

A study exploring new insights on postbiotics

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Abstract:

Postbiotics are soluble compounds from bacteria with beneficial effects on hosts, produced through fermentation processes, sometimes involving bacterial death that yields suboptimal compounds. They are utilized in foods for functionality, nutraceuticals for health improvements, and pharmaceuticals for disease diagnosis. Despite this, understanding and quantification of postbiotics are lacking. This review explores their production methods and therapeutic, industrial, and functional properties, emphasizing their role in food safety and applications. Postbiotics include secretions from living bacteria such as polysaccharides, teichoic acids, soluble proteins, and organic acids, many being bioactive compounds. Conversely, postbiotics can also emerge from cell-free supernatants, leading to lower bioactivity. Recently, they have garnered interest for extending shelf life, enhancing nutritional properties, and promoting health. The diverse bioactive components have broad applications across food, pharmaceuticals, nutraceuticals, and agrochemicals. Increased research focuses on different strains of Lactic Acid Bacteria, highlighting their postbiotics for food structuring applications. Breakthroughs in understanding postbiotics' biostabilization of hydrocarbons reveal new avenues for controlled structuring. Anticipation grows for food-grade, bio-based, and biodegradable alternatives to synthetic polymers, with postbiotics from whey protein concentrate also showing potential. Improved experimental techniques are enhancing comprehension of postbiotics' interactions in food matrices and microbiomes both in vitro and in vivo.

Keywords: Postbiotics, Fermentation, Functional foods, Mechanism of action

Introduction to Postbiotics

Functional foods are now gaining significant research attention due to their health endorsing properties from bioactive components present in the food, which may be living cells and/or nonviable cells. Foods that contain viable probiotics along with fermentable prebiotics are thought to provide health benefits. Among several functional bioactive compounds and components, 'Prebiotics' is the most studied one. The term 'Postbiotics' specifies any soluble substances that are secreted by living bacteria in the food matrix or released after the lysis or the death of bacteria (Oglio et al., 2022). Recent studies suggest that postbiotics may have vast potential in the pharmaceutical, food, and nutraceutical industries for health promotion and ailments prevention as they contain different signaling molecules that exhibit excellent antioxidant, immunomodulatory, anti-inflammatory, antihypertensive, antiproliferative, and other properties (Asif et al., 2023). Thus, postbiotics might be the options of choice instead of probiotics, as most of the diseases induced by probiotics occur due to the viability of probiotics. This review explicitly highlights the production and product features of postbiotics which can be utilized for the applications in the broadest field from food to therapy.

Probiotics have been at the forefront of academic and commercial interest. Since the advent of the concept, they have been marketed as health-enhancing foods, supplements, and medicines. Such preparations contain beneficial bacteria, and to be strictly termed probiotics, they must be administered in sufficient numbers with live microbes in mathematical proportion. Probiotic bacteria exert their health benefits in food, feed, and therapeutic applications using a multitude of mechanisms. The secretion of bioactive compounds (biomolecules) is one of the major mechanisms by which probiotics exert their bioregulatory effects. As degrading food matrices retain the majority of the probiotic metabolites and cell-specific structural products, there is now considerable market interest in postbiotics. In soil samples, a

large, purified postbiotics concentrate containing live bacteria and biotic components was given to maintain soil microbial communities and soil health. In the health food cosmetics sector, bioprocessed materials containing bacteria and/or metabolites from probiotic lactic acid bacteria used were marketed for applied skin-related benefits.

Historical Context of Postbiotics

An integral part of modern life, microbial interactions are critical for the development and maintenance of health in multicellular organisms. With illumination of these interactions, the number of human applications of microbes has exploded, for example, probiotics: live microbial food supplements having health benefits (Vinderola et al., 2023). Initially focused on lactobacilli, bifidobacteria and yeasts, probiotics have expanded to include other lactic acid bacteria, together with similar genera such as *Enterococcus*, *Naeronococcus*, *Propionibacterium*, and *Akkermansia*. Probiotic preparation on human health is burgeoning, generating a multitude of clinical trials and meta-analyses. With the market projected to reach USD128 billion in 2029, there are many product formulations available, with many different organisms and targets.

Discovery of new microbes, greater understanding of existing microbes, and insights from pharmaceutical developments have opened avenues for translating probiotic benefits to their non-viable counterparts. The term “postbiotics” is used to define non-viable probiotic microbes and/or their metabolites with beneficial effects on host health. Postbiotics are spore-forming bacteria or fermented products which are largely adopted in food applications. Knowledge about postbiotics is increasing dramatically, however published research on postbiotics is also scattered. Thus, a comprehensive analysis providing the most recent research across multiple fields is timely to promote this area of research going forward for food and nutraceutical applications. Postbiotics can broadly be grouped into three categories: a) dead microbial cells, b) cell surface components, and c) ferment metabolites.

