

## 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 With recent to~~ scientific advances ~~in the recent yearments~~, there is an ~~increased~~ growing understanding of ~~the~~ significance of microbiota in health and disease. The two-way communication between the host and the microbiota ~~by involves~~ the production of various ~~types of~~ metabolites, ~~which plays an important that play crucial~~ roles in host energy metabolism, influence nervous system function and ~~the 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, ~~includeing~~ short-chain fatty acids, exopolysaccharides, vitamins, teichoic acids, bacteriocins, enzymes and peptides that ~~conferprovide~~ health benefits to the host. Postbiotics can be stored under normal environmental conditions, have a prolonged shelf life, easy to transport, ~~storage, and handlinge~~ and overcome the limitations of probiotics use. ~~ConsequentlyAs a result,~~ postbiotics have immense potential ~~foras a~~ 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 ~~increasgrowing~~ interest in the field of host-microbiota interactions ~~especially in the context of, particularly concerning~~ human health and disease. ~~MostThe majority~~ of the intestinal microbiota ~~is locatedresides~~ in the intestinal lumen, with approximately  $10^{11}$ - $10^{12}$  microorganisms/ gram of intestinal content, ~~number of bacteria might be higher than\_ potentially outnumber\_ ofing~~ intestinal cells. Human gut is home to trillions of microbes belonging ~~to~~ over 1000 species of bacteria, ~~among whichwith~~ 95% ~~arebeing~~ anaerobic (Afzaal et al., 2022; Liu et al., 2023). The major gut microbial phyla ~~are:include~~ Firmicutes, Bacteroidetes, Fusobacteria, Actinobacteria, Proteobacteria, and Verrucomicrobia. ~~About 90% of all gut microbiota is dominated by~~ Bacteroidetes and Firmicutes ~~dominate about 90% of all gut microbiota~~ (Bourebaba et al., 2022; Scarpellini et

al., 2022; Xue et al., 2020; Afzaal et al., 2022). ~~The G~~gastrointestinal microbiota (GM) is ~~also often~~ referred to as a “metabolic organ” ~~w~~ ~~due to its~~ metabolic capacity ~~similar~~ ~~ame~~ ~~like~~ ~~to~~ that of the liver, ~~where~~ ~~producing~~ a wide range of bioactive molecules, ~~and~~ specialized metabolites ~~which stimulate that have~~ 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, g~~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~~ ~~by detecting host hormones and peptides, producing a variety of signalling molecules that~~ 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~~ ~~synthesis of essential~~ 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. ~~A B~~balanced microbiota ~~plays an important role in is essential for~~ maintaining human health, ~~whereas~~ dysbiosis can disrupt gut homeostasis and ~~has been linked to is associated with~~ the onset or exacerbation of certain diseases. ~~Due to t~~This link between ~~the~~ dysbiosis and ~~disease~~ development ~~of several diseases led many has prompted~~ -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~~ing~~ gut microbiota is ~~through~~ direct fecal microbiota transplantation ~~form~~ from a donor ~~into the intestinal tract of~~ a recipient. Another method involves the ingestion of nutrients ~~utilized by that~~ host microorganisms ~~utilize~~, known as prebiotics (Peri et al., 2022). Prebiotics ~~are food components beneficially affecting gut~~

~~microbiota includes, such as~~ 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 i~~Increasing the diversity and population of intestinal microbiota ~~are eat can be achieved by consuming~~ 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 ~~the~~ WHO/FAO, probiotics are “live microorganisms that, when administered in adequate amounts, confer a health benefit on the host” (Tomasik and Tomasik, 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). ~~When P~~Prebiotics are ~~very often~~ administered together with probiotics, beneficially affecting host health, ~~they~~ are called as symbiotics (Scarpellini et al., 2022).

