

Review Article

Pre and probiotics to postbiotics- A changing paradigm

Abstract: The relationship between microbiota and human health has long been stated by Hippocrates said, “Death sits in the bowls” in 400 B.C. Due to scientific advances in the recent years, there is an increased understanding of significance of microbiota in health and disease. The two-way communication between the host and the microbiota by the production of various types of metabolites, which plays an important role in host energy metabolism, influence nervous system function and gut brain axis, immune maturation and homeostasis, maintenance of mucosal integrity, treatment of metabolic disorders, anti-obesity, cholesterol lowering, antioxidant, anticancer, antiproliferative and anti-inflammatory properties. Postbiotics are inanimate microorganisms or their compounds include short-chain fatty acids, exopolysaccharides, vitamins, teichoic acids, bacteriocins, enzymes and peptides that confer health benefit to the host. Postbiotics can be stored under normal environmental conditions, have prolonged shelf life, easy to transport, storage, handling and overcome the limitations of probiotics use. Consequently, postbiotics have immense potential for safe and effective means of promoting health and well-being.

Key words: Postbiotics, microbial metabolites, probiotics, functional foods

INTRODUCTION

In the recent years, there has been an increasing interest in the field of host-microbiota interactions especially in the context of human health and disease. Most of the intestinal microbiota is located in the intestinal lumen, with approximately 10^{11} - 10^{12} microorganisms/gram of intestinal content, number of bacteria might be higher than number of intestinal cells. Human gut is home to trillions of microbes belongs to over 1000 species of bacteria, among which 95% are anaerobic (Afzaal et al., 2022; Liu et al., 2023). The major gut microbial phyla are: Firmicutes, Bacteroidetes, Fusobacteria, Actinobacteria, Proteobacteria, and Verrucomicrobia. About 90% of all gut microbiota is dominated by Bacteroidetes and Firmicute (Bourebaba et al., 2022; Scarpellini et al., 2022; Xue et al., 2020; Afzaal et al., 2022). Gastrointestinal microbiota (GM) is also referred to as a “metabolic organ” with metabolic capacity same like that of the liver, where a wide range of bioactive molecules, specialised metabolites which stimulate beneficial effects on the host (Rafique et al., 2023). It

is vital organ due to its multidirectional functions with other organs through immunological, endocrine, humoral and metabolic pathways (Afzaal et al., 2022). There are about 23000 genes in the human genome whereas the microbiome encodes over 3 million genes (150 times more genes than the entire human genome) producing thousands of metabolites, which replace many of the functions of the host and consequently influencing the host's fitness, phenotype, and health (Valdes et al., 2018; Afzaal et al., 2022).

There is a two-way communication between the host and the gut microbiota. By detecting host hormones and peptides, gut microbes interact with the intestinal cells. A wide range of signalling molecules were produced by the microbes and these signals from the microbial metabolites will have influence on the host immune maturation and homeostasis, energy metabolism and mucosal integrity maintenance (Martyniak et al., 2021). They also play a very crucial role in the making of some important amino acids, vitamins, breakdown and absorption of some undigested food components (Kavita et al., 2024).

Several factors influence the composition of the microbiota including age, sex, host environment, genetics and inheritance, geography, stress, infant feeding method, birth process, life cycle stages, pharmaceuticals, antibiotic intake, diet and illness. Balanced microbiota plays an important role in maintaining human health whereas dysbiosis can disrupt gut homeostasis and has been linked to the onset or exacerbation of certain diseases. Due to this link between the dysbiosis and development of several diseases led many scientists to find ways to modulate dysbiosis to counteract these pathologies (Peri et al., 2022; Cresci and Bawden, 2015; Afzaal et al., 2022; Aggarwal et al., 2022).

One of the approaches to modify gut microbiota is direct fecal microbiota transplantation from a donor into the intestinal tract of a recipient. Another method involves the ingestion of nutrients utilized by host microorganisms, known as prebiotics (Peri et al., 2022). Prebiotics are food components beneficially affecting gut microbiota includes human milk oligosaccharides, fructo-oligosaccharides, lactulose, and inulin. They have been extensively used in manufacturing of functional foods (Scarpellini et al., 2022; Martyniak et al., 2021). There are a number of ways to increase the diversity and population of intestinal microbiota are eating a variety of fermented foods, consumption whole grains, lots of vegetables, foods rich in polyphenols and other plant-based foods (Ryafique et al., 2023). Replacement of non-healthy, dysregulated microbiota with healthy microbiota is through the administration of probiotics is another way of modulation of gut microbiota. According to

