

# A Critical Analysis of Postbiotics: Exploring their Potential Impact on the Health and Food Industries

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## Abstract

Postbiotics are an emerging field in gut and gastroenterological research. Despite it being a vast field, limited scientific research has been conducted on this topic. Postbiotics are functional bioactive compounds generated in the cell wall matrix during fermentation that may be used to promote health. Postbiotics play a critical role in human immune development against communicable and noncommunicable diseases. This review focuses on the recent advances and future perspectives of postbiotics in health and food science. The review also discussed the criteria and different types of postbiotics and elucidated the significance of postbiotics. The paper further reviewed the role of postbiotics as preservatives, active ingredients in packaging systems, anti-biofilm agents, and decontaminant agents in food processing industries.

**Keywords:** Gut Microbes, Probiotic, Prebiotic, Secondary Metabolites, Cellular Components, Anti-Inflammatory, Immuno-modulation, Bio-preservative

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## INTRODUCTION

Postbiotics emerged after the terms prebiotic, probiotic, and synbiotic. Postbiotics are metabolic leftovers of probiotic bacteria or the gut microbiota.<sup>1</sup> To use a scientific term, they are byproducts of the primary metabolism of probiotics or gut microbes. Non-digestible carbohydrates have a major impact on postbiotics production in the gut, which is highly dependent on the living microbiota of the individual or host. Postbiotics synthesized in the body are metabolized by the liver, kidneys, and other organs. Postbiotics are like magic bullets<sup>2</sup> that control immune system effector molecules and signaling pathways.<sup>3</sup> Insufficient postbiotics availability in the human system results in the emergence of neurological disorders, damage to the intestinal wall, and unbalanced homeostasis, resulting in metabolic syndromes such as cancer.<sup>4</sup> Postbiotics can be readily and thoroughly extracted from the probiotic or gut microbiota. To maximize postbiotics production, it is helpful to provide prebiotics such as resistant starch and different dietary fibers to the gut microbes living in human digestive system.<sup>5</sup> Recently, the FDA approved Rebyota, a fecal microbiota, for the prevention of *Clostridioides difficile* infection.

Major sources of bibliometric information, such as Web of Science, Scopus, PubMed, and Google Scholar, were extensively searched with keywords on postbiotics, health benefits of postbiotics, and their mechanism of formation and actions. From various search engines, a database of 128 papers was obtained. Thirty-two publications met the review criteria. The main purpose of this article is to provide an overview of postbiotics, focusing on their usefulness in the food and pharmaceutical industries as well as their overall quality.

### Evolution of the term Postbiotics

Several hypotheses have been proposed to characterize postbiotics.<sup>6</sup> Figure 1 shows the developments in postbiotics.<sup>7</sup> According to previous researchers, Postbiotics are non-viable probiotics or non-toxic, and metabolomes of the oral cavity, skin, urogenital tract, and gut.<sup>8,9</sup> In contrast, postbiotics are defined by the International Scientific Association for Probiotics

and Prebiotics (ISAPP) as “the preparation of inanimate microorganisms and/or their components that confers a health benefit on the host”.<sup>1</sup> Inactive microbial cells or their cellular components with or without useful metabolites may also be included as postbiotics.<sup>6</sup>

### Criteria developed by ISAPP for the preparation of Postbiotics

The ISAPP developed the following criteria for the preparation of postbiotics.

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.

