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POSTBIOTICS – AN ACTIVE AND MULTIFUNCTIONAL BIOLOGICAL AGENT

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Abstract: Postbiotics are a type of metabolite produced by probiotic bacteria, which have numerous positive effects on health. These postbiotics are obtained from food grade microorganisms and offer health benefits when consumed in sufficient quantities. The term "biotic family" has recently emerged in the fields of health, nutrition and food technology. The family includes probiotics, prebiotics and synbiotics. Probiotics are live microorganisms that are claimed to improve or restore gut microbiota, thereby providing health benefits. Prebiotics are non-digestible food ingredients that promote the growth of beneficial microorganisms in the intestines. Synbiotics, on the other hand, are a combination of prebiotics and probiotics that work together to inhibit the growth of harmful bacteria. Postbiotics can be classified into different types based on various criteria, with the most prominent classification being the metabolites generated by the microbiota. These metabolites include short chain fatty acids, exopolysaccharides, enzymes, cell wall fragments, cell free supernatant and other similar substances. Postbiotics have gained attention due to their diverse range of signalling molecules. Postbiotics are naturally occurring in fermented foods like yogurt, sauerkraut, pickled vegetables and kombucha, postbiotics are commonly utilised in the food industry to prevent food spoilage.

Keywords: Postbiotics, Metabolites, Exopolysaccharides and Cell-free supernatants.

Introduction

Food, the essential need of human life has nutrients that support growth and health. In the last decade, the gastrointestinal microbiota has been recognised as being essential for health. Gastrointestinal tract microbiota involves valuable microorganisms that develop therapeutic effects and are characterised as probiotics. The investigations on appropriate probiotic strains have led to the characterisation of specific metabolic by-products of probiotics named postbiotics. Postbiotics are defined as functional bioactive compounds obtained from food-grade microorganisms that confer health benefits when administered in adequate amounts. Postbiotics include cell structures, secreted molecules or metabolic by-products and inanimate microorganisms. This heterogeneous group of molecules presents a broad range of mechanisms and may exhibit some advantages over traditional “biotics” such as probiotics and prebiotics. Due to their specific chemical structure, safe profile and long shelf-life, postbiotics may have

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anti-inflammatory, immunomodulatory, antihypertensive properties, inhibiting abnormal cell proliferation and antioxidative activities. These properties demonstrated that postbiotics can improve host health by creating specific physiological effects.

The biotic family

The term 'biotic' is derived from the Greek word biotikos, meaning 'pertaining to life' and essentially refers to the biological ecosystem made up of living organisms together with their physical environment. "The biotic family" refers to a set of terms that have emerged lately in the fields of health, nutrition and food technology. The first three members of this family are probiotic, prebiotic and synbiotic. Probiotics are live microorganisms which when consumed in adequate amounts confer a health effect on the host (Guarner and Schaafsma, 1998). Whereas prebiotics, are non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and activity of one or a limited number of bacteria in the colon (Gibson and Roberfroid, 1995). Synbiotics is the combination of both probiotics and prebiotics. The International Scientific Association for Probiotics and Prebiotics recently redefined the term "synbiotics" as a mixture consisting of living microorganism and substrate(s) specifically utilised by host microorganisms that provides a healthy benefit on the host (Swanson *et al*., 2020). Latest member of the biotic family is postbiotics, which refers to bioactive compounds that may confer health benefits on the host.

Postbiotics

Postbiotics refer to probiotic derived products obtained from food grade microorganisms that confer health benefits when administered in adequate amounts (Carmen *et al*., 2021). They include cell structures and secreted products or metabolic by products that are discharged by viable microbial cells or that are collected and isolated after the cell lysis (Wegh *et al*., 2019). Most postbiotics are derived from bacteria, commonly *Lactobacilli* and *Bifidobacterium* members; however, fungal origin postbiotics are also investigated. ISAPP defined Postbiotic are preparation of inanimate microorganisms and their components that confers a health benefit on the host, also includes inanimate microorganisms. At present, there are some commercial postbiotics, in the form of supplements or incorporated in food matrices, that are mostly intended for their use in gastrointestinal or immune-related pathologies (Cuevas-Gonzalez *et al*., 2020).