Probiotic-beneficial organisms (live or inert) can interact with the gut epithelia, and postbiotics often display the same health benefits. Discovered postbiotics now include inactivated probiotic strains with disclosed mechanisms. In the food area, tremendous technological innovations have been reported to better foods, including health-boosting probiotics with corresponding fridges or flavour-palettes. It is just at this time that health-boosting dead micro-organisms provide enhanced food products at less than 1% of the cost of their live counterparts, in ways oblivious to regulators. Postbiotics have a wider variety of applications, such as: GRA relinquished; lesser scrutiny of the food processes; faster time-to-market; potentially palatability; dilution with other active components.

Definition and Classification of Postbiotics

In recent years, postbiotics, which are fermentation products produced by probiotics and present in fermented foods, have attracted growing interest from consumers and product developers. In June 2021, the ISAPP held a symposium on postbiotics in Amsterdam, during which the ISAPP board of directors approved and published a consensus definition of the term postbiotics. This cohort of experts also compiled a Q&A document indicating how to define postbiotics and what other associated terms encompass. Aside from those directly involved in this symposium and working group, it is important to provide precision and clarity on this definition to different stakeholders in addition to just publication in peer-reviewed journals (Vinderola et al., 2023).

The growing interest in postbiotics is reflected by many recent and ongoing clinical trials investigating the potential health effects of postbiotics, and by the increased use of postbiotics in marketing and product development. To provide clarity and precision on the ISAPP definition of postbiotics and possible implications for the development and marketing of postbiotic products, the current position paper aims to address, explain, and illustrate in simple terms the questions most commonly asked about postbiotics among stakeholders in the medical field.

The term postbiotics can be understood at three levels: (1) genus definition of postbiotics, which applies independent of knowledge on the state of the microorganism; (2) operational understanding of postbiotics, which applies in the process or analytical lab; and (3) mechanistic understanding of postbiotics, which relates to the active form and function of the inanimate microorganism (A. M. Wegh et al., 2019). Each of these levels can be further categorized with increasing resolution and detail. This position paper primarily focuses on the necessary questions, implications, and requirements for the operation definition of postbiotics, while the other two levels of definition are discussed to a minor extent.

Mechanisms of Action

Over the past two decades, there has been a dazzling discovery of new microbes within the gut. This has reshaped our understanding on the links between the microbiome and human health and disease, resulting in targeted application of microbes for therapeutic purposes beyond the gut, including in immunotherapy and cancer treatment. The heightened awareness has led to a meteoric rise in investment and innovation targeting microbes, their products and surrounding environmental factors to promote metabolic health. Postbiotics are fermentations of live or dead bacteria and their by-products that are used similarly to probiotics. They are a complex mixture of molecules, containing many bioactive compounds with multiple mechanisms of action. It has been shown that live or dead probiotic bacteria have contrasting bioactivities, raising the concept of postbiotics. The applications of postbiotics were first developed in infant formula to largely complement the health promoting activities of probiotics by maintaining microbe diversity and gut microbiome resistance, while avoiding serious safety concerns associated with the unpasteurized delivery of living microbes. The non-viable probiotic bacteria and their by-products encapsulated in polysaccharide matrices are increasingly being formulated into food, beverages, pharmaceuticals, and cosmetics as consumer health products (Mosca et al., 2022). The mechanisms of action by which postbiotics exert their benefit and their role in human health

are not clearly understood in most instances. Two of the major mechanisms by which postbiotics could potentiate a clinical benefit are immune system modulation and enhancing intestinal barrier function. Experimental evidence shows that dead bacteria could enhance barrier function, through the stimulation of tight junctions, or by stimulating mucous production. Several dead *Lactobacillus* species have been shown to enhance the intestinal mucous production through the activation of nucleotide-binding oligomerization domain in experimental studies. Other groups have shown that autoclaved *Bifidobacterium animalis* subsp *lactis* for example could enhance barrier function through the stimulation of IL-10 and mucal production.

Metabolic Pathways

Probiotic bacteria in the human body mainly contribute to health by producing various metabolites during fermentation of functional food and food ingredients. Postbiotics encompass all metabolites that contribute to health, encompassing both cellular and acellular components of probiotic bacteria. Various recent studies regarding the health benefits of postbiotics are reviewed. Special emphasis is placed on the specific organic acids identified by metabolomic and transcriptomic techniques, demonstrating anxiolytic effects after oral postbiotic treatment in a novel mouse model of chronic stress. Finally, it is suggested that a more integrative research approach among clinical nutritionists, microbiologists, biochemists, and omics specialists will accelerate exploration on postbiotics with potential implications in chronic stress-related disorders (Palade et al., 2022). Probiotics are defined as living microorganisms that confer a health benefit to the host. The primary modes of operation involve fermentation metabolism of functional food leading to the generation of beneficial postbiotics. Until recently, most of the referees gave credit for the myriad of health benefits of probiotics to the fermentative metabolites produced (short-chain organic acids including acetate, propionate, and butyrate), and as such, the metabolites were regarded as the postbiotics. However, several investigations also support that larger cellular components, in the form of intact cells, as well as

cell membrane fractions with bioactive signaling molecules such as exosomes, also confer a myriad of health benefits (particularly anti-inflammatory effects). In such a case, the term postbiotics embraces all metabolites that contribute to health, regardless of cellular or acellular components. An acute stress imposed by 15 minutes of water immersion evokes a significant weight loss and suppresses locomotor activity, which is associated with increased anxiety. Chronic stress enhanced hypothalamic expression levels of corticotrophin-releasing hormone and the phosphorylation levels of c-jun NH2-terminal kinase, leading to the development of anxiety-depressive mood. On the other hand, chronic stress reduces the postbiotic-induced enhancement of the Phosphorylated form of protein kinase B (Akt) signaling and the mRNA levels of neuropeptide Y in the Wenyuan-portion, affecting the anti-anxiolytic effect of postbiotics. The present data indicate that fecal concentrations of organic acids postbiotically generated from administration of the postbiotics such as, or its combination with RSM have implications in targeting mechanisms of chronic stress-induced mood disorders.