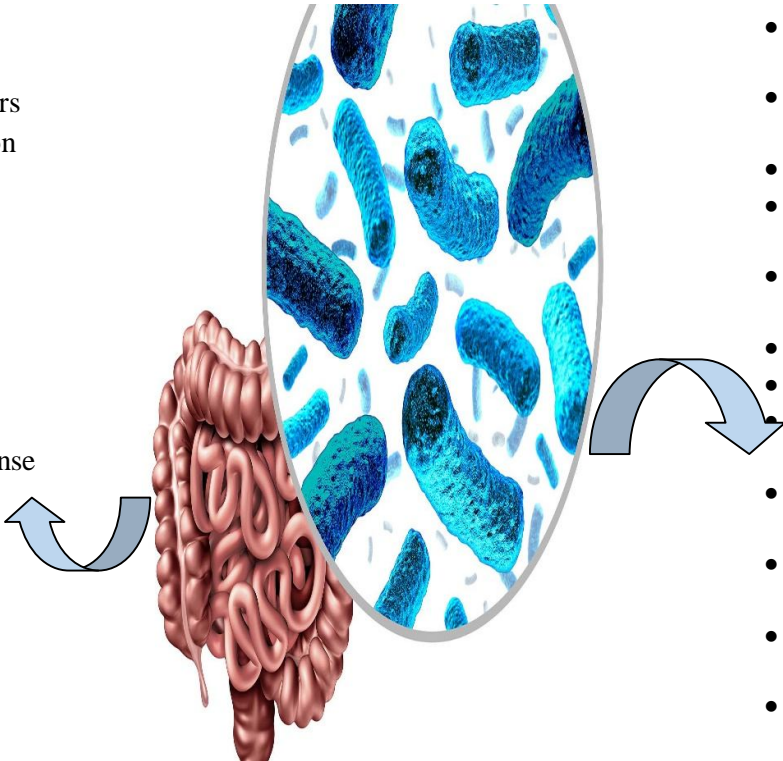
WHO/FAO, probiotics are “live microorganisms that, when administered in adequate amounts, confer a health benefit on the host” (Tomasik andTomasik, 2020; Martyniak et al., 2021; Scott, et al., 2022; Park et al., 2023; Gurunathan et al., 2024). Lactobacillus and Bifidobacterium are the most studied and generally recognized as safe (GRAS) probiotic bacteria (Monika et al., 2022) of the colon and they are suitable for amplification in the intestine, without any significant side effects (Moradi et al., 2021).Prebiotics are very often administered together with probiotics, beneficially affecting host health are called as symbiotics (Scarpellini et al., 2022).

Currently, there are two limitations that directly impact the use of probiotics effectively are: Firstly, probiotic therapies must be limited to use food safe, GRAS recognised or have Qualified Presumption of Safety (QPS) by the European Food Safety Authority (EFSA). Second limitation is when administering probiotics orally, viability of the probiotics in the upper GI track by stomach acids and bile salts may interfere with the adequate delivery of the number of viable cells. Therefore, oral administration of probiotics are not much successful in colonising health gut microbiota in the long term research studies (Scott et al. 2022).

But despite the potential health benefits of probiotics, knowledge on the safety, effectiveness, dosage and adverse effect when taken in excess amount is very limited(Tomasik andTomasik, 2020; Peri et al., 2022), necessitates careful consideration particularly in immunosuppressive individuals, having compromised gastrointestinal flora, abnormal mucosal barrier and those who are experiencing stress (Zhao et al., 2024). Even during production, maintenance of stability and viability are important and the presence of antibiotic resistance genes in the probioticscould be transmitted via horizontal gene transfer, and unpredictable strainspecific behaviours (Peri et al., 2022; Scarpellini et al., 2022).

Translocation of probiotics from gut lumen to the bloodstream and from there into vital organs, which could trigger systemic infections (Rad et al., 2021; Zhao et al., 2024). However, nowadays there is increasing evidence that health of the probiotics is due to the metabolites that they have produced rather than solely the presence of live microbes in the in host. Several evidences have also stated that health benefits associated with specific cell constituents of microbes (Scott et al., 2022). Hence, nowadays attention has shifted towards postbiotics as an alternative clinical approach of microbiota modulation (Peri et al., 2022; Scarpellini et al., 2022).

- Increases short chain fatty acids
- Increases antioxidant production
- Decreases inflammatory mediators
- Decreases pathogenic colonisation
- Improved lipid metabolism
- Increases healthy gut epithelium
- Decreases gut inflammation
- Increases insulin sensitivity
- Facilitates digestion
- Increases vitamin production
- Increases beneficial metabolites
- Increases immune response regulation
- Increases mucosal immunity
- Decreases body fat deposition
- Increases homeostasis



- Gut brain schizophrenia
- Gut brain hormonal and
- Gut lung axis
- Gut heart a hypertension,
- Gut liver inflammation
- Gut pancreas
- Gut bone axis
- Gut muscle fragility
- Gut skin axis-dermatitis
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- Gut bladder bladder

Healthy gut microbiota	Gut microbiota
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Fig 1. Gut microbiota

