kulmala, J., Salminen, S. & Salminen, E. Adhesion of inactivated probiotic strains to intestinal mucus. *Lett Appl Microbiol* **31**, 82–86 (2000).

24. Isolauri, E., Joensuu, J., Suomalainen, H., Luomala, M. & Vesikari, T. Improved immunogenicity of oral D x RRV reassortant rotavirus vaccine by Lactobacillus casei GG. *Vaccine* **13**, 310–312 (1995).

25. Bruce, A. W., Chadwick, P., Hassan, A. & VanCott, G. F. Recurrent urethritis in women. *Can Med Assoc J* **108**, 973–6 (1973).

26. Mann, G. V. & Spoerry, A. Studies of a surfactant and cholesteremia in the Maasai. *Am J Clin Nutr* **27**, 464–469 (1974).

27. Selwitz, R. H., Ismail, A. I. & Pitts, N. B. Dental caries. *The Lancet* **369**, 51–59 (2007).

28. Piwat, S., Sophatha, B. & Teanpaisan, R. An assessment of adhesion, aggregation and surface charges of Lactobacillus strains derived from the human oral cavity. *Lett Appl Microbiol* **61**, 98–105 (2015).

29. Blaabjerg, S., Artzi, D. & Aabenhus, R. Probiotics for the Prevention of Antibiotic-Associated Diarrhea in Outpatients—A Systematic Review and Meta-Analysis. *Antibiotics* **6**, 21 (2017).

30. Bjarnason, I., Sission, G. & Hayee, B. A randomised, double-blind, placebo-

controlled trial of a multi-strain probiotic in patients with asymptomatic ulcerative colitis and Crohn's disease. *Inflammopharmacology* **27**, 465–473 (2019).

31. Furrie, E. Synbiotic therapy (Bifidobacterium longum/Synergy 1) initiates resolution of inflammation in patients with active ulcerative colitis: a randomised controlled pilot trial. *Gut* **54**, 242–249 (2005).

32. Pal, R. *et al.* Probiotics: insights and new opportunities for Clostridioides difficile intervention. *https://doi.org/10.1080/1040841X.2022.2072705* **49**, 414–434 (2022).

33. Sun, Q., Yin, S., He, Y., Cao, Y. & Jiang, C. Biomaterials and Encapsulation Techniques for Probiotics: Current Status and Future Prospects in Biomedical Applications. *Nanomaterials (Basel)* **13**, (2023).

34. Sun, Q. *et al.* Progress and Current Limitations of Materials for Artificial Bile Duct Engineering. *Materials* **14**, 7468 (2021).

35. George, M. & Abraham, T. E. Polyionic hydrocolloids for the intestinal delivery of protein drugs: Alginate and chitosan — a review. *Journal of Controlled Release* **114**, 1–14 (2006).

36. Morales: Microencapsulation of probiotic cells: applicati... - Google Scholar. https://scholar.google.com/scholar_lookup?journal=Nutraceuticals&title=Microencapsulation+of+probiotic+cells:+Applications+in+nutraceutic+and+food+industry&author=M.E.+Morales&author=M.A.+Ruiz&volume=1&publication_year=2016&pages=627-668&.

37. Kaushik, P., Dowling, K., Barrow, C. J. & Adhikari, B. Microencapsulation of omega-3 fatty acids: A review of microencapsulation and characterization methods. *J Funct Foods* **19**, 868–881 (2015).

38. Kvakova, M., Bertkova, I., Stofilova, J. & Savidge, T. C. Co-Encapsulated Synbiotics and Immobilized Probiotics in Human Health and Gut Microbiota Modulation. *Foods* **10**, 1297 (2021).

39. Suvarna, V. C. & Boby, V. U. Probiotics in human health: A current assessment. *Curr Sci* **88**, 1744–1748 (2005).

40. Microbiol, M. L.-F.-I. R. J. & 2011, undefined. The use of probiotic in aquaculture: an overview. *researchgate.netM Lara-FloresInt Res J Microbiol, 2011•researchgate.net* **2**, 471–478 (2011).

