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Microbial Metabolites as Postbiotics: A Review

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Abstract:- Postbiotics are formulations containing dead/heat killed microbes or their components that provide health benefits to the host. The term "postbiotic" has captivated numerous researchers due to its extended shelf life, safe dosage limits, unique chemical structure, and the production of various signaling molecules that regulate immune-modulatory, anti-proliferative, antihypertensive, anti-inflammatory, anti-obesogenic, and hypocholesterolemic, antioxidant activities. This comprehensive analysis focuses on the practical application of probiotics and postbiotics as potential probiotic products, emphasizing their conceptual framework, mechanism of action, and practical implementation to enhance livestock and poultry productivity. The impact of postbiotics differs based on the particular therapeutic setting in which they are given, and they exert their influence by means of cellular and molecular mechanisms, including modulation of the immune and nervous systems.

I. INTRODUCTION

The animal microbiome is a complex ecosystem that houses a diverse range of microbial species, such as fungi, bacteria, viruses, and lower eukaryotes, that are vital for maintaining good health and nutrition. These microorganisms play a crucial role in the host's homeostasis by providing essential nutrients, aiding growth and development, regulating behavior, and protecting against harmful pathogens and toxins (Peixoto et al., 2021). Recent studies have revealed that the health-promoting effects of pre-probiotics are not directly linked to bacterial viability. Therefore, the viability of the microbiome is not a critical factor in achieving these benefits. This has led to further research and development of nonviable components of the microbiome for their potential health benefits. Lactobacillus, among the various lactic acid bacteria (LAB), is the most researched for the production of postbiotics (Aggarwal et al., 2022). To describe non-living microorganisms and their components that provide health benefits, the scientific community has proposed several terms, such as non-viable probiotics, ghostbiotics, paraprobiotics, heat-killed probiotics, and postbiotics (Vinderola et al., 2022). In 2021, ISAPP (International Scientific Association for Probiotics and Prebiotics) defined postbiotics as preparations of inanimate microorganisms and/or their components that confer health benefits on the host. This definition specifies that inactivated microbes, either whole or partial, must be present, with or without metabolic end products (Salminen et al., 2021). In comparison to prebiotics and probiotics, the concept of postbiotics is relatively recent. While postbiotic supplements may not be as readily accessible at present, they possess numerous advantages over probiotics. These advantages encompass ease of preparation, their purity, extended shelf life, precise action ability for mass production and capacity to elicit more targeted responses through specific ligand-receptor interactions. As research in this area progresses, the availability and utilization of postbiotics are expected to increase. (Nataraj et al., 2020). The concept of postbiotics is set to expand the range of functional microorganisms employed, encompassing species beyond the Bifidobacterium or Lactobacillaceae family. Some these species, which cannot be administered in live form due to safety concerns, have been investigated as potential postbiotics (Vinderola et al., 2022).

The focus of this review is on postbiotics, which encompass non-living microorganisms, their subcellular components, and metabolic byproducts that are produced by live bacteria during their growth or released after bacterial lysis. These postbiotics contribute to the host's nutritional and health benefits.

II. CLASSIFICATION OF POSTBIOTICS

Postbiotics are a diverse group of metabolites produced by the gut microrganisms. These metabolites include exopolysaccharides, short-chain fatty acids (SCFAs), fragments of cell walls, enzymes or proteins, and various other compounds. Additionally, postbiotics can be categorized as structural components, such as teichoic acids, plasmalogens and peptides. Another way to classify postbiotics is on their chemical composition basis, which includes carbohydrates (such as teichoic acids and polysaccharides rich in galactose), proteins (such as p40, p75 molecule, and lactocepin), lipids (acetate,butyrate, lactate,propionate, and plasmalogens derived from dimethyl acetyl), vitamins (specifically vitamin B-complex), organic acids (such as 3-phenyllactic acid and propionic acid), and other complex molecules (such as lipoteichoic acids and muropeptides derived from peptidoglycan). Furthermore, postbiotics can be classified based on their physiological effects, which include anti-obesogenic, anti-inflammatory, antioxidant, hypocholesterolemic, anti-proliferative properties and antihypersensitive, along with immune-modulatory effects (Thorakkattu et al., 2022).