Immune Modulation

Postbiotics are defined as non-viable microbial cells and/or products, secreted by or released from non-viable microorganisms, which confer health benefits on the host (Szydłowska & Sionek, 2022). The potential health effects of postbiotics may include stabilization of the host-microbiome balance, regulation of the immune response and metabolism, pathogen exclusion, antimicrobial activity, prevention of symptoms of gastrointestinal disorders, and relief of symptoms of allergies, respiratory problems, depression, and neurodegenerative diseases. Due to the variety of health properties postbiotics exhibit, they may be included in the group of functional food ingredients. There is a growing interest in food products containing bioactive postbiotic components, which act in a variety of ways on the human body, making them safe for consumption and eliminating the occurrence of antibiotic-resistant strains.

Postbiotics can bring potential health-promoting benefits for the host. The cellular and molecular mechanisms by which

postbiotics influence the immune system, as well as potential pro-health profits, are currently being studied intensively. The majority of the studies carried out so far were conducted with regard to different strains of *Lactobacillus*. However, postbiotics are also a product of various bifidobacteria. Products obtained from bifidobacteria may also bring potential health-promoting benefits for the host. The study also presents the methods used to generate postbiotics as well as those used to analyze them. The stabilization of the host-microbiome balance through the preservation of the intestinal mucosa barrier plays a key role in the protection against infections and in inhibiting the development of pathogens. It has been demonstrated that postbiotics may be a promising tool in preventing and alleviating gastrointestinal diseases. The benefits of postbiotics mainly stem from their effect on the digestive system, including relief of symptoms of gastrointestinal disorders.

Sources of Postbiotics

There is a significant difference in the scope of usable biomass between in vitro fermentation and subsequent in vivo outcomes of the fermentation. This is especially true when using infant feces as inoculum, given the opportunities for intergenerational differences in the gut microbiome or variation in bifidobacterial strains across different cultures (A. M. Wegh et al., 2019). In vivo studies are often designed with emphasis on controlling variation of adults feeding the same diet. In addition, for pragmatic considerations the added amount of postbiotic may sometimes be thrice the dose at which it was determined functional in vitro. Nevertheless, it is highly relevant to investigate whether these postbiotic preparations retained their functional properties when the fermentation was performed with a different substrate or more complex inoculum. Studies have already pointed out differences in the efficacy of postbiotic products produced with different substrates. It could also be envisaged that differences occur between non-functioning fermentation endproducts versus litogenic fermented end-products. Postbiotics are much less sensitive to degradation than probiotics and prebiotics; however, the position of functional moieties in

for instance a polysaccharide of 0.1 to 1 MDa could affect its digestion, stability and transport (Asif et al., 2023). Functional evaluations of cargo compounds under various conditions may elucidate the prospects of their development as food ingredients or supplementations. Often activity/structure studies focus on purely synthetic compounds, whereas it may be highly informative to explore the activity and structure of isolated fermentative end-products candidates. Such compounds are sometimes patented for their health benefits in food products, and thus new insights may yield candidates for commercial production that outperform synthetic options.

Fermented Foods

According to the World Health Organization, fermented foods are “produced through controlled microbial growth and fermentation of food components.” Fermentation converts elements of a complex food matrix into simpler, low molecular weight products. There are generally two types of fermentation: one where the genuine food matrix is altered, such as in the cheese and yogurt-making processes through the addition of milk starter cultures, and one where the matrix remains intact but the microbial and/or enzymatic activity affects the food microbiota and alters the functional properties of the food matrix (Tong et al., 2023). The most studied fermented foods are kombucha, kvass, kefir, and buttermilk.

Fermented foods have been studied for their health effects and have been shown to decrease the risks of several non-communicable diseases. Fermented foods are of interest to public health entities, the food industry, and food science researchers to improve disease prevention, health promotion, taste, and product safety. Although fermented foods generally contain probiotics, their microbial and metabolomic compositions are very complex. The health effects of fermented foods are mainly attributed to postbiotics and secondary metabolites produced during fermentation. Fermented foods are heterogenous food matrices that encompass “food components (nutrients, chemicals or food additives) or microbiota (bacteria, yeasts or viruses) resulting from a fermentation process capable of eliciting health benefit.” In

food science, there is also considerable interest in defining and characterizing new postbiotics from fermented foods and gaining insights into their underlying alteration processes. Fermentation holds great potential for the generation of new prebiotics. Prebiotics may be more efficient for food production, as addition of new raw substrates can be avoided. As an example, different postbiotics were characterized from bile-fiber fermented soy sauce broth. Prebiotics have been studied in greater detail compared to postbiotics.