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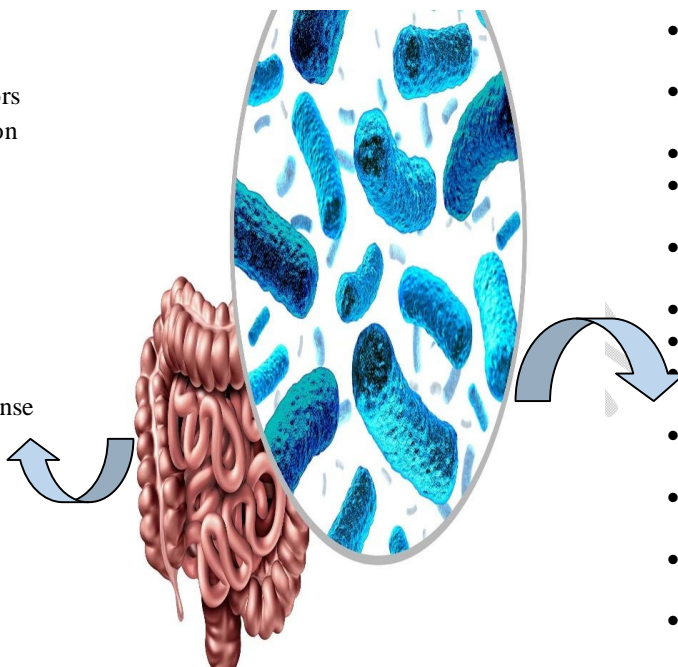
Currently, there are two limitations that directly impact the use of probiotics effectively are: Firstly, probiotic therapies must be limited to use food safe, GRAS recognised or have Qualified Presumption of Safety (QPS) by the European Food Safety Authority (EFSA). ~~The S~~Second limitation is ~~when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

~~But d~~Despite the potential health benefits of probiotics, ~~limited~~ knowledge ~~on exists~~ regarding their safety, effectiveness, dosage and adverse effects when taken in excess ~~amount is very limited~~ (Tomasik and Tomasik, 2020; Peri et al., 2022), ~~necessitates careful consideration~~ particularly in immunosuppressed individuals, ~~having those with~~ compromised gastrointestinal flora, abnormal mucosal barriers and ~~those who are individuals~~ experiencing stress (Zhao et al., 2024). ~~Even d~~During production, maintenance ~~of~~ stability and viability ~~are important and is crucial, as~~ the presence of antibiotic resistance genes in ~~the~~ probiotics could be transmitted ~~via through~~ horizontal gene transfer, ~~and leading to~~ unpredictable strain-specific behaviours (Peri et al., 2022; Scarpellini et al., 2022).

The ~~T~~ranslocation of probiotics from ~~the~~ gut lumen to the bloodstream and ~~from~~ ~~there into vital organs, which could~~ vital organs could potentially trigger systemic infections (Rad et al., 2021; Zhao et al., 2024). However, ~~nowadays there is increase~~emerging evidence suggests that ~~the health of the benefits of~~ probiotics ~~is due~~ may be attributed to the metabolites ~~that they have~~y produced rather than solely the presence of live microbes in the ~~in~~ host. ~~Several evidences have also stated that health benefits assoc~~ome studies have indicated ~~with~~ specific cell constituents of microbes are associated with health benefits (Scott et al., 2022). ~~Hence, nowadays~~As a result, attention has shifted towards postbiotics as an alternative clinical approach ~~to~~ microbiota modulation (Peri et al., 2022; Scarpellini et al., 2022).

UNDER PEER REVIEW

- 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 **Formatted Table** schizophrenia, autism, cognitive decline
- Gut brain endocrine axis-Metabolic, re hormonal and behavioural disorders
- Gut lung axis-Chronic obstructive pulmonary
- Gut heart axis-Thrombotic events, athero hypertension, cardiovascular diseases,
- Gut liver axis-Hepatocellular carcinom inflammation
- Gut pancreas axis-Pancreas cell inflammation,
- Gut bone axis-Osteoporosis, bone demineralis
- Gut muscle axis-Muscle impairment, sa fragility
- Gut skin axis-Psoriasis, acene, wrinkling, agir dermatitis
- Gut kidney axis-Acute kidney injury, chroni disease, nephropathy
- Gut reproductive axis-Ovarian dysfunction, i ovarian cancer, postmenopausal osteoporosis
- Gut bladder axis-Urinary tract infection, bladder