Classification of postbiotics

Postbiotics are classified into different type depending on various criteria's. The most prominent classification is based as the metabolites generated by the microbiota, such as short chain fatty acids, exopolysaccharides, enzymes, cell wall fragments, cell free supernatant and other metabolites (Hernandez-Granados and Franco-Robles, 2020).

a. Short chain fatty acids (SCFA): Short chain fatty acids are an important group of metabolites that are produced by intestinal bacteria through fermentation from plant polysaccharides. Prebiotics like fructooligosaccharides and inulin are fermented to produce SCFAs, particularly acetate, propionate and butyrate. These are present in an estimated molar ratio of 60:20:20 in the colon and feces. Butyrate is one of the most important trophic sources for enterocytes as it aids in regenerating the intestinal epithelium. It also has anti-inflammatory characteristics because it prevents nuclear factor-kappa B (NF-B) from becoming activated by reducing the expression of pro-inflammatory cytokines (Kim *et al*., 2018).

Metabolic functions of Butyrate: Butyrate serves not only as a primary nutrient that provides energy to colonocytes but also a cellular mediator regulating multiple functions of gut cells. Plate 1 is the schematic illustration of major metabolic functions of butyrate. It boosts the antioxidant system in the liver, which reduces inflammation and cholesterol synthesis, increasing insulin sensitivity while decreasing hypercholesterolemia and hepatic stetosis. In the adipose tissues, the production and secretion of Leptin (which is a hormone secreted by fat cells that reduces appetite) is increased which reduces food intake thereby helps in reducing weight, also there is increase in beta oxidation and decrease in inflammation of the cells. In the pancreas, insulin secretion is increased while glucogon is decreased which helps in blood sugar management (Coppola *et al.*, 2021).

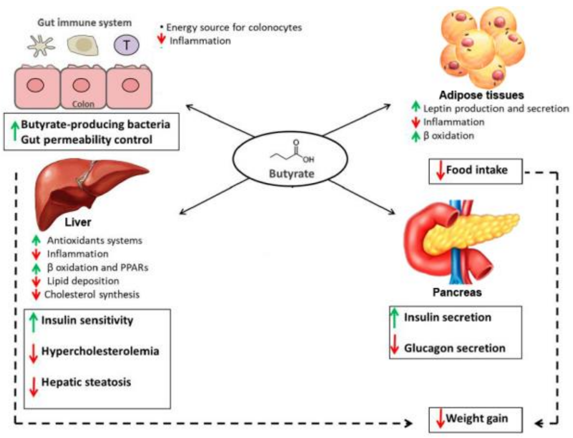

Plate 1: Metabolic functions of butyrate

b. Exopolysaccharides (EPS): Exopolysaccharides (EPS) are branched, repeating units of sugars or sugar derivatives that are long-chain, high-molecular-weight polymers and are produced by mostly lactic acid bacteria (LAB). EPS can be classified into two depending on the chemical composition as homopolysaccharides, contain only one type of monosaccharide unit (examples include cellulose, levan, curdlan, pullulan, and dextran, *etc*.) and heteropolysaccharides are those which contain repeating units of several different monosaccharides (examples include xanthan, gellan, galactan, and kefiran, *etc*.). They wrap most bacterial envelopes and play a major role in cell adhesion and protection. EPS produced by LAB have a wide range of structural variations, and this diversity allows the polymers to have a variety of bioactivities (Chaisuwan *et al*., 2020). EPS have the potential to be used in biomedical and pharmaceutical applications due to their immunomodulatory, antitumor, antioxidant, anti-inflammatory, antihypertensive, antibacterial and antiviral activity.

c. Enzymes: Enzymes can be defined as proteins that catalyse biochemical reactions. Based on their activity or function, enzymes have been categorised into six broad groups: oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases. Enzymes possess a variety of functions including physiological, biochemical, and regulatory. Enzymes are primarily derived from a small group of bacterial strains mainly *Bacillus subtilis* and *Bacillus licheniformis*; fungal strains mainly *Aspergillus niger*, and *Aspergillus oryzea* industrially. Two strains of *Lactobacillus fermentum* were reported to contain a significant amount of glutathione peroxidase which was later discovered to have strong *in vitro* antioxidant activities (Singhania *et al*., 2017). Lata *et al*. (2022) evaluated the enzymatic profiling of probiotic yeast like *Pichia kudriavzevii* Y33 isolated from traditional home-made mango pickle. This probiotic yeast was found to produce various enzymes like phytase, amylase, lipase and protease. Theses enzymes are used as flavouring agents in food industry and increases the mineral availability in fermented food products. These enzymes are helpful in the digestion of macromolecules in the human body.