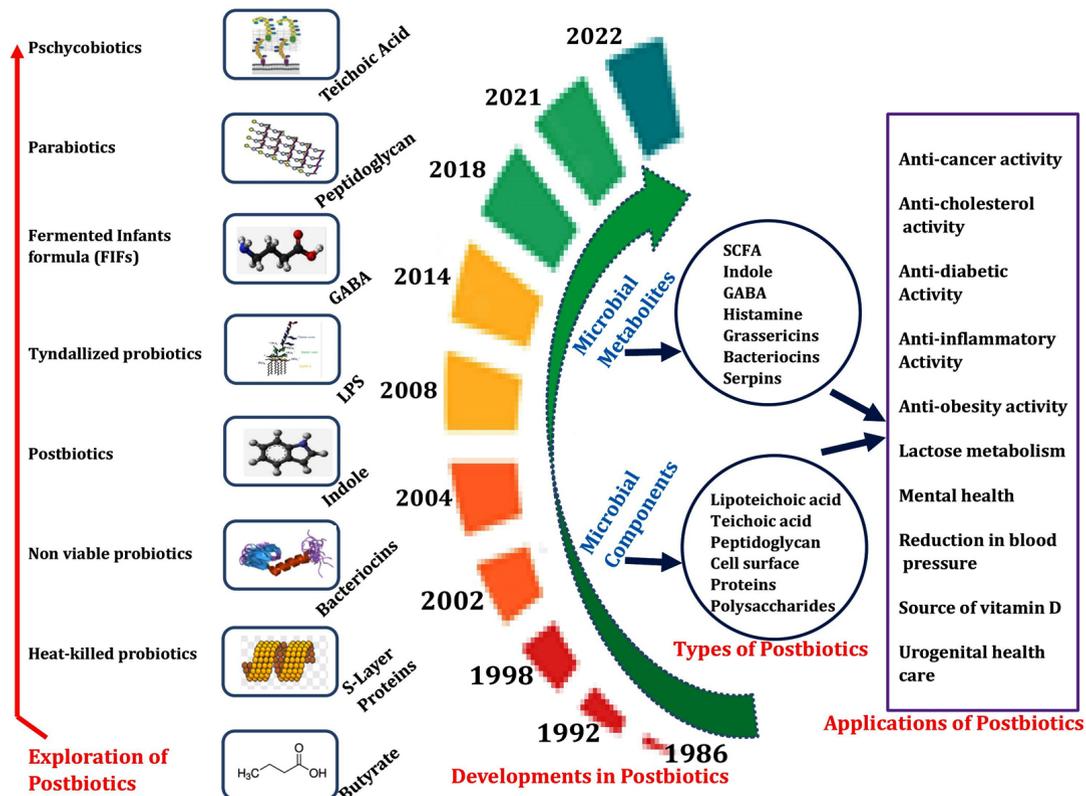
### Production of postbiotics

Non-digestible dietary fibers, resistant starches, and non-starch polysaccharides are fermented by colonic microbes under anaerobic conditions in the digestive systems of humans and animals (Figure 2).<sup>12</sup> Such a beneficial microbes are known as probiotics,<sup>13</sup> where their metabolic products are known as postbiotics.

### Types of postbiotics

Many hypotheses have been proposed in previous studies regarding the types of postbiotics. According to Ailioaie *et al.*, postbiotics can be broadly classified as paraprobiotics and Fermented Infant or follow-on formula (FIF).<sup>14</sup> Paraprobiotics – otherwise known as ghost probiotics, non-viable probiotics, or inactivated probiotics, are now often defined as non-viable or inactivated microbial cells, which, when administered in sufficient amounts, confer benefits to the host (Salminen *et al.*). Fermented Infant Formulation (FIF) are infant or follow-on formulae that have been fermented with lactic acid-producing or other bacteria,<sup>15</sup> and in most cases do not contain viable bacteria.

However, according to the ISAPP guidelines, postbiotics can be either,



**Figure 1.** Developments in postbiotics study modified from Bourebaba et al.<sup>10</sup> and Muskan et al.<sup>11</sup>

- (a) Probiotic organisms and inactive probiotic/ gut microbes
- (b) Cell-free supernatants (a mixture of compounds produced by microbes)<sup>4</sup>
- (c) Primary and secondary metabolites

Table shows the functional products of microbial metabolites which can act as postbiotics

**Functional role of commonly known postbiotics (probiotic secretions)**

**Short-chain Fatty Acids (SCFAs)**

Short-chain fatty acids are monocarboxylic acids containing two to six carbon atoms. SCFAs are produced in the large intestine of the digestive system through anaerobic fermentation of indigestible carbohydrates, such as those found in foods high in dietary fiber (which are typically high in cellulose, pectin, hemicellulose, lignin, and resistant starch). It improves the metabolic efficiency of the host microbiome by increasing the transfer of carbon from the diet

to the microbiome.<sup>29</sup> The movement of SCFAs from colonococci to the bloodstream is typically facilitated by H<sup>+</sup>-dependent or sodium-dependent monocarboxylate transporters (MCTs and SMCTs).<sup>30</sup> Eg: Acetate, Butyrate, Propionate, Formic acid, Valeric acid, Caproic acid, β-hydroxybutyric acids;<sup>30</sup> Short-chain galacto-oligosaccharides (scGOS) and long-chain fructo-oligosaccharides (IcFOS).<sup>5</sup>