Microbial Fermentation

Microbial fermentation can be utilized to produce a wide range of postbiotics due to the diversity of microbial, fermentation, and byproduct types. Fermentation of different substances produces different postbiotics with different effects. New products can be explored and increased with diverse microbes and fermentation methods. Liquid state fermentation is commonly used due to the widespread application of this method in industrial processing. However, solid-state fermentation has shown superior characteristics and unique products too, and applications of solid-state fermentation methods in postbiotics production are timely and noteworthy (Tong et al., 2023).

The fermentation processes of *Lactobacillus* and *Bacillus* sp. are explored using black rice bran, which is abundant, inexpensive, and nutritious as a substrate. Bacteriocins, postbiotics with antimicrobial activity, and various fermentation conditions are screened to optimize production using the response surface methodology. The supernatant of fermented black rice bran is demonstrated to relieve oxidative stress, inflammation, and injury to Caco-2 and RAW264.7 cells similar to that of probiotics. HPLC-QTOF-MS is used to explore bioactive metabolites and 64 candidates with potential health benefits are identified. It is anticipated that screening the world of fermentation substrates and microbes would provide practical, effective, and low-cost postbiotics for human health.

Health Benefits of Postbiotics

Despite growing interest in postbiotics, research is still in its

infancy and many questions remain unanswered. Further research is likely to extend experimental strategies that have been published for probiotics and prebiotics. Postbiotics are defined as preparations of inanimate probiotics and/or their cellular components, and/or their metabolites/end products that convey health benefits to the host. Recent publications provide an increasingly large number of possible postbiotics and report on their beneficial interactions with gut-associated immune system; for example, in studies with mice models of immune disorders or irritable bowel syndrome there is considerable scope for further investigation of postbiotics for these conditions in various species including humans. If the desirable modulation of the immune system would be established, possible use as food compounds or supplements in animal or human nutrition is also an area of research that warrants further attention. The concept of postbiotics is rapidly maturing in human nutrition. In January 2019, WHO members published a review that shows the caveats of this assumption. In fact, a wide variety of inanimate or non-viable products obtained during probiotic metabolic activity, but per se not considered “postbiotics” are mentioned (A. M. Wegeh et al., 2019). The authors define “postbiotics” as preparations of inanimate probiotics and/or their cellular components, and/or their metabolites or end products that confer health benefits to the host. Increasing studies are demonstrating the beneficial effects of postbiotics on human health highlighting the immunomodulatory, anti-inflammatory, antioxidant, and anti-viral properties (Oglio et al., 2022). This review outlines the classification of the postbiotics and describes some recent inventions on isolation and recovery, preparations, efficacy assessment, molecular mechanisms of action, and technological applications of the postbiotics, all aimed at palliative or preventive therapy or supplements for prevention of disease. These beneficial effects of postbiotics could be related to the activities of their components, including lipopolysaccharides (LPS), lipoteichoic acid (LTA), peptidoglycans, bacteriocins, enzymes, nucleotides, peptides, or short-chain fatty acids (SCFAs). Most postbiotics in these studies are based upon *Lactobacillus* species and hence are also referred to as “milk fermented with *Lactobacillus* species”

in which the specific health beneficial functional substratum in the “postbiotics” is often not identified. Postbiotics are also produced using various types of bacteria including *Bifidobacterium*, *Bacteroides*, *Enterococcus*, thermophilic lactic acid bacteria, and propionic acid bacteria. Some components of the postbiotics interact with the host’s immune system via Toll-like receptors (TLRs). LPS of Gram-negative bacteria are recognized by TLR4 and induce the production of cytokines. LTA and peptidoglycans are recognized by TLR2, and also induce the production of cytokines. In addition to TLRs, flagellin is a major structural component of the flagella of many bacteria and it is recognized by TLR5. On the other hand, bacteria RNA can bind TLR3, TLR7, and TLR8, whereas bacterial CpG-DNA is recognized by TLR9. Another relevant postbiotics component is the SCFA butyrate. It binds the G protein-coupled receptors such as GPR41, GPR43, GPR109, and other nuclear receptors. The activation of TLRs or G protein-coupled receptors by the postbiotics components induces innate immune response and thus indirectly activates adaptive immunity. Such advantages may be especially relevant in case of the food chain, and situations where production and storage conditions are hard to maintain, for example in developing countries, may serve as an alternative to provide the beneficial effects of other “biotics” or biotic products in vulnerable population.