d. Cell wall fragments: A number of components of the cell wall of bacteria are immunogenic, that are substances which produce an immune response which includes teichoic acids, lipoteichoic acids *etc*. Lipoteichoic acids and teichoic acids are primary constituents of the cell wall of Gram-positive bacteria and constitute about 60% of the cell wall mass in a Gram-positive bacterium. In a study conducted by Jung *et al*. (2022), the lipoteichoic acid was extracted from K8, K88, K5-5, and K55-5 strains of *Lactobacillus plantarum* and the authors also found that different lipoteichoic acid structures across the four strains of *L. plantarum* led to different immunological responses from immune cells. Teichoic acids play a crucial role in pathophysiology and antibiotic resistance processes. Both teichoic and lipoteichoic acids demonstrate a range of bioactivities including anticancer, immunomodulatory and antioxidant properties.

e. Cell free supernatant (CFS): Cell free supernatants (CFS) are liquids that contain the metabolites left behind from microbial growth and any unabsorbed nutrients from the growth medium. CFS which are formed when microorganisms are fermented, have anti-inflammatory, antioxidant and antitumor properties and are also used to treat diarrhoea. CFS produced by LAB can have an antibacterial effect due to organic acids, proteinaceous molecules and fatty acids. Along with other compounds, Lactic and acetic acid are primarily responsible for the antibacterial activity of CFS from LAB (Lee *et al*., 2002).

Health benefits of postbiotics

Recent investigations have reported that the intestinal microbiome community possesses a significant health promoting effect on the host physiology by the secretion of small biomolecules that exerts a unique ability to modulate the host cellular pathways. These small molecules act as a beneficial tool for host microbial interactions and communication. Based on current evidence postbiotics have the potency to be a safe and appropriate alternative to prevent side effects pertinent to probiotics besides the enforceable benefits such as treatments of some human complications. Postbiotics in the first step, communicate with the gut environment and intestinal cells, exert their primary health effects by modifying cellular processes and subsequently in the second step with frequent consumption of probiotic and postbiotic products confer further secondary health effects through modifying metabolic pathways in the host.

a. Anti-inflammatory: Two mechanisms explain how postbiotics can stimulate and modulate the host's anti-inflammatory response, involving both elicit immune and acquired host response. The initial response is related to the innate immune system, and it consists of a series of pattern recognition receptors able to associate with microorganisms. Two of these pattern recognition receptors involved in the host response to postbiotics are the nucleotide binding and oligomerization domain (NOD)-like receptors (NLRs) and the toll-like receptors (TLRs).

b. Anti-proliferative: Anti-proliferative effect of postbiotics is opposing the proliferation of tumour cells. Anti-cancer effect of postbiotics depends on the type of probiotic from which it is extracted. Molecular mechanisms of postbiotics in cancer prevention and treatment are involved in pathways such as modulation of immune response, inhibition of mutagenesis and carcinogens, activation of pro-apoptotic pathways, decreased bacterial translocation and increased apoptosis (Fong *et al*., 2020).

c. **Anti-microbial:** Postbiotics address the potential bacterial hazards of living cell probiotics, based on the idea that bacterial survival is not essential for human health. Although the postbiotics investigation is still generally later, antimicrobial properties show up to be one of their benefits. Studies have shown that postbiotic compounds have antibacterial (pathogenic and spoiler bacteria) effects, thus preventing infectious diseases and food spoilage. These compounds prevent the colonisation of pathogenic bacteria in the intestine and prevent intestinal diseases such as bad-tempered bowel disorder or provocative bowel illness (Chen *et al*., 2019).

d. **Immunomodulation:** Postbiotics are also associated with immune modulatory activities. This action is mediated by various mechanisms which involves maintaining the integrity of the intestinal mucosal barrier, antagonising pathogens with antimicrobial compounds and stimulating the innate and adaptive immune system. Postbiotics increases anti-inflammatory cytokine and decreases pro inflammatory cytokines. It prevents penetration of pathogens into epithelial cells thus preventing bacterial translocation. They were antagonizing pathogens, stimulating expression in Toll Like Receptors and mediate regulation of Th-1 and Th-2 mediated immune responses (Yesilyurt and Cakmak, 2021).

e. **Anti-hypertensive:** Hypertension is a significant risk factor for cardiovascular and cerebrovascular diseases and its development involves multiple mechanisms. Gut microbiota has been reported to be closely linked to hypertension. Short-chain fatty acids (SCFAs), the metabolites of gut microbiota participate in hypertension development through various pathways, including specific receptors, immune system, autonomic nervous system, metabolic regulation and gene transcription. Plasma SCFAs at basal concentrations (0.1–0.9mmol) could activate GPR41 to induce vasodilation and lower blood pressure; conversely, SCFAs with a higher concentration (0.9mmol) could activate Olfr78 to increase renin release and blood pressure levels (Wu *et al*., 2021).