**Healthy gut microbiota**

**Gut microbiota**

**Gut dysbiosis**

Fig 1. Gut microbiota

UNDER PEE

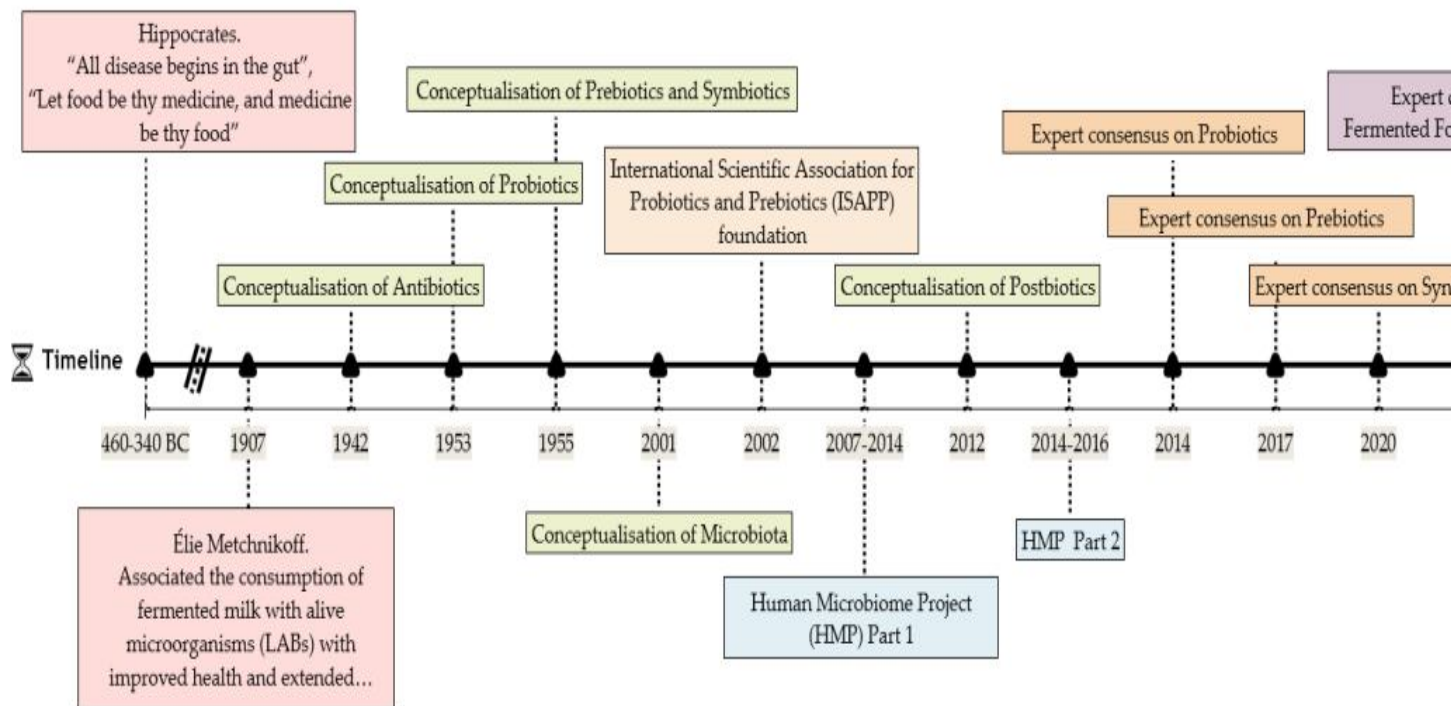


Fig 2. Timeline illustrating the main historical milestones in microbiology (Cabello-Olmo et al., 2021)

### Concept of postbiotics

More than Over 2000 years ago, Hippocrates stated 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, initiated studies on intestinal microbiota and is known as the "Founding Father of Probiotics". The 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 marked the beginning of modern microbiome science, with significant advancement in postbiotics development (Gurunathan et al., 2024).

The term “postbiotics” ~~has been is~~ derived from the Greek words ~~for ‘post’, refers to~~ “post”, ~~meaning after, and “-bios”-, meaning life 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 refers to~~ all metabolic bioactive components ~~generat~~produced by bacteria during fermentation ~~or microorganism’sbial~~ metabolism ~~or breakdown of bacteria~~, which ~~have~~act beneficial ~~to effects on bothe~~ microbes ~~as well asnd~~ humans (Tomasik and Tomasik, 2020; Wegh et al., 2019). In 2021, ~~ISAPP (The~~International Scientific Association of Probiotics and Prebiotics) (ISAPP) proposed a consensus definition of postbiotics ~~to replace previously proposed definitions. Tas~~ 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, with postbiotics being the most~~ commonly used ~~term~~. 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~~byproducts 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, ~~discharge~~released biosurfactants, amino acids, flavonoids ~~determined postbiotics and lower organic acids, for instance, lactic acid, organic acids, neuropeptides, terpenoid and phenolic derived compounds, and lower organic acids like lactic acid~~ (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 e~~Enzymes and vitamins produced by ~~the~~ gut microbes are more consumer- friendly ~~when compared to the synthetic one. Different types of metabolites produced have the ability to than synthetic ones and can help~~ 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 ~~signalingsignalling~~ 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 ~~found~~ in ~~any~~ fermented foods ~~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~~ metabolic ~~sm~~ products (Martyniak et al., 2021). ~~However,~~ ~~as many species share the same metabolic pathways~~ (Scott et al., 2022). ~~Certain f~~oods like buttermilk, fermented pickles, yogurt, cottage cheese and high-fiber foods like oats, flaxseed and garlic ~~maycan~~ increase ~~the~~ postbiotics in the gut (Aggarwal et al., 2022).