Important functions of SCFAs include signaling for TREG (Regulatory T Cells) differentiation to reduce inflammation, which is important in brown tissue activation, functional regulation of liver mitochondria, and regulation of neutral intracellular signal mechanisms in the peripheral nervous system, central nervous system, and gut-associated immune tissues. During the cell cycle, SCFAs play important roles in stimulating the activity of histone deacetylase (HDAC) by increasing the acetylation of lysine residues of nucleosomal histones. Epigenetic cofactors/substrates produced by gut microbiota<sup>31</sup>

cause DNA methylation and histone modifications. A recent study has summarized the role of SCFAs in inhibiting SARS-CoV-2 surface proteins.<sup>11</sup>

SCFAs are naturally produced through host metabolic pathways, especially in the liver; however, the colon is the primary site of production and requires the presence of certain colonic bacteria. At concentrations of 500-600 mM, acetate, propionate, and butyrate were the most abundant SCFAs in the feces of healthy humans. Extremely high concentrations (70-140 mM) are released in the proximal colon, with lower concentrations (20-70 mM) in the distal colon and (20-40 mM) in the distal ileum. Non-metabolized SCFAs, except acetate, in colonocytes are utilized in the liver to provide energy to hepatocytes helps to increase gut-brain communications.

SCFAs in the human gut typically cause mucus production in the colorectal region and aid in the protection of the intestinal barrier through their anti-inflammatory effects. Cancer of the colon is present in this area. Irritable bowel syndrome (IBS), inflammatory bowel disease (IBD), obesity, metabolic syndrome, type 2 diabetes,

cancer, and autoimmune diseases are all linked to insufficient SCFA availability, which in turn disrupts homeostasis and causes functional disorders. SCFAs inhibit histone deacetylases and function as G protein-coupled receptors.<sup>30</sup>

Recent research has shown that butyrate and propionate can increase the levels of hormones in the digestive tract, leading to diminished hunger. Bacteroidetes, on the other hand, primarily produce acetate and propionate, and Firmicutes, butyrate.<sup>30</sup> Because acetate acts as a lipogenic substrate and propionate inhibits lipogenesis by downregulating fatty acid synthase in the liver, the acetate/propionate ratio is thought to be significant for de novo lipogenesis.<sup>32</sup>

Loss of body weight and adiposity may be facilitated by propionate and butyrate, which may activate intestinal gluconeogenesis and induce the expression of gluconeogenesis-related genes.<sup>3</sup> Both Brown and White adipose tissues respond to acetate by increasing browning activity. The importance of the bacterial production of SCFAs in the physiology of the gastrointestinal tract has led to the hypothesis that alterations in the abundance

**Table.** List of secondary metabolites (Postbiotics)

Microbial metabolites	References
Bioactive peptides secreted proteins, peptides	Cuevas-González et al. <sup>16</sup> Koleilat <sup>17</sup>
Secreted biosurfactants (Laurostearic acid), Aggregation promoting factor (APF)	Drolia et al. <sup>18</sup>
Neurotransmitters (GABA) polyunsaturated fatty acids (Arachidonic acid, docosahexaenoic acid, linoleic acid, linolenic acids, Omega-3-fatty acids)	Chudzik et al. <sup>19</sup> Nazarii et al. <sup>20</sup>
Polypeptides (Bacitracin, Nisin, Reuterin) Bacteriocins: lanthionine-containing bacteriocins (class I) and the nonlanthionine-containing bacteriocins (class II)	Cabello-Olmo et al. <sup>21</sup> Jastrząb et al. <sup>22</sup>
Vitamin B complex (Biotin, cobalamin, folate, niacin, pantothenic acid, pyridone, riboflavin, thiamine)	Qing et al. <sup>23</sup>
Aromatic amino acids, Polyamines (Putrescine, cadaverine, spermidine, and spermine)	Maria et al., <sup>24</sup>
Lactocepin	Pandey et al. <sup>7</sup>
Retinoic acid, Long chain fatty acids, Trimethylamine – N-Oxide (TMO), Conjugated linoleic acid (cla)	Hamid et al. <sup>25</sup>
Superoxide dismutase, catalase, Enterocins, Serpins, Histamine	Pandey et al. <sup>7</sup>
Functional proteins, teichoic acids, p40 and p75 proteins.	Walhe et al. <sup>26</sup> , Lee et al. <sup>27</sup> , Bauerl et al. <sup>28</sup>

of acetate, propionate, and butyrate are linked to a decline in health.