Anti-obesogenic: Obesity is a major health issue worldwide. Various probiotic strains and bioactive components produced by their metabolic activity reduce body fat. SCFAs, a postbiotic activate G protein, inhibit excessive energy and insulin-mediated fat accumulation by white adipose tissue, and regulate energy balance by promoting fat consumption. Postbiotics inhibit pancreatic lipase (PL) for management of obesity (Park *et al*., 2023).

f. **Hepatoprotective:** Bioactive components of probiotics reduce oxidative stress, inflammation and fibrosis all of which play a role in the aetiology of a variety of liver diseases. Organic acids that are produced by probiotic bacteria can infiltrate the cell membrane, inhibiting nutrient transport and ATPase activity. Postbiotics prevent hepatic necro-inflammation by restoring gut barrier integrity. It activate the host endogenous antioxidant system and trigger antioxidase activities (Dewanjee *et al*., 2022).

g. Hypocholesterolemic: Cholesterol accumulation is one of the leading causes of cardiovascular disease, resulting in a high number of deaths. Therefore, lowering blood cholesterol levels is critical for preventing cardiovascular disease. HDL cholesterol removes cholesterol from the bloodstream and prevents arteriosclerosis, while low-density lipoprotein (LDL) cholesterol causes cholesterol to accumulate in blood vessels. Postbiotics reduce cholesterol levels in the body through direct or indirect mechanisms. In an experiment where *Bifidobacterium longum* SPM1207 is disrupted by sonication and postbiotics are isolated and used to analyse their effect on high-cholesterol fed rats (Shin *et al*., 2010) compared to the control, serum total cholesterol and LDL cholesterol levels are significantly reduced and HDL cholesterol levels are slightly increased in high-cholesterol postbiotic-fed rats. In recent years, dietary supplementation with probiotic metabolites such as EPSs has gained popularity to lower blood cholesterol levels. Bhat and Bajaj (2019) have observed that the EPSs of *Lactobacillus paracasei* M7 have high cholesterol lowering activity (70.78%) *in vitro*. Thus, the intake of probiotics may help manage mild hypercholesterolemia.

Potential food sources and food industry applications of postbiotics

Postbiotics, which are produced by various bacterial and fungal species including *Lactobacillus, Bifidobacterium, Streptococcus, Eubacterium, Faecalibacterium* and *Saccharomyces*, can be found naturally in fermented foods like yogurt, sauerkraut, pickled vegetables and *kombucha* (Barros *et al*., 2020). While some foods like yogurt, kefir, pickled vegetables and *kombucha* are already abundant in postbiotics or their precursors (Chaluvadi *et al*., 2015), certain postbiotics are deliberately added to specific foods instead of relying on their natural production by the original strain. For instance, the cell free supernatant from *L. pantarum* YML007 has been researched as a biopreservative for soybean grains (Rather *et al*., 2014). Exopolysaccharides (EPS) that contain rare sugars have been studied for their potential applications in the food industry. These EPS have the ability to improve the physical and chemical characteristics of food products, such as viscosity, stability and water binding capacity. They also enhance sensory properties, like palatability. However, except for dextran, EPS from lactic acid bacteria have not been commercially used as food additives due to their low yields (Torino *et al*., 2015). Nisin, a lantibiotic produced by specific strains of *Lactococcus lactis subsp. lactis*, is the only bacteriocin approved for use as a food preservative. Nisin is used in various food products, including canned soups, ice for storing fresh fish, baby foods, baked goods, mayonnaise and dairy products, particularly cheeses (Chen and Hoover, 2003).

Gomez-Sala *et al*. (2016) in their study explored the effects of a marine bacteria called *Lactobacillus curvatus* BCS35, in two different forms (solution and lyophilized), as a food ingredient in fresh fish. They discovered that both forms of postbiotics can be considered as a biopreservative for refrigerated fish. The lyophilized postbiotics from *Leuconostoc spp*. effectively reduced the growth of background microorganisms and *E. coli* O157:H7 in ground meat (Koo *et al*., 2015). Instead of directly adding postbiotics to milk, the researchers found that *in situ* production of postbiotics by lactic acid bacteria LAB showed significant antilisterial activity in refrigerated milk (Pujato *et al*., 2014). In a comprehensive study, Leyva *et al*. (2019) examined the potential of various bioprotective cultures to produce antifungal metabolites such as organic acids, free fatty acids and volatile compounds in sour cream, yogurt, cheese model and semi hard cheese. The quality and quantity of antifungal metabolites produced by protective cultures can be influenced by the food matrix and the type of culture. One way to enhance the antimicrobial potential of packaging is by incorporating bacterial cells into the material. This can lead to the production and release of antimicrobial substances, as well as the competition of probiotics with spoilage microorganisms and pathogens on the surface of food (Moradi *et al*., 2020).