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

~~They~~Postbiotics have unique characteristics such as safety, stability, non-toxicity, definitive chemical structure, ~~faileasy~~ transportation ~~ability~~, and low-cost maintenance, ~~and are considered~~ safe substitutes for certain populations ~~and~~ provid~~ing~~es 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~~However, there is currently no specific regulatory framework ~~specific to for~~ postbiotic preparations for human application and ~~their~~ safety assessments. ~~For~~

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Characterization 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 can help develop standardized concentrations for postbiotic therapies and expand research efforts (Scott et al., 2022). Therefore, currently Research is currently focussing on the isolation and characterizing of postbiotics for its potential use as therapeutic agents. Postbiotics like Nyaditumresea, Cytoflora, and Lacteol are the postbiotics already have been approved to be for 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).

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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 systemically on other organs through the communication axis between gut and target organs like the Gut-brain axis, gut-liver axis and Gut-lung axis (Nataraj et al., 2020).

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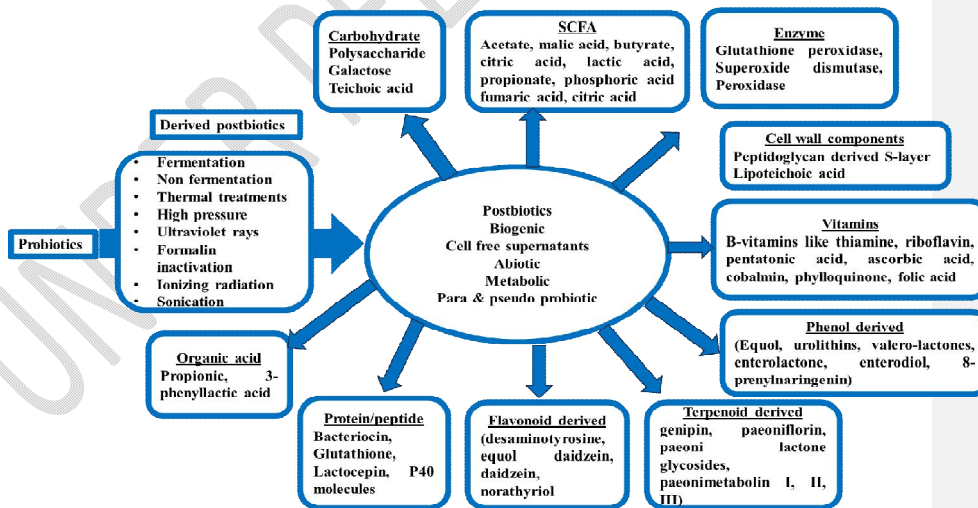