UNDER PELL

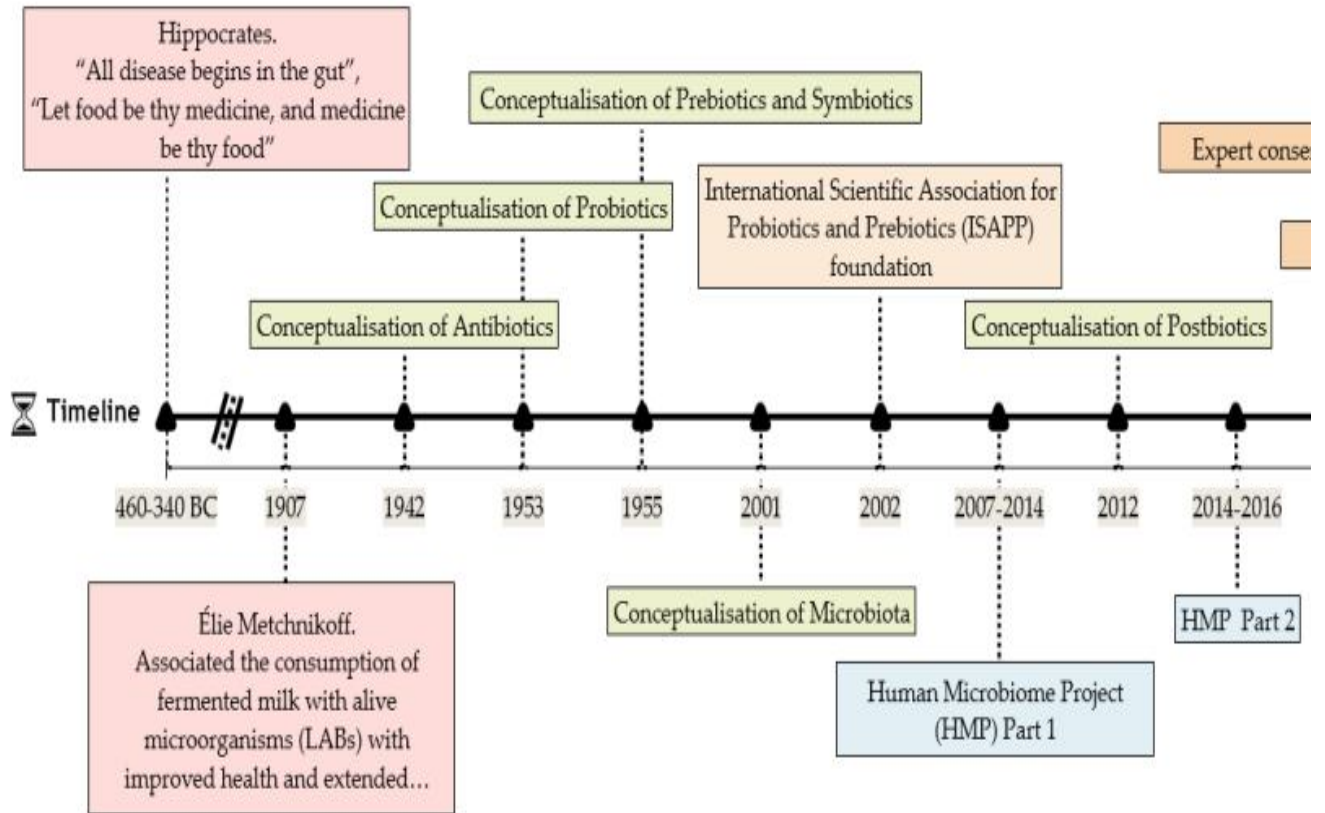


Fig 2. Timeline illustrating the main historical milestones in microbiology(Cabello-Olmo et al., 2021)

Concept of postbiotics

More than 2000 years ago, Hippocrates said that “all disease begins in the gut”. Today, it was found that pathogenesis of many inflammatory and auto immune disorders was associated with intestinal microbiota. In the early 1900s, the emergence of studies on intestinal microbiota was initiated by Elie Metchnikoff, a Russian microbiologist who began working with Louis Pasteur at the Pasteur Institute in Paris. He was the “Founding Father of Probiotics”. Human Genome Project is the “birth” of the modern age of microbiome science. The current state of microbiome science has significantly benefited from the development of postbiotics (Gurunathan et al., 2024).

The term postbiotics has been derived from the Greek word for ‘post’, refers to after, and ‘bios’, refers to life (Rafique et al., 2023; Vinderola et al., 2022; Park et al., 2023). The term postbiotics is an umbrella term defines combination of all metabolic bioactive

components generated by bacteria during fermentation/microorganism's metabolism or breakdown of bacteria, which acts beneficially to the microbes as well as humans (Tomasik and Tomasik, 2020; Wegh et al., 2019). In 2021, ISAPP (International Scientific Association of Probiotics and Prebiotics) proposed a consensus definition of postbiotics to replace previously proposed definitions. The "preparation of inanimate microorganisms and/or their components that confers a health benefit on the host" (Scott et al., 2022; Vinderola et al., 2022; Kvakova et al., 2022; Zhao et al., 2024; Cabello-Olmo et al., 2021; Park et al., 2023; Gurunathan et al., 2024). Paraprobiotics, ghost probiotics, inactivated probiotics, non-viable microbial cells, biogenic, supernatant, pseudobiotic, abiotic, metabolic, and postbiotic are the different terms used for the cell components and metabolites produced by the probiotics. Among these terms, postbiotics is the very commonly used. In some studies, cell wall components of the probiotics are categorized as paraprobiotics (Rad et al., 2021; Teame et al., 2020; Teame et al., 2020; Vinderola et al., 2022; Zhao et al., 2024; Park et al., 2023; Aghebati-Maleki et al., 2022). The different postbiotic particles incorporate metabolic results of live probiotic microorganisms.

