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Secondary Metabolites of Probiotics: Recent Advances, Mechanisms, Health Benefits and Future Perspectives

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Abstract

Probiotics are living microorganisms that confer health benefits when administered in adequate amounts, but an increasing body of evidence indicates that many of their biological effects are mediated by secreted metabolites and other microbial effector molecules rather than by persistent colonization itself. These compounds include organic acids, short-chain fatty acids (SCFAs), bacteriocins and other antimicrobial peptides, exopolysaccharides (EPS), bioactive peptides, enzymes, vitamins, amino-acid-derived metabolites, gamma-aminobutyric acid (GABA), and redox-active molecules. Collectively, these metabolites influence intestinal microbial ecology, epithelial barrier integrity, immune signalling, pathogen exclusion, oxidative stress and host metabolic pathways. Recent research has also connected microbial metabolites with host receptors, including G-protein-coupled receptors and the aryl hydrocarbon receptor, thereby providing molecular explanations for probiotic–host interactions. Importantly, the terminology surrounding these products requires precision: the International Scientific Association for Probiotics and Prebiotics (ISAPP) defines a postbiotic as a preparation of inanimate microorganisms and/or their components that confers a health benefit; purified microbial metabolites alone are not postbiotics under this definition. Recent studies have highlighted opportunities for metabolite-focused therapies because purified or standardized microbial products may offer advantages in stability, formulation and safety compared with live microorganisms. However, strain specificity, production conditions, dose, bioavailability, interactions with the resident microbiota and limited human clinical evidence remain important challenges. This review summarizes current knowledge of major probiotic secondary metabolites, their mechanisms of action, applications in food and medicine, emerging technologies for their discovery and production, and priorities for future research.

Keywords: probiotics; secondary metabolites; postbiotics; bacteriocins; short-chain fatty acids; exopolysaccharides; bioactive peptides; GABA; gut microbiota; immunomodulation

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1. Introduction

Probiotics have traditionally been investigated as viable microorganisms capable of modifying the intestinal ecosystem and producing beneficial effects in the host. However, probiotic activity is increasingly understood as a consequence of complex molecular interactions involving microbial metabolites, cell-associated molecules and host signalling pathways. Recent reviews describe these substances collectively as probiotic effector molecules, including small-molecule metabolites, secreted peptides, enzymes, extracellular polymers and extracellular vesicles. Their production is frequently strain-specific and is strongly affected by nutrient availability, pH, oxygen, fermentation conditions and interactions with other microorganisms.

The expression "secondary metabolites of probiotics" is useful but requires clarification. In microbial physiology, primary metabolites are generally associated with growth and essential metabolism, whereas secondary metabolites are not necessarily required for growth and may provide ecological or competitive advantages. Many compounds discussed in probiotic research—including lactic acid and SCFAs—are therefore better described as **microbial metabolites or bioactive effector molecules** rather than strictly secondary metabolites. Furthermore, not every metabolite is a post biotic. The 2021 ISAPP consensus defines postbiotics as preparations of inanimate microorganisms and/or their components that confer a health benefit; substantially purified metabolites such as butyrate, lactate or other isolated compounds are not postbiotics by themselves.

This distinction has become increasingly important as research moves from whole-organism probiotics toward defined microbial products. Recent literature suggests that metabolite-centered approaches may enable more reproducible mechanisms, formulation and dosing, although clinical validation remains considerably less developed than the preclinical evidence.

2. Major Classes of Probiotic-Derived Metabolites

2.1 Organic acids and short-chain fatty acids

Lactic acid is one of the characteristic products of lactic acid bacteria and contributes to pathogen suppression by lowering environmental pH and creating conditions unfavourable to many competing microorganisms. Acetic acid and other organic acids can additionally influence microbial competition and epithelial physiology.

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SCFAs—including acetate, propionate and butyrate—are among the most extensively investigated microbial metabolites. Although SCFAs in the human colon are generated predominantly by complex communities fermenting dietary substrates rather than simply by administered probiotic strains, probiotic organisms can contribute directly or indirectly to their production through metabolic interactions and cross-feeding. A 2025 *Nature Reviews Microbiology* review emphasizes that SCFAs act locally in the gut and systemically through multiple mechanisms involving epithelial, immune and metabolic processes.

Butyrate is particularly important as an energy source for colonocytes and as a regulator of epithelial and immune functions. Acetate and propionate also participate in host metabolic signaling. SCFAs can activate G-protein-coupled receptors and influence histone deacetylase activity, providing mechanisms by which microbial metabolism can affect gene expression and inflammation. However, their physiological effects depend on concentration, tissue location, receptor expression and the broader microbial environment.