Fig 3. Main postbiotics generated by microbiota in colon and cecum

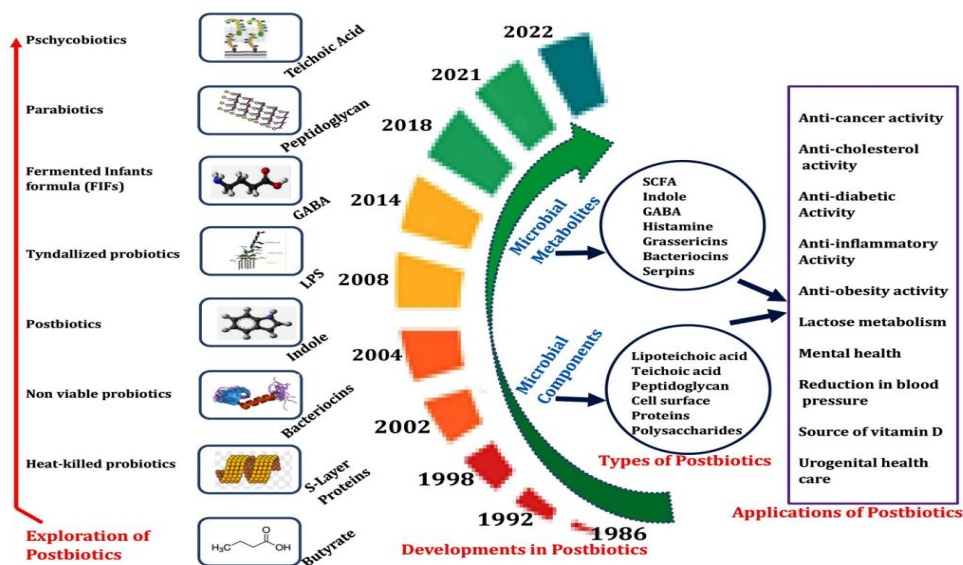


Fig 4. Developments and applications of postbiotics (Babu et al., 2023)

#### 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

Postbiotics can be produced both by through natural and or laboratory methods (Park et al., 2023). There are various techniques used employed for the isolation and purification of postbiotics from the probiotic species. Fermentation is an important source of production of key method for producing postbiotics, in which where microbial cells utilize prebiotics to generate range of and produces a variety of postbiotics with diverse different biological properties, resulting in the enrichment of food with beneficial

substances. ~~Along with the~~In addition to natural production, ~~of postbiotics, a variety of,~~ laboratory techniques can ~~be used to improve~~enhance the nutritional profile, shelf life, and health-promoting properties of a variety of foods (Rafique et al., 2023; Park et al., 2023).

Isolation ~~of~~ng postbiotic metabolites involves cell ~~dis~~ruption techniques ~~including such as~~ enzymatic, thermal, solvent extraction, radiation, ohmic heating, high pressure processing, sonification, pulse electric field and drying. ~~Whereas in case of~~For the production of cell component postbiotics, microbial cells should be exposed to factors without destroying the cell structure. ~~Remaining extraction and~~ ~~clean up~~purification steps include ~~centrifugation,~~ dialysis, lyophilization and column purification (Teame et al., 2020; Malashree et al., 2019). ~~Both f~~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~~develop innovative techniques for the commercial ~~level production of postbiotics~~scale postbiotic production that maintain functional benefits while being cost- and time-effective (Park et al., 2023).

~~Many probiotics are known to produce post~~Numerous probiotics, including ~~the~~genera ~~such as~~ *Lactobacillus*, *Bifidobacterium*, *Streptococcus*, *Bacillus*, and *Faecalibacterium* ~~genera, are known to produce postbiotics~~ (Park et al., 2023; Gurunathan et al., 2024). ~~The~~ *Lactobacillus* ~~is a large bacterial~~ genus ~~compr~~isest about ~~approximately~~ 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~~studied species, ~~has been studied for~~ exhibits postbiotic characteristics in vitro, ~~by~~ 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:

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**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 both Gram-negative and Gram-positive bacteria shows anticancer, antiproliferative, and immunomodulatory effects, and also inhibits 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 enhancing the proliferation of T and NK lymphocytes (Zólkiewicz et al., 2020; Chen et al., 2024). Exopolysaccharides 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 the Gram-positive bacteria possessing 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, known as kefiran, delayed the development of atherosclerosis in a rabbit model (Zólkiewicz et al., 2020). Wang et al (2014) reported that exopolysaccharides (EPS) derived from *Lactobacillus spp* exhibited significant antiproliferative activities against colonic carcinoma cells. As Peptides present in the form of peptidoglycan, an important component of the bacterial cell wall, peptides present in the form of peptidoglycan reduced insulin resistance and adipose tissue inflammation in an obese mouse model (Wu et al., 2023). EPS xopolysaccharides 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 (Thorakkattuet al., 2022). Exopolysaccharides made produced 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 the food industry (Thorakkattuet al., 2022).