Postbiotics are classified into intra and extracellular compounds (Malashree et al., 2019). Postbiotics can include cell supernatant, nutrients, natural acids, bacterial lysates with cell surface proteins, bacterial enzymes and peptides, metabolites produced by bacteria such as short-chain unsaturated fats, emitted proteins/peptides, bacteriocins, synapses, teichoic acids, cell wall fragments, peptidoglycan-derived neuropeptides, exopolysaccharides, polysaccharides, discharged biosurfactants, amino acids, flavonoids determined postbiotics and lower organic acids, for instance, lactic acid, organic acids, neuropeptides, terpenoid and phenolic derived compounds (Tomasik and Tomasik, 2020; Monika et al., 2022; Scott et al., 2022; Mishra et al., 2024; Liang and Xing, 2023; Rad et al., 2021; Patel et al., 2020; Fesseha et al., 2022; Kim et al., 2021; Mantziari et al., 2020; Kvakova et al., 2022; Gurunathan et al., 2024).

Various types of enzymes and vitamins produced by the gut microbe are more consumer friendly when compared to the synthetic one. Different types of metabolites produced have the ability to counter gut dysbiosis by directly modifying the gut microbiota (Mishra et al., 2024). The ISAPP stated postbiotic action by five mechanisms such as modulation of the resident microbiota; enhancement of epithelial barrier functions; modulation of local and systemic immune responses; modulation of systemic metabolic responses; and systemic signaling via the nervous system (Salminen et al., 2021). Postbiotics

can exert antioxidant, immunomodulatory, anti-inflammatory, anti-obesogenic, anticancer, antitumor, antiproliferative, antibiofilm, anti-adhesion, antihypertensive, hypocholesterolaemic, hepatoprotective, cardioprotective, anti-atherosclerotic, and anti-ulcerative effects (Cabello-Olmo et al., 2021).

Postbiotics are found naturally in any fermented food found such as tempeh, yogurt, kefir, kimchi, sauerkraut, and certain pickles, as well as inside the human body. Similar to probiotics, the production of postbiotics depends upon the types of microorganism, the strain and the metabolism product (Martyniak et al., 2021). However, as many species share the same metabolic pathways (Scott et al., 2022). Certain foods like buttermilk, fermented pickles, yogurt, cottage cheese and high-fiber foods like oats, flaxseed and garlic may increase the postbiotics in the gut (Aggarwal et al., 2022).

Various internal and external factors might affect the activity of postbiotics. Interaction between resident microbiota, various food compounds and enzymes (proteolytic enzymes) with postbiotics can inhibit its metabolic functions. External factors like PH of the food also alter the antimicrobial activity of postbiotics, optimal PH for the activity of postbiotics is between 4-9. Among the studies conducted on the use of postbiotics in the food found that both pasteurized milk and ground meat have an optimal pH, therefore they cause no disturbance in the function of postbiotics (Aggarwal et al., 2022).

They have unique characteristics such as safety, stability, non-toxicity, definitive chemical structure, facile transportation, and low-cost maintenance, safe substitutes for certain populations and provides health benefits similar to probiotic cells (Pirhadi et al., 2021; Rad et al., 2021; Mishra et al., 2024; Cabello-Olmo et al., 2021; Mishra et al., 2024). Postbiotics are deemed safer than probiotics and therefore, it has great potential value in the field of food (Ruan and Serventi, 2022). In order to confirm the safety of the postbiotic products from microbial species requires ISAPP consensus statement. Till date, there is no regulatory framework specific to postbiotic preparations for human application and their safety assessments. For characterisation of postbiotic products, they could be further processed to isolate and analyse particular health promoting compounds. Characterisation of postbiotics enables postbiotic therapies to be developed using standardised concentrations and could thus create a basis for extending and broadening postbiotic research efforts (Scott et al., 2022). Therefore, currently research is focussing on the isolation and characterisation of postbiotic for its potential use as therapeutic agents. Nyaditumresea, Cytoflora, Lacteolare the

postbiotics already approved to be used as immunomodulators, supplements and for infection control agents (Mishra et al., 2024). Lactobacillus is the most studied bacteria among the various Lactic acid bacteria for the production of postbiotics (Aggarwal et al., 2022; Barros et al., 2020).

There is no interaction between the postbiotics metabolites and food. Therefore, there is no chance of undesirable changes in the sensory quality and no risk of acquiring antibiotic resistance genes. Hence, postbiotics are used as a safe alternative in immune suppressive individuals and for newborns. Postbiotics can act both locally and systematically on other organs through the communication axis between gut and target organs like Gut brain axis, gut-liver axis and Gut lung axis (Nataraj et al., 2020).