Gut Health

There are areas where postbiotics have potential efficacy, for example the liver. The gut-liver axis is a bidirectional relationship between the gut, its microbiome, and the liver. A healthy microbiome maintains gut-liver axis homeostasis. Liver cirrhosis is associated with a profoundly altered microbiome and damaged intestinal barrier. There is increasing evidence that microorganism-derived metabolites including trimethylamine, SCFAs and ethanol have a pathogenic role in non-alcoholic fatty liver disease. Postbiotics are considered a potential new therapeutic avenue for these liver diseases, but more research is needed to confirm their benefits. Establishing the interaction mechanisms between postbiotics and commensal microorganisms will improve the

understanding of the potential clinical benefits of postbiotics and possibly lead to targeted postbiotic therapy. Optimization of postbiotic production from *Bacillus amyloliquefaciens* J and *Lactiplantibacillus plantarum* SN4 fermentation it is essential to identify economically effective alternatives to chemically synthesized antibacterial/regulatory agents that can induce resistance mechanisms in pathogens. In that regard, a growing trend is to use fermented food or plant extracts and natural compounds to select probiotics with beneficial properties that promote gut health. In brief, we developed postbiotics through solid-state fermentation with *Bacillus amyloliquefaciens* J and *Lactiplantibacillus plantarum* SN4. These postbiotics exhibited significant antibacterial activity against pathogenic bacteria, enhanced the viability and proliferation of human intestinal epithelial cells via targeting pathways involved in epithelial homeostasis and mucosa development, and reduced lipopolysaccharide-induced inflammatory responses in vitro and in vivo. With potent gut health-promoting effects and environmentally sustainable production, we expect that the developed postbiotics can be used as novel dietary supplements or functional food ingredients for gut health maintenance (Tong et al., 2023).

Anti-inflammatory Effects

As bacteria ferment prebiotics, specific metabolites are produced which may regulate a variety of metabolic events. Short-chain fatty acids (SCFA) such as acetate, butyrate, and propionate are the main fermentation products and are known to modify metabolic events in the gut. SCFAs are actively absorbed into the bloodstream, with >80% of butyrate uptake occurring in the colon, the primary site of butyrate production (Martyniak et al., 2021). SCFAs are involved in several metabolic processes, modulating inflammation, mucosal integrity, motility, and secretion. SCFAs are critical for the health of the gut and systemic immunity. The anti-inflammatory properties of SCFAs have been linked to the reduction of cytokines such as IFN- γ and IL17A. It was found that butyrate administration in ex vivo cultures of human colonic biopsies reduced lipopolysaccharide (LPS)-induced IL-6, IL-12, TNF- α , and IL-23 production. In a

mouse model of colitis, butyrate administration significantly decreased the levels of several pro-inflammatory cytokines, prostaglandin E2, and cytochrome P450 (CYP). In addition, butyrate ameliorated renal inflammation by downregulating IL-6, IL-1 β , TNF- α , and MCP-1 levels in serum and kidney tissues. The anti-inflammatory activity of SCFAs has been recently correlated with the upregulation of GlcN-6-SO4 and consequently GAGs production in SKCO-15 and Caco-2 cells. However, it has not yet been studied whether butyrate or other particular SCFAs induce GlcN-6-SO4 production and correlate with the activation of the GAG biosynthetic pathway in macrophage cells or other intestinal epithelial cell lines (Sornsene et al., 2021). Macrophages are another major component of the immune system and have also been studied in biocompatibility and toxicology tests. They are classified into different phenotypes according to their activity and pro-inflammatory mediators generated. Nautiyal and co-workers demonstrated that GAGs extracted from marine organisms can suppress the inflammatory response in RAW 264.7 macrophage cells through NO limit production, IL-6 and TNF- α mRNA downregulation and iNOS inhibition. However, postbiotic analysis of butyrate/SCFAs production and GAG biosynthetic pathway activation in macrophage cells has little been reported.

Metabolic Health

Sugar consumption and obesity rates have been implicated in the risk of developing type 2 diabetes, metabolic syndrome and cardiovascular disease. Their mechanisms of action are poorly understood. Recent reports suggested that high-sugar diets at levels found in modern society might influence energy homeostasis and glucose metabolism through alteration in gut microbiota and gut-brain axis signaling pathways. This review provides a wealth of new information on how high-sugar diets remodel complex microbiota, gut bacterial metabolic function, how gut microbiota connect with the host via alteration of the gut-brain axis such as vagal signaling and blood circulation, outlining that both alterations are required for the metabolic imbalance and cardio-metabolic disease phenotype induced by high-sugar diets. Gut microbiota and

the by-products, the microbiota metabolism, are recognized as important factors in the pathogenesis of metabolic diseases. Fecal microbiota transplantation from high-fat diet-obesogenic mice into germ-free mice led to development of gut microbiota, visceral fat deposition and metabolic disorders associated with obesity and fatty liver. Recent reports showed that diet-induced microbiota by high-sugar diet were different from that by high-fat diet. Mice model of metabolic disorders induced by high-sugar diet showed lower body weight together with resistance to insulin than obesity. Biochemical, histological, genomic and metabolomics results determined that high-fat and high-sugar diets induced gut permeability, low-grade inflammation, and cardiovascular disease risk factors. plasma metabolites were identified to be significantly altered by high-sugar diets which may play a role in the development of metabolic disorders. gut microbes were found diminished by high-sugar diet, which produced butyrate and suggested to play an important role in regulating host gut barrier, metabolism, and inflammation. These changes may be applicable to the obesogenic diet-induced and cardiovascular disease risk also.