2.2 Bacteriocins and antimicrobial peptides

Bacteriocins are ribosomally synthesized antimicrobial peptides produced by numerous probiotic and food-associated bacteria. Lactobacillaceae, *Lactococcus*, *Pediococcus* and related organisms produce diverse bacteriocins with activity against competing Gram-positive bacteria and, in some cases, broader antimicrobial effects.

Their mechanisms include membrane permeabilization, pore formation, interference with cell-wall biosynthesis and disruption of essential cellular processes. Because bacteriocins can inhibit foodborne pathogens, they are being investigated as natural biopreservatives and as possible alternatives or complements to conventional antimicrobial agents. Recent research emphasizes their potential in food safety but also identifies unresolved questions concerning gastrointestinal metabolism, toxicity, immunogenicity and unintended effects on beneficial members of the microbiota.

Bacillus probiotics represent another important source of antimicrobial secondary metabolites. Recent research has summarized lipopeptides, peptides and other metabolites from probiotic *Bacillus* species as potential candidates against multidrug-resistant pathogens. Their antimicrobial diversity makes spore-forming probiotics particularly interesting for biotechnology and antimicrobial discovery.

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2.3 Exopolysaccharides

EPS are extracellular polysaccharides synthesized by numerous probiotic bacteria, particularly species of *Lactobacillus* sensu lato and *Bifidobacterium*. Their biological properties depend strongly on molecular weight, monosaccharide composition, branching and three-dimensional structure.

Probiotic-derived EPS can contribute to biofilm formation, adhesion and protection against environmental stress. From the host perspective, they have been associated with immunomodulatory, antioxidant and anti-inflammatory activities. Recent research on *Bifidobacterium* EPS has emphasized their potential to influence immune responses and intestinal health while also highlighting engineering approaches for modifying their physicochemical and functional properties.

EPS may therefore be particularly valuable as functional food ingredients. Nevertheless, activity cannot be generalized across all EPS preparations because structural differences between strains can produce substantially different biological responses.

2.4 Bioactive peptides and enzymes

Probiotic microorganisms possess extensive proteolytic systems capable of transforming food proteins and peptides into smaller bioactive molecules. These products may have antioxidant, antimicrobial, immunomodulatory or enzyme-inhibitory activities. Recent research has examined interactions between food-derived bioactive peptides and probiotic bacteria, particularly the roles of peptide transport systems such as oligopeptide permease and di-/tripeptide transporters.

Proteolytic activity is particularly relevant during fermentation because probiotics can convert relatively inactive dietary proteins into biologically active peptide fractions. However, many studies remain in vitro, and the extent to which specific peptides survive digestion, reach target tissues and produce clinically meaningful effects remains uncertain.

2.5 Amino-acid-derived metabolites and GABA

Amino-acid metabolism represents another important route for production of bioactive compounds. GABA is a prominent example. Several lactic acid bacteria convert glutamate to GABA through glutamate decarboxylase. *Lactobacillus brevis*, *L. plantarum* and selected *Bifidobacterium* strains have been investigated as GABA-producing microorganisms.

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GABA-producing fermentation is being developed for functional foods and nutraceutical applications. Production can be optimized by manipulating pH, substrate concentration, fermentation mode, co-culture, immobilization and metabolic engineering. Although experimental evidence suggests potential effects on neurological and cardiometabolic parameters, claims regarding human health benefits require appropriately designed clinical trials.

3. Molecular Mechanisms of Action

Probiotic metabolites influence health through several interconnected pathways. The first is **microbial competition**. Organic acids and bacteriocins can suppress pathogens directly, while nutrient consumption and ecological interactions alter the composition and function of the resident microbiota.

The second mechanism involves the **intestinal epithelial barrier**. Metabolites can affect tight-junction proteins, mucus production, epithelial energy metabolism and inflammatory signaling. SCFAs are particularly important because they influence epithelial cells directly and modify immune-cell behaviour.

The third mechanism is **immune modulation**. Microbial metabolites can regulate macrophages, dendritic cells, regulatory T cells and other immune populations. Recent work shows that gut microbial molecules, including SCFAs, secondary bile acids and tryptophan metabolites, participate in several stages of T-cell biology, from differentiation to activation and exhaustion.

The fourth mechanism involves **host receptor signaling**. SCFAs can signal through G-protein-coupled receptors, whereas certain tryptophan-derived metabolites interact with the aryl hydrocarbon receptor. Such pathways provide molecular links between microbial metabolism and host immunity.

Finally, probiotic metabolites can influence **oxidative stress and cellular metabolism**. Antioxidant enzymes, EPS and certain metabolites may modulate reactive oxygen species and endogenous antioxidant defences. The combined effects of these pathways may explain why a single probiotic strain can produce multiple physiological effects rather than acting through one mechanism alone.