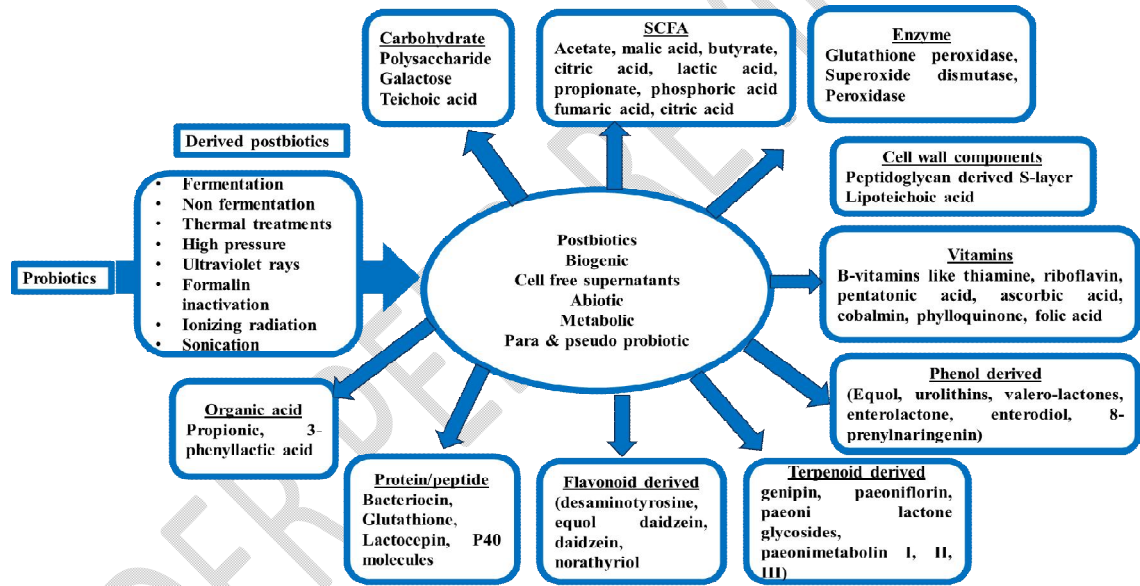


Fig 3. Main postbiotics generated by microbiota in colon and cecum

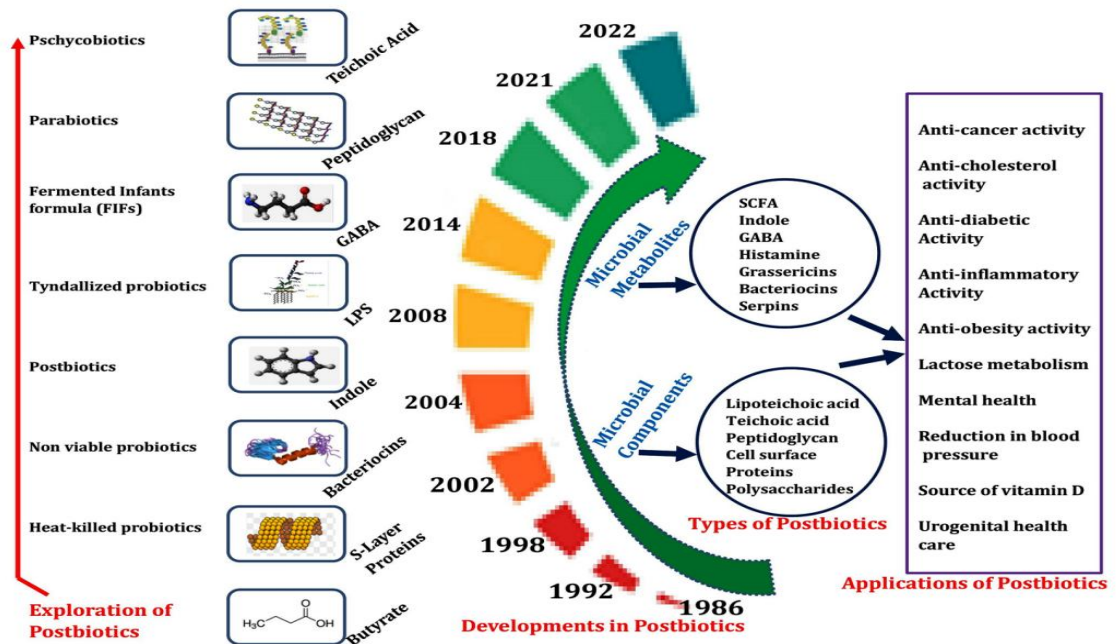


Fig 4. Developments and applications of postbiotics (Babu et al., 2023)

ISAPP Criteria for the preparation of Postbiotics are:

1. Molecular characterization of the progenitor microbes by sequencing their genes.
2. Detailed description of the inactivation methods and the matrix.
3. Confirmation that inactivation has occurred.
4. Evidence of health benefits in the host from a controlled, high-quality trial.
5. Detailed description of the composition of the postbiotic preparation.
6. Assessment of safety of the postbiotic preparation in the target host for the intended use (Babu et al., 2023).

Sources and production of postbiotics

Postbiotic can be produced both by natural and laboratory methods (Park et al., 2023). There are various techniques used for the isolation and purification of postbiotics from the probiotic species. Fermentation is an important source of production of postbiotics, in which microbial cells use prebiotics and produces a variety of postbiotics with different biological properties, resulting in the enrichment of food with beneficial substances. Along with the natural production of postbiotics, a variety of laboratory techniques can be used to improve

the nutritional profile, shelf life, and health-promoting properties of a variety of foods (Rafique et al., 2023; Park et al., 2023).