Postbiotics in Disease Prevention

The discovery of microbial processes on gut-brain signaling has led to several assessments regarding the sending of messages to different brain areas, and commonly targeted diseases to treat using live probiotics have also been outlined. Translational studies in mice and rats by several strains of lactic acid bacteria and bifidobacteria also corroborate critically on potential effectiveness in preventing and controlling depression-related symptoms. Overall consensus suggests targeting of these bacteria mainly on the mouse striatum. This approach on human gut-brain axis has built on publicly available human gut microbiota-associated g-proteins coupled receptors and their second-messengers on a behavior score-based MAPT. Drugs used against all mainly target the accumbens. A meta-analytic superreboot of gut microbiota infers on the different capabilities and somewhat opposite unlikely efficacies of faecal transplant vs postbiotic consumption on rotavirus vaccination efficiency in

the early stages of life (Scarpellini et al., 2021). Meanwhile, SNPs assigned a critical period in gene age on receptor L. rhamnosus GAD65, GAD2 S125A, and NLGN1 in GATAdg2-Abl, Defb1 -5' region, and Zbtb20 domain.

Postbiotics regulate many immune-mediated processes, but it is challenging to summarize many studies. Most documented mechanisms of action are related to immunomodulatory effects in health and disease. Generally referred to as metabolites, a broad definition includes dead or non-viable bacteria or their lysates, capsules, enzymes, or any other debris after probiotic fermentation. Postbiotics exert beneficial effects on immune response, and several mechanisms have been clarified in detail. Metabolites are more likely to act on lymphatic macrophages than on gut epithelium, thus modulating mucosal immunity through a “top-down” mechanism. Butyrate boosts the immunosuppressive profile of lymphatic macrophages, and SCFAs promote GPR43+ macrophages that drive Treg expansion. Another critical mode of action is modulation of efferent neural pathways from gut to brain, e.g., through inhibition of the immune pathway in gut-associated molestation and then the vagus way. Postbiotics used in clinical studies aimed at controlling respiratory tract colonization and infections. Bacterial peptidoglycan promoted dendritic cell maturation towards a Th2-dependent immune response through Nod2 recognition. Supernatants of bifidobacteria activated Th2 activation to control respiratory syncytial virus. The newly studied metabolite of *Lactobacillus paracasei* CRL-431 applied in recurrent wheezing children promoted tolerogenic dendritic cells and regulatory T cell expansion and reduced exacerbations of asthma.

Obesity

Obesity constitutes a major risk factor for many illnesses including diabetes, metabolic syndrome, cardiovascular diseases, and certain types of cancer (Noor et al., 2023). Many studies have been done over the past few decades to learn about the different risk factors for obesity. The gut microbiome varies widely among individuals and ethnicity. Loss of microbiome diversity (increased Firmicutes and decreased Bacteroidetes) is linked to obesity. Gut microbiomes may play a pivotal

role in the development of obesity and metabolic disorders. Gut microbiome composition and functions vary with host age, genetic background, and meal composition, which may all affect host metabolism. Until relatively recently, obesity was viewed as a consequence of an energy imbalance. The discovery of the extensive capacity of gut microorganisms to influence host energy capacity to extract energy from food, microbiome-derived metabolites that induce insulin resistance and inflammation, and gut-brain signaling pathways, all led to the re-evaluation of the first law of energy conservation and meticulously understudying microbes in the gut for their impact on energy balance. A balanced gut microbiota relies on a combination of external and internal factors. The gut microbiome is involved in carbohydrate metabolism, bile acid metabolism, and short-chain fatty acid metabolism. Different SCFAs influence different parts of metabolism through differential activation and binding to other receptors. Factors such as excessive energy intake, malnutrition in early years of life, and changes in lifestyle will disrupt the balance of gut microbiota. The diversity of microbial composition, reduced metabolic genes, and nutrient intake will lead to changes in SCFAs, promoting obesity.

Diabetes

Diabetes mellitus (DM) is a chronic, heterogeneous metabolic disorder characterized by alterations in the secretion of insulin from pancreatic β cells or insulin resistance by target organs, such as the liver, muscle, and adipose tissue. The two major forms of DM are distinguished as type 1 diabetes (T1D) and type 2 diabetes (T2D). The prevalence of DM has increased dramatically around the world, which has serious health consequences. Little research effort has been directed at the potential health benefits of postbiotics in relation to diabetes. The scientific literature examining the effects of postbiotics in DM is scarce compared with the number of publications focusing on probiotics, prebiotics, or fermented foods.

Most eligible studies investigated compounds from probiotic bacteria, including Gram-positive and Gram-negative species. Only one study considered postbiotics obtained from probiotic yeasts. Despite the different animal models of

DM addressed in the literature, studies on T1D outnumber studies on T2D. The type of outcome measure varied among the different types of postbiotics. Few studies investigated postbiotics produced by bacteria growing in the presence of sugar extracts. While research on CHO metabolic enzymes in human health is abundant, this review only highlights some aspects linked to postbiotics in regards to diabetes and its associated comorbidities. The majority of studies used homogenates of the cell walls of probiotic bacteria, which may not faithfully represent their true metabolic activity. Many studies have focused on key enzymes implicated in the breakdown of carbohydrates and carbohydrates metabolism. Most of the studies performed screening of some hundreds of strains regarding their inhibitory activity against α -glucosidase.