### Neurotransmitters

Gut bacteria like *Bifidobacterium*, *Bacillus subtilis*, *Lactobacillus plantarum* and *Lactobacillus brevis* etc., produce various neurotransmitters like catecholamines, norepinephrine, dopamine, and acetylcholine, which plays vital role in the brain functioning through by modulation of nerves signalling through the gut-brain axis. Acetylcholine and catecholamines are involved in emotions, motor control, memory and learning processes. While serotonin is known for its mood-elevating effects in elevating the mood (Patterson et al., 2014). Gamma amino butyric acid (GABA) acts as an inhibitory neurotransmitter-inhibitor, promoting relaxation, modulating sleep disorders and decrease anxiety. These metabolites play a crucial role as antidepressants and psychiatric related disorders management (Patterson et al., 2014). GABA can also induce stimulate insulin production, enhance glucose tolerance and insulin sensitivity, and exert have anti-inflammatory and immunomodulatory effects, all of which help in making it beneficial for management of diabetes (Cabello-Olmo et al., 2021).

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**Bacteriocins:** Bacteriocins are ribosomally-synthesized, heat-stable antimicrobial proteins or peptides produced by the gut microbiota (Wu et al., 2023; Prajapati et al., 2023). They exhibit bacteriostatic or bactericidal effects of bacteriocins that inhibit the activity of pathogenic microorganisms such as *Listeria monocytogenes*, *Clostridium perfringens*, *Salmonella enterica*, and *Escherichia coli* (Mantziari et al., 2020). The antimicrobial activity of bacteriocins is directly related to their effects on bacterial peptides' structure, function and impact on the structure and function of bacterial peptides, as well as their ability to inhibit spores and pore formation on the cell membrane of pathogens (Rad et al., 2021; Mishra et al., 2024). Bacteriocins have the potential to suppress or eliminate drug-resistant organisms in contrast to traditional antibiotics due to their ability to by disrupting bacterial cell membranes and causing the leakage of internal components (Lou et al., 2023). Bacteriocins are like They are considered the next generation of antimicrobials and found potential for use in show promise for use in food preservation. Nisin, the first bacteriocins approved for commercial use as a food preservative by the European Food Safety Authority (EFSA), the Food and Drug Administrative (FDA), and Health Canada approved Nisin, first bacteriocin for commercial use as food preservative. Currently, more

~~than~~, is currently used in over 80 countries ~~using it~~ as a food additive. ~~Some of Other~~ bacteriocins include ~~nisin~~, subtilisin, lactococcin G&Q, enterocin, lactocyclin, bovicin, plantaricin, and lacticin (Gurunathan et al., 2024).

**Short-chain fatty acids (SCFAs)** ~~like such as~~ acetate, propionate and butyrate are produced in the large intestine by the microbiota during ~~the~~ fermentation of non-digestible polysaccharides (Wu et al., 2023; Chudzik et al., 2021; Babu et al., 2023). These are ~~the~~ 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 influences have a~~ directly or indirectly ~~on the influence on~~ brain function (Chudzik et al., 2021). They are absorbed by colonocytes, ~~and~~ influence intestinal mucosal immunity, barrier integrity and function. ~~They~~ SCFA 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  $\gamma$ -aminobutyric acid (GABA) (Silva et al., 2020). SCFAs also ~~regulate~~ the liver mitochondrial function, ~~regulate~~ insulin secretion and ~~increases the~~ whole-body energy homeostasis by entering ~~into~~ the systemic circulation and ~~reaching~~ other tissues (Silva et al., 2020; Psichas et al., 2015; Larraufie et al., 2018). SCFAs, ~~including~~ formic acid, ~~and~~ propionic acid, act against the activity of pathogenic bacteria by interfering with the cytoplasmic membrane structure, nutrient transport and macromolecular synthesis. They also ~~have possess~~ antibacterial and antifungal properties (Mantziari et al., 2020). SCFAs ~~facilitates~~ the growth of beneficial bacteria by creating ~~a~~ 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, ~~and helps~~ manage hunger and weight in the central nervous system (Park et al., 2022). Propionate ~~regulates~~ hepatic gluconeogenesis, carbohydrate metabolism, and inhibits cholesterol synthesis (Liu et al., 2023). Butyrate is the essential SCFA and ~~the~~ primary source of energy for colonocytes. It also possesses anticancer ~~properties~~ and anti-inflammatory properties through ~~the~~ inhibition of proinflammatory cytokines (Afzaal et al., 2022; Szydłowska and Sionek, 2023; Vrzácková, et al., 2021). SCFAs have the ability to cross ~~the~~ blood brain barrier and can reach ~~the~~ central nervous system, ~~regulatesing~~ the immune response (Szydłowska and Sionek, 2023). Butyrate salt participates in intestinal cell nutrient metabolism, ~~and~~ promotes epithelial regeneration. ~~It also~~ increases the expression of immune suppressive cytokines and ~~downregulates~~ 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).