Isolation of postbiotic metabolites involves cell destruction techniques including enzymatic, thermal, solvent extraction, radiation, ohmic heating, high pressure processing, sonification, pulse electric field and drying. Whereas in case of production of cell component postbiotics, microbial cells should be exposed to factors without destroying the cell structure. Remaining extraction and clean up steps include centrifugation, dialysis, lyophilization and column purification (Teame et al., 2020; Malashree et al., 2019). Both formalin and thermal treatment are the most popular techniques for the production of postbiotics with beneficial microbial's health-promoting qualities during the generation of postbiotics (Rafique et al., 2023). Magnetic resonance spectroscopy (NMR) and chromatographic techniques are used for identification and characterisation of postbiotics (Malashree et al., 2019). The most ideal method for detecting postbiotics in complex biological systems is metabolomics (Rafique et al., 2023). Presently, these inactivation methods are useful only for the bench scale production of postbiotics. Therefore, further research is required to create new innovative techniques for the commercial level production of postbiotics that maintain functional benefits while being cost- and time-effective (Park et al., 2023).

Many probiotics are known to produce postbiotics, including *Lactobacillus*, *Bifidobacterium*, *Streptococcus*, *Bacillus*, and *Faecalibacterium* genera (Park et al., 2023; Gurunathan et al., 2024). *Lactobacillus* is a large bacterial genus consists about 60 species. Lactic acid bacteria produce a wide variety of metabolites like short chain fatty acids, fructooligosaccharides, exopolysaccharides, conjugated linoleic acids, bacteriocins, and selenoproteins, which have demonstrated promising health benefits (Zhao et al., 2024). *Lactococcus lactis*, a commonly used species, has been studied for its postbiotic characteristics in vitro, stimulating immune cells to produce cytokines, which are beneficial for overall health. Postbiotics minimize the requirement for exposure to live microbes, which is crucial for kids with developing immune systems and leaky intestinal barriers (Park et al., 2023).

Classification and functional role of postbiotics:

Exopolysaccharides (EPS): During growth, exopolysaccharides are produced by the microorganisms to the outside of the cells act as virulence factors for pathogens. In addition,

EPS have antioxidant, anti-infective and anticancer effects (Ying et al., 2023). Peptidoglycan is a complex component of the cell wall of Gram-negative and Gram-positive bacteria shows anticancer, antiproliferation, and immunomodulatory effects, and also inhibit proinflammatory cytokines (Silva et al., 2020; Park et al., 2022). Exopolysaccharides protect the intestinal epithelial cells from pathogenic microbes by forming protective biofilms on the cells. They have immunomodulatory, anti-inflammatory, anti-tumour and anti-mutagenicity, antioxidant, anti-bacterial, and anti-viral effects (Szydłowska and Sionek, 2023). They interact with macrophages, dendritic cells and modulate the immune response by enhanced proliferation of T and NK lymphocytes (Zólkiewicz et al., 2020; Chen et al., 2024). Exopolysaccharide from lactobacilli and bifidobacteria plays an important role in inhibiting pathogenic bacteria such as *E. coli* and *Citrobacter rodentium* (Mantziari et al., 2020).

The lipoteichoic and teichoic acids are the essential components of cell wall of Gram-positive bacteria possess immunomodulatory, antioxidant and anticancer properties. Lipoteichoic acid releases anti-infectious peptides like cathelicidin and defensin and promotes non-specific anti-inflammatory response (Szydłowska and Sionek, 2023). Teichoic acids have antioxidant, anticancer and immunomodulatory capabilities (Jose and Elena, 2020). Exopolysaccharides inhibit the cholesterol absorption and regulate the lipid metabolism (Zólkiewicz et al., 2020; Prajapati et al., 2023). Exopolysaccharides from *Lactobacillus kefiranofaciens* called kefiran delayed the development of atherosclerosis in a rabbit model (Zólkiewicz et al., 2020). Wang et al (2014) reported that exopolysaccharide (EPS) derived from *Lactobacillus spp* exhibited significant antiproliferative activities against colonic carcinoma cells. As an important component of bacterial cell wall, peptides present in the form of peptidoglycan reduced insulin resistance and adipose tissue inflammation in obese mouse model (Wu et al., 2023). EPS derived from *Lactococcus lactis* showed antioxidant activity by increasing antioxidant enzymes like catalase, glutathione peroxidase, and superoxide dismutase activities and lowering lipid peroxidation levels in serum and in the livers of mice (Thorakkattu et al., 2022). Exopolysaccharides made by lactic acid bacteria, particularly *Lactocaseibacillus rhamnosus*, enhance the physicochemical and sensory properties of milk products (Gurunathan et al., 2024). Currently, EPS are used as emulsifying, stabilizing, and water-binding agents in food industry (Thorakkattu et al., 2022).

Neurotransmitters

Gut bacteria like Bifidobacterium, Bacillus subtilis, Lactobacillus plantarum, Lactobacillus brevis etc., produce various neurotransmitters like catecholamines, norepinephrine, dopamine, acetylcholine, which plays vital role in the brain functioning through modulation of nerves signalling via gut-brain axis. Acetylcholine and catecholamines involved in emotion, motor control, memory and learning process. While serotonin involved in elevating the mood (Patterson et al., 2014). Gamma amino butyric acid (GABA) is an inhibitory neurotransmission inhibitor, promotes relaxation, modulate sleep disorders and decrease anxiety. These metabolites play a crucial role as antidepressants and psychiatric related disorders management (Patterson et al., 2014). GABA can induce insulin production, enhance glucose tolerance and insulin sensitivity, and exert anti-inflammatory and immunomodulatory effects, all of which help in management of diabetes (Cabello-Olmo et al., 2021).