41. Shi, X. *et al.* Electrospinning of Nanofibers and Their Applications for Energy Devices. *J Nanomater* **2015**, 1–20 (2015).

42. Valizadeh, A. & Mussa Farkhani, S. Electrospinning and electrospun nanofibres. *IET Nanobiotechnol* **8**, 83–92 (2014).

43. Noruzi, M. Electrospun nanofibres in agriculture and the food industry: a review. *J Sci Food Agric* **96**, 4663–4678 (2016).

44. Bharali, D. J. *et al.* Nanoparticle Delivery of Natural Products in the Prevention and Treatment of Cancers: Current Status and Future Prospects. *Cancers (Basel)* **3**, 4024–4045 (2011).

45. Denyer, S. P. & Stewart, G. S. A. B. Mechanisms of action of disinfectants. *Int Biodeterior Biodegradation* **41**, 261–268 (1998).

46. Lloyd-Price, J. *et al.* Multi-omics of the gut microbial ecosystem in inflammatory bowel diseases. *Nature* **569**, 655–662 (2019).

47. Arnold, J. W., Roach, J. & Azcarate-Peril, M. A. Emerging Technologies for Gut

Microbiome Research. *Trends Microbiol* **24**, 887–901 (2016).

48. Terekhov, S. S. *et al.* Ultrahigh-throughput functional profiling of microbiota communities. *Proceedings of the National Academy of Sciences* **115**, 9551–9556 (2018).

49. Terekhov, S. S. *et al.* Microfluidic droplet platform for ultrahigh-throughput single-cell screening of biodiversity. *Proceedings of the National Academy of Sciences* **114**, 2550–2555 (2017).

50. Tao, L. *et al.* Database and Bioinformatics Studies of Probiotics. *J Agric Food Chem* **65**, 7599–7606 (2017).

51. Luo, G. *et al.* Major Traditional Probiotics: Comparative Genomic Analyses and Roles in Gut Microbiome of Eight Cohorts. *Front Microbiol* **10**, (2019).

52. Spacova, I. *et al.* Future of Probiotics and Prebiotics and the Implications for Early Career Researchers. *Front Microbiol* **11**, (2020).

53. Smits, T. H. M. The importance of genome sequence quality to microbial comparative genomics. *BMC Genomics* **20**, 662 (2019).

54. Lebeer, S. *et al.* Identification of probiotic effector molecules: present state and future perspectives. *Curr Opin Biotechnol* **49**, 217–223 (2018).

55. Lebeer, S., Claes, I. J. J., Verhoeven, T. L. A., Vanderleyden, J. & De Keersmaecker, S. C. J. Exopolysaccharides of Lactobacillus rhamnosus GG form a protective shield against innate immune factors in the intestine. *Microb Biotechnol* **4**, 368–374 (2011).

56. Allain, T. *et al.* Engineering Lactic Acid Bacteria and Bifidobacteria for Mucosal Delivery of Health Molecules. in *Biotechnology of Lactic Acid Bacteria* 170–190 (Wiley, 2015). doi:10.1002/9781118868386.ch11.

57. Astó, E. *et al.* Effect of the Degree of Polymerization of Fructans on Ex Vivo Fermented Human Gut Microbiome. *Nutrients* **11**, 1293 (2019).

58. Bein, A. *et al.* Microfluidic Organ-on-a-Chip Models of Human Intestine. *Cell Mol Gastroenterol Hepatol* **5**, 659–668 (2018).

59. Greenhalgh, K. *et al.* Integrated In Vitro and In Silico Modeling Delineates the Molecular Effects of a Synbiotic Regimen on Colorectal-Cancer-Derived Cells. *Cell Rep* **27**, 1621-1632.e9 (2019).