### Extracellular Vesicles (EVs)

EVs or membrane vesicles are present in the lipid bilayers of cells. It facilitates interkingdom crosstalk and has immunomodulatory and anti-inflammatory responses against pathogens. They are the main therapeutic indicators of functional brain disorders, such as mood disorders<sup>33</sup> and neuropathological conditions. It is one of the key segments of probiotic microbes and is involved in TREG (Regulatory T Cells) cell activation. Beneficial bacteroids have been identified in EVs.<sup>34</sup> *Lactobacillus casei* BL23 EVs have been identified to contain lipoteichoic acid and P40 and P75 proteins with proinflammatory activity and induce epidermal growth factor receptors. EVs from *Akkermansia muciniphila* have been shown to regulate colitis.<sup>35</sup>

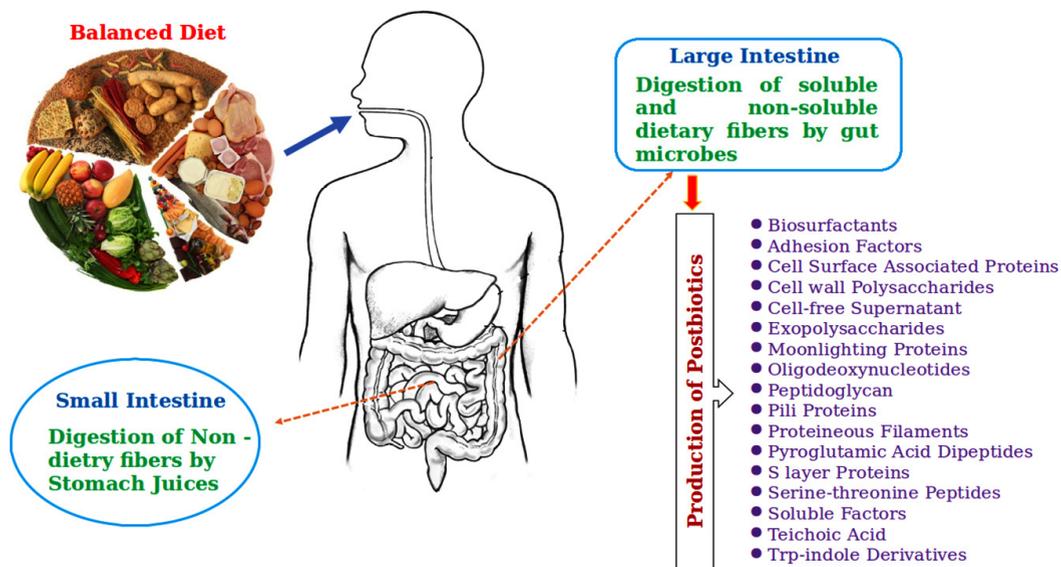
### Anti-microbial Peptides (AMPs)

Bacteriocin is a widely studied AMP. They are produced by both Gram-positive and Gram-negative bacteria;<sup>36</sup> and have broad-spectrum antimicrobial activity.<sup>37</sup> The benefits of bacteriocins are based on six pillars, namely spectrum, stability, bioengineering, diversity, production, and safety.

There are four classes of bacteriocins: Class I: bacteriocins include proteolytic and heat-resistant small peptides substantially modified by specific enzymes at the transcriptional level, lantibiotics, sactypeptides, glycoamines, and loop peptides. Class II: bacteriocins are subdivided into four subtypes (at least): pediocin-like, two peptides, circular, and linear non-pediocin-like. They are comprised of small temperature- and pH-resistant peptides without minor or minor post-transcriptional processing, such as disulfide bonds. Class III: Bacteriocins or bacteriolysins incorporate large thermolabile proteins > 10 kDa. Class IV bacteriocins consist of complex proteins conjugated to lipids or carbohydrates.<sup>2</sup>

### Health benefits of postbiotics

Postbiotics can act as effector molecules to trigger molecular signals in the immune system.<sup>3</sup> They improve nutrient absorption and release biologically active molecules as needed to maintain metabolic, immune, and neural networks in the body in balance.<sup>22</sup> Inadequate postbiotic production disturbs the homeostatic equilibrium of host organisms.<sup>38</sup> However, their instigation and potential activity are linked to the host organism.<sup>39</sup> With the help of postbiotics, nutrients can be delivered directly to the intestines, where they