Bacteriocins: They are ribosomal-synthesized heat-stable antimicrobial proteins or peptides produced by the gut microbiota (Wu et al., 2023; Prajapati et al., 2023). Bacteriostatic or bactericidal effect of bacteriocins inhibit the activity of pathogenic microorganisms such as *Listeria monocytogenes*, *Clostridium perfringens*, *Salmonella enterica*, and *Escherichia coli* (Mantziari et al., 2020). Antimicrobial activity of bacteriocins is directly related to their effects on bacterial peptides' structure, function and inhibits spores and pore formation on pathogenic cell membrane (Rad et al., 2021; Mishra et al., 2024). Bacteriocins have the potential to suppress or kill drug-resistant organisms in contrast to traditional antibiotics due to their ability to disrupt bacterial cell membranes and cause the leakage of internal components (Lou et al., 2023). Bacteriocins are like next generation antimicrobials and found potential for use in food preservation. European Food Safety Authority (EFSA), Food and FDA, and Health Canada approved Nisin, first bacteriocin for commercial use as food preservative. Currently, more than 80 countries using it as a food additive. Some of the bacteriocins include nisin, subtilisin, lactococcin G&Q, enterocin, lactocyclin, bovicin, plantaricin, and lactacin (Gurunathan et al., 2024).

Short-chain fatty acids (SCFAs) like acetate, propionate and butyrate are produced in the large intestine by the microbiota during fermentation of non-digestible polysaccharides (Wu et al., 2023; Chudzik et al., 2021; Babu et al., 2023). These are most studied postbiotics and the molar ratio of acetate:propionate:butyrate is approximately 60:20:20 (Vrzácková, et al., 2021; Park et al., 2022). SCFA produced have influence directly or indirectly on the brain function (Chudzik et al., 2021). They are absorbed by colonocytes, influence intestinal

mucosal immunity, barrier integrity and function. They promote indirect signalling to the brain via systemic or vagal pathways by stimulating the production of gut hormones such as glucagon-like peptide 1 (GLP1) and peptide YY (PYY) as well as neurotransmitters like serotonin and γ -aminobutyric acid (GABA) (Silva et al., 2020). SCFAs also regulate the liver mitochondrial function, regulate insulin secretion and increase the whole-body energy homeostasis by entering into the systemic circulation and to other tissues (Silva et al., 2020; Psichas et al., 2015; Larraufie et al., 2018). SCFAs, formic acid, propionic acid act against the activity of pathogenic bacteria by interfering with the cytoplasmic membrane structure, nutrient transport and macromolecular synthesis. They also have antibacterial and antifungal properties (Mantziari et al., 2020). SCFAs facilitate the growth of beneficial bacteria by creating more acidic intestinal environment (Park et al., 2022).

Propionic acid is the main source of gluconeogenesis and prevents long-term weight gain. Acetic acid has antioxidant properties, manage hunger and weight in the central nervous system (Park et al., 2022). Propionate regulate hepatic gluconeogenesis, carbohydrate metabolism, and inhibits cholesterol synthesis (Liu et al., 2023). Butyrate is the essential SCFA and primary source of energy for colonocytes. It also possesses anticancer properties and anti-inflammatory properties through inhibition of proinflammatory cytokines (Afzaal et al., 2022; Szydłowska and Sionek, 2023; Vrzácková, et al., 2021). SCFAs have the ability to cross blood brain barrier and can reach central nervous system, regulates the immune response (Szydłowska and Sionek, 2023). Butyrate salt participate in intestinal cell nutrient metabolism and promotes epithelial regeneration. It also increases the expression of immune suppressive cytokines and downregulate pro-inflammatory genes (Liu et al., 2023). SCFAs influence blood pressure in humans through various mechanisms. Butyric acid diminishes angiotensin II-induced hypertension in mice (Park et al., 2022).

When compared to butyric and propionic acid, acetic acid is less abundant but it possesses anti-inflammatory and analgesic properties. It may manage hunger and weight in the central nervous system, implying that it may help prevent cardiovascular disease (Park et al., 2022). The acetate acts as a lipogenic substrate and propionate inhibits lipogenesis by downregulating fatty acid synthase in the liver. Therefore, the acetate/propionate ratio is thought to be significant for de novo lipogenesis (Babu et al., 2023). SCFAs may play a key role in regulating inflammatory diseases by controlling the movement and activity of immune cells to sites of inflammation, and allowing the number of pathogens to decrease quickly (Lou et al., 2023). SCFAs produce mucous production in the colorectal region and provides

protection of the intestinal barrier through their anti-inflammatory effects. Insufficient availability of SCFAs is linked to many diseases including irritable bowel syndrome (IBS), inflammatory bowel disease (IBD), obesity, metabolic syndrome, type 2 diabetes, cancer, and autoimmune diseases, which in turn disrupts homeostasis and causes functional disorders (Babu et al., 2023). Autoimmune disorders like rheumatoid arthritis is often associated with intestinal barrier dysfunction. Hence, restoration of the intestinal barrier has shown potential in reducing the onset of arthritis (Ying et al., 2023).