4. Therapeutic and Food Applications

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The metabolite-centered concept is increasingly attractive for functional foods, nutraceuticals and pharmaceutical development. Live probiotics may experience viability losses during food processing, storage and gastrointestinal transit. Temperature, oxygen, water activity and food matrix characteristics can substantially affect probiotic survival. Consequently, functionalization of foods with stable microbial metabolites may overcome some limitations associated with maintaining live-cell viability.

Clinical research is beginning to support this transition. A 2024 randomized, double-blind crossover trial involving 69 young adults evaluated a postbiotic preparation in chronic diarrhea. The intervention was associated with improvements in stool characteristics, defecation frequency, urgency and anxiety, alongside changes in gut microbial composition and metabolite profiles.

Another 2024 clinical trial investigated postbiotic supplementation in patients with cerebrovascular disease and reported changes in inflammatory and oxidative-stress biomarkers, although several clinical outcomes and overall microbiota measures did not differ significantly between groups. These findings illustrate both the promise and the limitations of current clinical evidence: measurable biochemical effects do not necessarily translate into broad clinical improvement.

Food preservation is another major application. Bacteriocins and antimicrobial metabolites may reduce foodborne pathogens and spoilage organisms while responding to consumer demand for naturally derived preservatives. However, regulatory approval, stability during processing and possible effects on the resident microbiota remain important considerations.

5. Emerging Technologies and Research Directions

Metabolomics, comparative genomics, transcriptomics and synthetic biology are transforming the discovery of probiotic metabolites. Rather than studying one strain and one metabolite at a time, researchers can now combine genomic biosynthetic-potential analysis with mass spectrometry and functional assays to identify previously uncharacterized compounds.

A particularly important development is the shift toward **strain-resolved metabolite profiling**. Two strains belonging to the same species may possess substantially different biosynthetic capabilities. Consequently, future probiotic products are likely to require molecular characterization at strain level rather than relying on species-level labels.

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Bioprocess optimization is also becoming increasingly sophisticated. Fermentation pH, carbon and nitrogen sources, oxygen availability, co-culturing and controlled feeding can redirect metabolic flux toward desired compounds. Encapsulation and other formulation technologies may subsequently improve metabolite stability and bioavailability. Recent reviews emphasize that such technologies could help transform probiotic-derived metabolites into more standardized therapeutic and food ingredients.

Precision nutrition represents another emerging direction. Because individual microbiomes differ substantially in substrate utilization and metabolite production, identical probiotic administration may generate different metabolic outcomes between individuals. Future interventions could therefore combine baseline microbiome profiling, metabolomics and host biomarkers to identify individuals most likely to respond to particular microbial metabolites.

6. Current Limitations and Safety Considerations

Despite rapid progress, several barriers prevent routine clinical translation. First, metabolite production is highly strain- and environment-dependent. Results obtained using cell-free culture supernatants cannot automatically be extrapolated to the human gastrointestinal tract.

Second, dose and bioavailability remain poorly defined for many compounds. A metabolite may demonstrate strong activity at concentrations achievable in vitro but fail to reach comparable concentrations at the relevant anatomical site.

Third, metabolite preparations may contain multiple active molecules, making it difficult to determine which component is responsible for an observed effect. The ISAPP definition is therefore important because it separates defined metabolites from the broader category of postbiotic preparations.

Finally, safety requires systematic evaluation. Antimicrobial metabolites should be assessed for their effects on beneficial microorganisms, while bacteriocins and other peptides require evaluation of gastrointestinal metabolism, cytotoxicity and immunogenicity. Recent literature continues to identify the lack of standardized production protocols, characterization methods and regulatory frameworks as major obstacles to commercialization.

7. Conclusion

Research on probiotic secondary metabolites is moving the field from a microorganism-centered concept toward a **molecule- and mechanism-centered model of probiotic action**.

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Organic acids, SCFAs, bacteriocins, EPS, bioactive peptides, GABA and other microbial products can influence pathogen exclusion, epithelial integrity, immune responses, oxidative balance and host metabolism. The emerging postbiotic field further demonstrates that health effects may sometimes be delivered without administration of viable microorganisms.

Nevertheless, enthusiasm should be matched by rigorous characterization and clinical validation. Metabolite activity is strain-, matrix-, dose- and host-dependent, and many reported effects still originate from in vitro or animal studies. Future research should prioritize standardized metabolomics, mechanistic validation in humans, dose-response studies, long-term safety evaluation and well-designed randomized clinical trials. The integration of multi-omics, synthetic biology, precision fermentation and personalized nutrition may ultimately enable the development of defined microbial metabolites as reproducible ingredients for functional foods and targeted therapeutics.

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