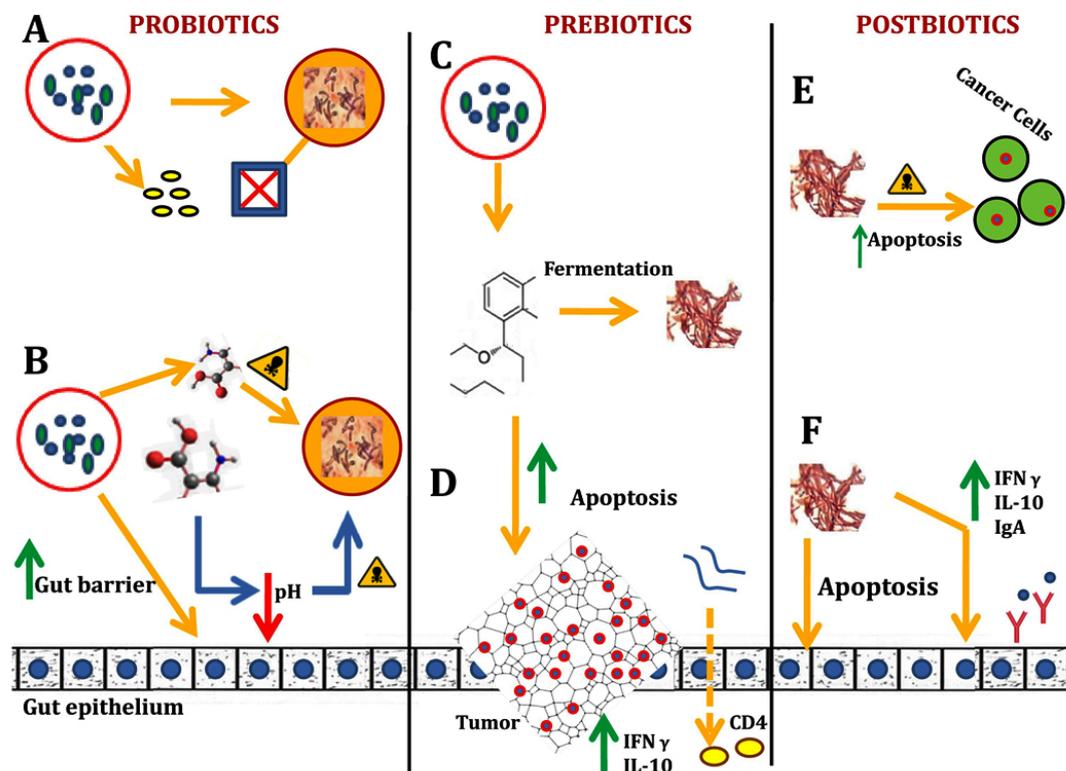
**Figure 2.** Postbiotics production in human gut system

have the greatest impact, extending their shelf life and reducing logistical burdens. Intestinal microbes and their hosts communicate via molecular interactions following the production of bioactive metabolites (short-chain fatty acids) or via interactions with the host immune cells via cell-surface molecules.<sup>40</sup> Postbiotics are effective for treating IBS and related syndromes,<sup>41</sup> reducing lactose intolerance, enhancing the absorption of essential amines<sup>42</sup> and maintaining steady mucin secretion.<sup>43</sup> Postbiotics have the potential to replace antibiotics in the treatment of both communicable<sup>9</sup> and noncommunicable.<sup>44</sup>

Investigations from clinical demonstrations have revealed that a host's health status and disease complications are significantly associated with lifestyle factors, especially diet.<sup>45</sup> Phenolic and bio-active components such as probiotics, prebiotics, synbiotics, and postbiotics, in addition to macro and micro nutrients, play a vital role as health-promoting ingredients in functional foods.<sup>46</sup>

Figure 3 shows the proposed mechanism of action of probiotics, prebiotics, and postbiotics. In maintaining gut homeostasis, organisms/probiotics like *Lactobacillus*, *Bifidobacterium* and *Clostridiales* plays a major role (A) Colonization and proliferation of pathogens is prevented by competing for microenvironment and nutrients. (B) By lowering luminal pH antimicrobial peptides are released with direct bactericidal effect. Probiotics increase of mucin production, expression of tight junctions are enhanced and also promote epithelial restoration. From probiotics prebiotics are derived. (C) By the process of fermentation postbiotics are produced. (D) Gut epithelium directly absorbs oligosaccharides. To release IL-10, IFN-g they stimulate CD4+ and T-cell. (E) Apoptosis increased, against cancer cells cytotoxicity increased. (F) Inhibition of apoptosis and the level of Ig A, IFN-g and IL-10 increased.<sup>47</sup>