Bacterial lysates (BL): Chemical or mechanical degradation of Gram-positive and negative bacteria results in the production of bacterial lysates. Bacterial lysates have been sold on the market for decades as an immune product for respiratory infections. BL recognize and resist pathogenic bacterial infections and stimulates the bacterial infections (Liu et al., 2023).

Cell-free supernatants: contains metabolites produced by the microbes. Supernatants of different microbes possess different activities. Supernatants of *Lactobacillus acidophilus* and *Lactobacillus casei* have anti-inflammatory and antioxidant effects on intestinal epithelial cells, macrophages, and neutrophils by increasing the secretion of the anti-inflammatory cytokine interleukin 10 (IL-10) (Fesseha et al., 2022). Supernatants from *Bifidobacterium shorteri* can reduce the release of various inflammatory mediators in dendritic cells and thus safeguarding the immune system against pathogenic bacteria (Ying et al., 2023).

Vitamins like thiamine, riboflavin, niacin, pyridoxine, pantothenic acid, biotin, folate, cobalamin and menaquinone are produced by the gut microbiota helps in DNA replication and repair, RBC formation, enhance immune functioning, works as enzymatic cofactor (Nicholson et al., 2012; Forster et al., 2017; Liu et al., 2023). Folate produced by the microbiota has an important role in the methylation and DNA synthesis, reparation, and is also an excellent antioxidative agent (Zólkiewicz et al., 2020). Niacin induces anti-inflammatory properties in macrophages and dendritic cells and suppress colonic inflammation (Puccetti et al., 2020). Vitamin K produced by the gut microbiota plays an important role in blood clotting and bone health (Prajapati et al., 2023). Microorganisms such as *Viridans Streptococci*, *B. subtilis natto*, *L. lactis*, *L. reuteri*, *Pichia pastoris*, and *Flavobacterium sp.* produces vitamin K2 (Ying et al., 2023). Cobalamin is a vitamin that is only synthesized by anaerobic bacteria and not by animals, plants, or fungi (Park et al., 2022). Vitamin B12 by *Propionibacterium feldsponenum*, *Salmonella*, and *Lactobacillus roehlis* (Ying et al., 2023).

Enzymes: Various physiological, metabolic and regulations functions were performed by the enzymes produced by the microorganisms. At industrial level, enzymes were produced from different fungal and bacterial strains. Enzymes such as peroxide dismutase (SOD), NADH-oxidase, glutathione peroxidase (GPx), and catalase act as antioxidants. In vitro studies found highest glutathione peroxidase in two strains of *L. fermentum* (Rafique et al., 2023). The genus *Bacillus* produces high yield of proteolytic enzymes which have stability against adverse conditions such as temperature, pH, organic solvents, oxidizing compounds, and detergent. Enzyme catalase from genetically modified *Lactobacillus lactis* was inhibitory property against chemically induced colon cancer in mice (Thorakkattu et al., 2022).

Organic acids: Organic acid-based postbiotics have antimicrobial properties. Citric acid, acetic acid, and tartaric acid are the most important acids produced by probiotic bacteria have strong antibacterial effects. Lowering intracellular pH and reducing membrane integrity are the main mechanisms responsible for the antibacterial mechanism of organic acids (Aghebat-Maleki et al., 2022). Among organic acids, lactic acid and acetic acid have very strong antibacterial activities and so, they can be used in the food sector for biopreservation (Rad et al., 2021).

Secondary bile acids: In liver, cholesterol combines with taurine or glycine and results in the formation of primary bile acids. They are stored in the gall bladder and released into the duodenum feeding to aid in the emulsification of dietary fats. Most of the primary bile acids produced are reabsorbed in the intestine, a small percentage is degraded by anaerobic bacteria in the gut, resulting in the production of secondary bile acids, are mainly excreted in feces, with a small portion entering circulation. These active metabolites possess bioregulatory activity and act as signaling molecules within the human body and plays regulating role in the host metabolism (Chen et al., 2024).

Challenges of postbiotics:

As emerging field, there are numerous challenges that need to be addressed. Firstly, definition and scope of postbiotics still need to be refined and expanded. Most of the postbiotic compounds are still in the experimental stage. There is no scientific evidence available to prove their efficacy of postbiotics against various pathological conditions. Metabolic signalling pathways are not well elucidated. Though animal models demonstrated the efficacy of postbiotics, FDA regulatory approval of postbiotics requires human trials. Recommended

intake, toxicity and dosage of these products should be well documented and scientifically proven. Shelf life and viability of these products need to be tested both in vitro and in vivo.

Conclusion

Postbiotics are superior to pre and probiotics because of ease of preparation, mass production, purity, precise action, targeted responses and long shelf life. When compared to pre and probiotics, postbiotics are not very common and available. But in the recent years, there is an increase in interest in the utilisation of postbiotics as functional bioactive compounds, which may contribute to improvement of host health. Hence, researchers are focusing on utilising postbiotics to modulate nutrition, health and disease. They can best be used as an alternative to probiotics in premature neonates, children, immunosuppressive individuals and as a therapeutic agent for the treatment of diseases. They can also be used in food preservation, food packaging, functional food and food supplements. As postbiotics are inanimate microorganisms, they cannot colonise the host but can modify the function and composition of gut microbiota. Regulation of the term postbiotics is still in the infancy stage. Hence, postbiotics are like next generation therapeutic agents for various health disorders.

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