❖ *Types*

➢ *Volatile Organic compounds*

The vast majority of compounds produced by intestinal bacteria during fermentation are collectively known as SCFAs (short chain fatty acids). These compounds are generated when prebiotics are metabolized by bacteria and dietary fibers are fermented by human enzymes to release energy. Numerous studies have linked SCFAs to a range of potential health benefits, including protection against tumors, immunological disorders, and inflammation in the colon epithelium. Additionally, SCFAs may help manage weight, regulate glucose levels, and address cardiovascular issues (Rafique et al., 2023).

➢ *Peptides*

Postbiotics, specifically peptides produced by the microbiota, and Bacteriocins, a well-studied type of antimicrobial peptide (AMP), possess diverse antibacterial properties. These compounds are synthesized by both Gram-positive as well as Gram-negative bacteria, and they have the potential to function as endotoxin neutralizers and immunomodulatory drugs, thereby enhancing the innate immune response (Hols et al., 2019; Rafique et al., 2023).

➢ *Cell wall fragments*

The immune responses of the host can be regulated through the interaction between cell wall fragments and the immune system. These fragments function as PAMPs (Pathogen-Associated Molecular Patterns), which are specifically recognized by PRRs (Pattern Recognition Receptors) found on immune cells (Liu et al., 2023). These macromolecules are highly effective and play a crucial role in stimulating the immune system and releasing a variety of cytokines such as Interleukin-1, 6,8,12, TNFa, and IFNa (Aggarwal et al., 2022).

➢ *Cell-free supernatants*

During the process of microbial growth, a multitude of biologically active metabolites are synthesized. These metabolites can be extracted from microbial cells using centrifugation and have been discovered to contain abundant antioxidants, flavonoids, phenolics, antimicrobics, and anticancer compounds (Hamad et al., 2020). The biological effects on the health of the host are more pronounced in CFS due to the synergistic interaction of different biomolecules, as opposed to purified bio molecules (Hartmann et al., 2011). The culture filtrate of *Lactobacillus* and *Pediococcus* species was found to contain significant amounts of hydrogen peroxide, lactic acid, diacetyl and protein, all of which exhibit inhibitory effects against *Escherichia coli*, *Staphylococcus aureus*, *Aspergillus flavus* and *Aspergillus niger* (George-Okafor et al., 2020).

➢ *Exopolysaccharides*

In the last few decades, the therapeutic potential of exopolysaccharides (EPS) has captured the attention of the medical and food industries. These extracellular carbohydrate polymers, produced and secreted by microorganisms, have a high molecular weight and have been extensively studied for their applications in medical treatments and the food sector (Zhong et al., 2022). EPS displays a plethora of biological activities, ranging from immunomodulation, anti-tumor effects, prevention of mutations, antioxidant properties, and anti-inflammatory actions to control of hypertension, antibacterial and antiviral activities, cholesterol-lowering effects, and gastrointestinal benefits. These diverse attributes position EPS as a promising candidate for biomedical and pharmaceutical applications (Makino et al., 2016).

➢ *Other postbiotic metabolites*

Phenolic metabolites, vitamins, and aromatic amino acids are among the essential metabolites that can be synthesized by the gut microbiota for the host. These metabolites play a role as signaling molecules and substrates for animal metabolic reactions, influencing a range of physiological and pathological processes (Liu et al., 2023).

III. MECHANISM OF ACTION

Salminen et al. (2021) has reported that the International Scientific Association of Probiotics and Prebiotics (ISAPP) consensus statement has shed light on the enigma of postbiotic action. Various mechanisms are involved in this process, such as the improvement of epithelial barrier functions, the modification of resident microbiota, the adjustment of local and systemic immune responses, the regulation of systemic metabolic responses, and the transmission of signals through the nervous system.The interaction between host and microbial products through MAMPs (microbe-associated molecular patterns) of commensals with specific PRRs (pattern recognition receptors) on host cells is the basis of the signaling mechanism of postbiotics. MAMPs encompass a range of surface molecules, including cell wall proteins, teichoic acids, nucleic acid, polysaccharides and many more. The key PRRs involved in this mechanism consist of CTLRs (C-Type Lectin-Like Receptors), TLRs (Toll-Like Receptors), GPCRs (G-Protein-Coupled Receptors) and NLRs (Nucleotide-Binding Oligomerization Domain-Like Receptors) (Teame et al.,2020). Through the gut-brain axis, postbiotics have the capacity to enhance the structure of gut microbiota and contribute to the prevention and management of different diseases. Microbes possess the ability to produce diverse neuroactive substances, including neurotransmitters like acetylcholine, serotonin, GABA, dopamine and compounds capable of binding to receptors expressed in the brain, like bile acids and indoles. These substances have the potential to impact both the gut and the central nervous system, thereby governing behavioral and cognitive functions in animals and humans (Haque et al., 2022). The health-promoting effects of some postbiotics are shown in table 1.