Postbiotics derived from certain probiotic cultures have the ability to reduce the expression of harmful factors (such as enterotoxins and enzymes) in drug-resistant foodborne pathogens, without exhibiting antagonistic behavior (Ramezani *et al*., 2019). This capacity is specific to certain strains. Hartmann *et al*. (2011) emphasised the importance of testing the effectiveness of postbiotics on culture media containing foodborne pathogens. Their study revealed that the anti-*Listeria* effects of postbiotics were temporary, as *Listeria* growth resumed later.

A study was conducted to examine the use of postbiotics derived from three LAB in low heat milk and milk permeate on a pilot scale. The goal was to determine if these postbiotics could act as an antifungal ingredient, inhibiting the growth of fungal spoilage in sour cream and semi-hard cheese (Garnier *et al*., 2019). The results showed that the postbiotics were effective in reducing the fungal population in the cheese, while still maintaining sensory acceptance. Additionally, the antifungal capabilities of postbiotics were tested on bread by spraying them onto the bread loaves. The postbiotics derived from *Lactobacillus reuteri* were able to prevent mold contamination for 15 days when stored at 25^{o}C, and they also improved the texture of the bread (Jonkuviene *et al*., 2016).

A study conducted by Shehata *et al*. (2019) examined the impact of postbiotics from a *Lactobacillus* strain that produces antifungal substances on *Aspergillus*, a producer of aflatoxin, in wheat grains. The findings revealed that the fungal growth was completely halted, resulting in a 15 days extension of the grains' shelf life. Similarly, Muhialdin *et al.* (2011) discovered that postbiotics derived from various *Lactobacillus strains* delayed fungal growth and prolonged the shelf life of processed cheese, commercial bread and tomato puree when stored at both 4 and 30^{o}C. Interestingly, the presence of specific ingredients in the bacterial culture medium or food can trigger the production of valuable substances in postbiotics that the bacterium would not typically generate. For example, certain strains of *L. plantarum* have the ability to produce 10-hydroxy-cis-12-octadecenoic acid (HYA) from linoleic acid, which has therapeutic potential for managing colitis and diabetes (Miyamoto *et al*., 2019). HYA has a great therapeutic value for controlling colitis and diabetes. As a promising idea, co-addition of linoleic acid rich vegetable oil and *L. plantarum* into dairy products could lead to the production of such a therapeutic agent. Recently, the antimicrobial activity of HYA has been reported against *Helicobacter pylori* (Matsui *et al*., 2017).

Postbiotics offer an alternative to using live bacteria in antimicrobial active packaging systems. There are various methods for incorporating postbiotics into food packaging systems. These include applying a thin layer of postbiotics onto the polymer surface, immobilizing individual postbiotics on polymers through ion or covalent linkages, directly incorporating postbiotics into the packaging polymer matrix and laminating postbiotic loaded active film between two external layers to enhance stability and control migration (Hartmann *et al*., 2020).

Sharaf *et al*. (2019) developed chitosan nanoparticles containing postbiotics derived from seven LAB strains (*L. plantarum, L. helveticus, L. rhamnosus, L. reuteri, Streptococcus thermophiles, Enterococcus faecium*, and *L. lactis*). The antibacterial and antifungal activity of these nanoparticles was confirmed in Egyptian cheese. Additionally, Ma *et al*. (2020) utilised postbiotics from *L. lactis* ATCC 11454, a nisin-producing strain, as an amino acid source to prepare active films based on sodium alginate, collagen, sodium carboxymethylcellulose or collagen based active films.

Conclusion

Several scientific studies have provided evidence of the beneficial effects of metabolites derived from probiotics on human health. Postbiotics, which are bioactive compounds produced through fermentation and synthesised in laboratory settings, have been extensively researched and found to possess various native biological activities. These activities include anti-inflammatory, anti-proliferative, anti-microbial, immunomodulatory, anti-hypertensive, anti-obesogenic, hepatoprotective and hypocholesterolemic effects. Postbiotics, with their unique properties, show promise in controlling harmful microorganisms and can serve as a new bio preservative agent in functional food formulations. The concepts of probiotics, prebiotics, synbiotics, and postbiotics remain an ongoing trend and further studies are needed to better understand the complex relationships between these concepts and their mechanisms of action.

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