Previous studies on probiotics have demonstrated that some strains present an inhibitory effect on α -glucosidase activity, with the heaviest molecules retaining the highest inhibitory activity. Supernatants from three LABs also exhibited DPP-IV inhibitory activity, which is a promising result since DPP-IV inhibitors are currently used as antidiabetic drugs. Preliminary data indicate that *Lactobacillus* species are over-represented in products from the gastrically treated, tumor-bearing rats. Future research may explore the potential role of LAB postbiotics as natural AGI and DPP-IV inhibitors. Postbiotics may also represent a line of research that has been proposed for the development of anti-T2D compounds on genetically modified organisms. Regarding intestinal permeability and integrity, DM is frequently associated with **a leaky gut and gastrointestinal complications.**

Cardiovascular Diseases

Postbiotics include any substance released by or produced through the metabolic activity of the microorganism, which exerts beneficial effects on the host. Postbiotics show benefits on host health via mechanisms similar to probiotics. The currently available classes of postbiotic substances include cell-free supernatants, exopolysaccharides, antioxidant enzymes, cell wall fragments, SCFAs, bacterial lysates, and metabolites produced by gut microbiota. These postbiotics

display many beneficial effects on the human body, such as immunomodulatory effects, antitumor effects, infection prevention, and accelerated wound healing. It is of interest that postbiotics have antiatherosclerotic effects. For example, an exopolysaccharide from *Lactobacillus kefirianofaciens* prevented atherosclerosis in hypercholesterolemic rabbits. In addition, fragmented lactic acid bacterial cells from *Lactobacillus amylovorus* might be beneficial for the prevention and treatment of dyslipidemia by attenuating LDL cholesterol and triglycerides while increasing HDL cholesterol in an obese mouse model. The beneficial roles of postbiotics in this disease are intriguing.

Fatty streaks are the first visible lesions that present themselves in atherosclerosis, and they form within 1–2 months of birth in humans. A 3-hydroxy-3-methyl-glutaryl coenzyme A reductase inhibitor is effective for atherosclerosis treatment; however, statin use is contraindicated in some populations, including patients with statin-induced myopathy, sarcopenia, and liver diseases. Moreover, prolonged use could lead to an increased risk of type 2 diabetes in healthy subjects. Therefore, postponing the occurrence of atherosclerotic plaques is needed in the future. For the diet and gut microbiota, regularly consuming fermented foods may help ameliorate this disease and thus require extensive research. The experimental results from postbiotic treatments of these animals were exciting; however, their specific mechanisms of action on host cells should still be investigated. Understanding how postbiotics are metabolized and ingested into the bloodstream, as well as whether translocation occurs in the superior mesenteric lymph node, liver, and brain, is needed. Utilizing omics analysis and real-time imaging approaches may be helpful for these mechanisms.

Postbiotics and Mental Health

Mental disorders affect a significant part of the population. The gut-brain axis was identified as a crucial conduit for interaction between the gut microbiome and the brain. Increasing evidence showed that gut microbiota modulate the brain function and affect mood, memory, cognition, and behavior. This effect appears to be mediated via the

modulation of the hypothalamic-pituitary-adrenal (HPA) axis and through the gut-brain signalling pathways including the neuroendocrine, neuroimmune, neuromodulatory, and indirectly the gut microbiota-gut-brain. These microbe-host interactions may also have physiological consequences such as compositional changes in gut microbiota, the permeability of the gut and blood brain barrier, and the production of different transmitter systems with concomitant neurochemical reactions (Mosca et al., 2022).

Lactobacillus gasseri CP2305 was shown to exert anxiolytic-like and antidepressant-like effects in rodent models of stress or anxiety. Well-controlled clinical results showed that the long-term use of *L. gasseri* CP2305 prevented the decline in mental state and sleep quality seen in the placebo-group in a study of healthy adults under stressful conditions. In a clinical trial, daily intake of CP2305 significantly reduced anxiety, sleep disturbance, and salivary chromogranin A levels compared with the placebo group, providing preliminary evidence of the efficacy of postbiotics as an agent that relieves mood disturbances (C Bested et al., 2013).

In addition to the gut-brain axis, other nervous system-related diseases are associated with bile acid metabolism, malabsorption of polar lipids or amino acids, and gut microbiota alterations as well. A growing body of evidence suggests a potential use of postbiotics in these contexts. For example, a clinical trial showed that daily intake of plasmalogens, a type of ether phospholipids, inhibited memory loss and improved cognitive function in patients with mild-to-moderate Alzheimer's disease. Meanwhile, new approaches to the treatment of depression, targeting the gut-brain axis using probiotics or short-chain fatty acids (SCFAs), are being studied. Additionally, multiple sclerosis (MS) has been suggested to arise in genetically predisposed individuals following environmental trigger(s).