Table1 The postbiotics and their *in vitro* bioactivities.

Postbiotic components	Producer organism	Mode of action	Benefits	Reference
bacteriocin	*Pediococcus pentosaceus*	Antimicrobial activity	Improves the growth performance of broiler chickens	Jayaraman et al., 2013
SCFA	*L.reuteri* AN417	Controlling the expression of genes implicated in the creation of biofilm.	Antibiofilm activity	Yang et al., 2021
Exopolysaccharide	*Lactobacillus plantarum* NCU116	To stimulate the upregulation of Fas/Fasl and initiate apoptotic signaling.	Anticancer activity	Zhou et al., 2017
CFS	*L. acidophilus*, *L. plantarum*, *L. rhamnosus*,and *L. reuteri*	Antimicrobial activity	prevent vaginal infection in cows	Spaggiari et al., 2022
Cell wall-derived	Yeast	Mycotoxin remediation	limiting the bioavailability and toxin absorption,	Kudupoje et al., 2022
Bacterial DNA	Bifidobacterium	decrease of IL-1β and increase of IL-10	anti-inflammatory effect	Lammers et al., 2003

- *Production Technologies for Postbiotics*

The production of postbiotics greatly benefits from the fermentation process, which serves as a crucial source. Metabolomics, a potent method for quantifying micromolecules in complex biological systems, proves to be an ideal tool for detecting these valuable postbiotics (Gueniche et al., 2022). In addition to the natural process of postbiotic formation, a range of laboratory techniques, including ultraviolet light, high pressure, thermal treatments, formalin inactivation, sonication and ionizing radiation, can be utilized to enhance the nutritional profile, prolong the shelf life, and improving overall well-being for various types of foods, whether fermented or non-fermented. Apart from pH modifications, ohmic heating, supercritical CO_2, pulsed electric fields, and drying techniques, there could be more efficient methods available for the inactivation and generation of postbiotics (de Almada et al., 2016). The most prevalent approach for the preparation of postbiotics involves the use of temperature and formalin.

- *Characterization of Postbiotics*

The analysis of postbiotics, characterized by their complex composition and varying polymerization levels and glycosidic bonds, demands the use of advanced equipment and multiple concentration/purification steps. Commonly utilized methods such as spectroscopy (FTIR, NMR), chromatography (thin-layer chromatography, liquid-phase, gas-phase), and spectrophotometry are employed for both qualitative and quantitative assessment (Barros et al., 2020; Moradi et al., 2021).

- *Future prospects*

The latest biotics research suggests that postbiotics are a preferable alternative to probiotics, as they offer comparable health benefits with minimal risk of introducing live microorganisms. Furthermore, the development of "precision postbiotics" for targeted therapeutic and preventative medicine is an exciting area of research, which could lead to more effective treatments for specific illnesses in particular patient subgroups (Goswami et al., 2018). The future of postbiotics research holds promise in harnessing the power of microorganisms as functional ingredients. This interdisciplinary field has the potential to merge the realms of food science, microbiology, healthcare, and personalized treatment, opening up new avenues for exploration and innovation. (Aggarwal et al., 2022).

IV. CONCLUSION

The realm of postbiotics is currently in its nascent phase when compared to prebiotics and probiotics. Further exploration and investigation in this domain hold the potential to enhance physiological functioning and promote the well-being of the host. It is becoming increasingly evident that non-living microorganisms and/or their components can offer advantageous effects on the well-being of a recipient when administered in adequate quantities. Further exploration into the biological reactions of metabolites and the interactions between hosts and postbiotics through diverse - omic methodologies will uncover additional uses for postbiotics in both domains i.e. clinical and non - clinical fields. Research is needed to develop a set of appropriate regulatory and safety parameters for postbiotic products until the EFSA (European Food Safety Authority) and FDA (Food and Drug

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Administration) can develop a regulatory framework for postbiotics.

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➢ *Competing Interests*

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