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When compared to butyric and propionic acid, acetic acid is less abundant but it possesses anti-inflammatory and analgesic properties. It may help manage hunger and weight in the central nervous system, implying that it may help prevent cardiovascular disease (Park et al., 2022). Acetate acts as a lipogenic substrate and propionate inhibits lipogenesis by down-regulating 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 promote 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 disrupting homeostasis and causing functional disorders (Babu et al., 2023). Autoimmune disorders like rheumatoid arthritis is often associated with intestinal barrier dysfunction. Hence, the 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. Bacterial lysates recognize and resist pathogenic bacterial infections and stimulates the bacterial infections (Liu et al., 2023).

**Cell-free supernatants:** Cell-free supernatant 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).

Comment [U23]: Italic

**Vitamins:** The gut microbiota produces essential 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 which are crucial for DNA replication, RBC~~ formation, ~~enhance~~ immune functioning, ~~works as, and~~ enzymatic ~~cofactor processes~~ (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~~, and acts as an ~~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 ~~produces~~synthesized by the gut microbiota plays an important role in blood clotting and bone health (Prajapati et al., 2023). Various Mmicroorganisms 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 on, exclusively~~ synthesized by anaerobic bacteria, ~~and not~~ not produced by animals, plants, or fungi (Park et al., 2022). Vitamin B12 is produced by *Propionibacterium feldspoenenum*, *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 haveith high stability against adverse conditions such as temperature, pH, organic solvents, oxidizing compounds, and detergent. Additionally, Eenzyme catalase from genetically modified *Lactobacillus lactis* ~~whas~~ shown inhibitory ~~propertesy~~ 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 (Aghebati-Maleki et al., 2022). ~~Among organic acids, I~~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).

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**Secondary bile acids:** In the liver, cholesterol combines with taurine or glycine ~~and results in the formation of to form~~ primary bile acids. ~~They which~~ are stored in the gall bladder and released into the duodenum ~~feeding~~ to aid in the emulsification of dietary fats. ~~While M~~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 leading to~~ production of secondary bile acids. ~~These secondary bile acids are are main~~primarily excreted in feces, with a small portion entering circulation. These active metabolites possess bioregulatory activity and act as ~~signalingsignalling~~ molecules ~~with~~in the human body and plays regulating role in the host metabolism (Chen et al., 2024).

### Challenges of postbiotics:

~~As emerging field, there are numerous~~The field of postbiotics faces several challenges that ~~need to must~~ be addressed. ~~Firstly, The~~ definition and scope of postbiotics ~~still need to be need further~~ refinement and expanded ~~Most of the as most~~ postbiotic compounds are still in the experimental stage. ~~There is no s~~Scientific evidence ~~availableis lacking~~ to prove their efficacy of postbiotics against various pathological conditions. ~~Mand m~~ metabolic signalling pathways are not well ~~elucidatedunderstood~~. Though animal models demonstrated the efficacy of postbiotics, FDA regulatory approval of postbiotics requires human trials. ~~It is crucial to establish R~~recommended intake, toxicity and dosage of ~~these products should be well documented and scientifically proven. postbiotic products based on scientific evidence.~~ ~~Additionally, the S~~shelf life and viability of these products need to be tested in both in vitro and in vivo.

### Conclusion

Postbiotics are considered superior to pre and probiotics ~~because of due to~~ ease of preparation, mass production, purity, precise action, targeted responses and long shelf life. ~~When comparison to pre and probiotics, postbiotics are not very common and available.~~ But in the recent years, there is an increase interest in the utilisation of postbiotics as functional bioactive compounds, may contribute to improvement of host health. Hence, researchers are focusing to utilise postbiotics to modulate nutrition, health and disease. They can best use as an alternative to probiotics in premature neonates, children's, immunosuppressive individuals and as therapeutic agent for the treatment of diseases. They can also be used in the food preservation, food packaging, functional food and food supplements. As postbiotics are inanimate microorganisms, they cannot colonise the host buy modify the function and

Comment [U27]: rewrite the sentence

composition of gut microbiota. Regulation of the term postbiotics is still in the infancy stage. **Hanceene**, postbiotics are like next generation therapeutic agents for various health disorders.

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