Current Research Trends

Research on postbiotics has gained momentum over the last eight years as they are being increasingly marketed as functional food ingredients. Research in the area is divided

into two strands—one focusing on their functionality while the other on their safety. Although some studies examine the mode of action elucidating the beneficial effects of postbiotics at the cellular or molecular level and/or elucidate mechanisms of how postbiotics influence gut microbiota composition, many studies do not focus on the underlying mode of action of postbiotics but rather evaluate their bioactivity based on gut microbiota elucidation by sequencing methods or other molecular microbiological methods (Asif et al., 2023). Research on innovative ways to deliver and analyze the vectors used for delivery is the key aspect but not much attention is paid to this aspect. It is not surprising that of the studies published, the majority were conducted with one or several strains of lactobacilli. Lactic acid bacteria and bifidobacteria are easily grown in media where they produce high amounts of lactic acid and thus exhibit inhibitory effects. However, non-lactic acid-producing bacteria also exert beneficial effects and should be further examined in food ingredients or functional foods either directly or as postbiotics. Especially in fermented foods, bacteria survive in food matrices even if lactic acid-producing bacteria exert inhibitory effects on their growth. With the rising interest in category III on the path to becoming a health claim, research on non-digestible oligosaccharides has gained further interest. One of the interesting aspects of the growing postbiotics literature will be the examination of new ingredient categories not previously studied extensively. With the future setting of standards, it will at last be possible to compare studies more meaningfully. However, it is important to keep in mind that postbiotics are heterogenic and complex mixtures that differ greatly in composition depending on the production organism and physical treatments/deliveries. Hence, even when similar production conditions are used, a difference in health-promoting activities will be noticed, especially when different genera or species are tested. These factors need to be taken into consideration when setting standards. Overall, the growing body of postbiotic literature is expected to provide deeper insights into ways in which the complexity of the postbiotic ome can be divided into simpler categories suitable for health-benefiting applications. Moreover, know-how brought in from other fields will lead to the identification of new postbiotics.

Challenges in Postbiotic Research

Recently, postbiotic, a novel biosource metabolite, has garnered widespread attention in various research fields. Postbiotics have been regarded as the metabolic end products of probiotics and their fermentation process, and deliver beneficial biological activities to the host (Tong et al., 2023). In some commercial products, the term postbiotics is mainly used for heat-killed probiotics, but in recent years, different types of postbiotics have been proposed. Candidates of postbiotics include dead cells, heat-inactivated cells, lysate of probiotics, cell wall polysaccharides, bacteria-produced extracellular polysaccharides, bacteria-produced metabolites including SCFA, exopolysaccharides, bacteriocins, and proteins, and fermented food containing bioactive bacterial metabolites. The understanding of postbiotics is still far from being comprehensive. Academic and/or industrial groups worldwide are increasingly pursuing research on the potential applications of postbiotics. Significant therapeutic or beneficial effects of a variety of postbiotics have been demonstrated on multiple disease models. The postbiotic market is expanding with great potential in pharmaceuticals, functional foods, and nutraceuticals development. Although postbiotics hold great promise for further research and development, there are also challenges and obstacles in this emerging field. In this review, the definition and classification of postbiotics, the methods used for isolation and identification of postbiotics, and representative postbiotics with potential applications are summarized. This review aims to provide an overview of current progress and highlight the important knowledge gaps of postbiotics for researchers, industry, and health authorities. The primordial definition of postbiotics is low-molecular-weight products secreted by live bacteria that confer health benefits to the host. Current research has moved beyond metabolites secreted by live cells, and some heat-killed probiotics or cell lysates are now also regarded as postbiotics. The concept and catalytic activity of postbiotics are being actively explored and developed by numerous researchers globally. Postbiotics are considered ancient cellular structures or metabolites that may precede the human experience of probiotics.

Conclusion

The probiotics and prebiotics powerfully influence the evolution of gut microbiota, becoming more and more diverse over time. However, a positive effect on gut microbiota cannot be guaranteed when it comes to postbiotic treatment. In this regard, upon review of different randomized controlled trials, treatment with ingestible SCFAs has been associated with a decrease in biodiversity of the gut microbiota, with considerable changes in some bacterial families (Scarpellini et al., 2021). It is likely that the same dose, route, and duration of treatment with postbiotics can have different effects on gut microbiota composition. In some clinical trials testing lactic acid bacteria fermented milk, positive effects on symptoms and gut microbiota dysbiosis were reported. In contrast, when the same postbiotics were administered for about a year, improvements on quality of life were not found, but a decrease in gut microbiota diversity occurred.

The immunomodulatory effects of postbiotics have been extensively reported in the past thirty years. This can be due to the functional properties of the compound per se, or to an indirect effect because of its ability to reshape, repair, and regulate microbiota dysbiosis. However, most information on postbiotics concerns SCFAs. Short-chain fatty acids can regulate blood levels of various messenger molecules. Penetration in the endothelial layer of blood vessels is still under debate, but with a predominant action at colon epithelium. Butyrate can be absorbed at a much higher rate compared to acetate and propionate. On entering the cell, all three SCFAs can maintain a local acidic pH, which may affect cellular targets directly. Concerning interferons, SCFAs are toxic for many cancer cell lines. Propionate, for instance, has been found to selectively induce apoptosis in gastric cancer cell lines.

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