In addition to optimizing the health benefits attributed to probiotics, a new



**Figure 3.** Assumed mechanism of action of Probiotics, Prebiotics and Postbiotics modified from Michela et al.<sup>47</sup>

concept of postbiotics or nonbiotics has been introduced.<sup>45</sup> By stimulating the immune system, postbiotics are associated with anti-inflammatory development in the intestine and bowel, as well as immunomodulatory, anti-obesogenic, antihypertensive, anti-proliferative, antioxidative, and hypocholesterolemic activities. It has been reported that the fermentation of intestinal bacteria or its structural fragments generates postbiotics that greatly influence host physiology and have an impact on health. In view of the enzymatic effect on microorganisms, it has been reported that enzymes such as subtilisin-like protease and glutamyl endopeptidase are efficient against degradation and eradication of extracellular polymeric substances and biofilms generated by *Serratia marcescens*, respectively.<sup>48</sup>

However, the impacts of macro- and micronutrients from postbiotic sources have also gained significant importance in health. Microbiota-secreted vitamins seem to be protective against Coronary Artery Disease and osteoporosis.<sup>49</sup> This implies that the potential mechanism of the effect of postbiotics is anti-inflammatory, immunomodulatory, anti-cancer, anti-tumor, and anti-proliferative activities, which include regulation of the central nervous system and anti-atherosclerotic, hypocholesterolemic, cardioprotective, and anti-hypersensitive effects.<sup>50</sup>

In addition to the above-mentioned mechanisms, the hepatoprotective and anti-ulcerative properties are also health benefits of postbiotics.<sup>50</sup> In addition, intestinal permeability and modulation of microbial activity, along with prebiotic effects, are maintained by the action of postbiotics. As a regulatory mechanism, the inhibition of pathogens, antibiofilm, and anti-adhesion effects are taken care of by the effect of postbiotics to promote health.<sup>51</sup>

An important emphasis has been placed on the similarity between postbiotics and probiotics in terms of their properties, owing to species and strain specificity. In addition, microbial progenitors that are used for their formulation act as dependency factors for postbiotic actions. In addition, the substrate or matrix, which are the sites for postbiotic production, also turn out to be a deciding factor for the resulting postbiotic. The mechanisms of action of postbiotics must be correlated with the release of a myriad of products,

including metabolites and cellular components from resident microbes.<sup>52</sup> This adds up to the major significance of the host, while these myriad products behave as messengers in microbiota-host interactions.<sup>33</sup> Carbello-Olmo *et al.* inferred that the gastrointestinal microbiota (GM) could be a major source of postbiotic constituents.<sup>53</sup>

### The function of postbiotics in Diabetes Mellitus (DM)

Resident gastrointestinal microbiota is among the set of environmental factors that are involved in the development and progression of *diabetes mellitus* (DM).<sup>53</sup> A chronic disease such as DM is implicated in an increase in blood glucose levels (hyperglycemia).<sup>54</sup> This condition is caused either by beta-cell destruction (Type 1 *Diabetes mellitus*-T1DM) or the loss of pancreatic function.<sup>54</sup> Inadequacy in insulin secretion is attributed to insulin resistivity imposed by peripheral tissues such as the liver, muscle, and adipose tissue (Type 2 *Diabetes mellitus*-T2D).<sup>54</sup>

Resident microbes play an interesting role in correlating host energy balance and T2D metabolism, whereas the connectivity between resident microbes and immunity is involved in the development of T1D. However, inflammation is associated with both T1D and T2D.<sup>55</sup> Therefore, the reports of Clarke *et al.* have stated the role of resident microbes in host-energy balance, metabolism, inflammation, and immunity on a widely accepted note.<sup>56</sup> Homeostasis in the host occurs through the absorption of nutrients, intestinal permeability, or controlled gene expression. These functions are influenced by gut microbiota.<sup>57</sup>

A cross-sectional study has inferred the unfriendly composition and activity of diabetic GMs shared among patients with T1D and T2D patients. The actions of this “diabetic microbiota” are associated with the decline of butyrate-producing species, enhancement of opportunistic organisms, and an abundant reduction in gene count attributed to the impairment of metabolic activities, alterations in the transport of nutrients, and enzymatic activity. These fluctuations affect SCFA concentrations and oxidative stress responses.<sup>58</sup>

In view of animal studies (in rodents) based on microbial exopolysaccharides (EPs) in

*diabetes mellitus*, it was found that the alloxan or streptozotocin (65 mg/Kg BW) induced rodents possessed an increase in serum insulin levels.<sup>59</sup> Clinical trials have been performed on T1D models of Sprague-Dawley rats that involved microbial Eps.<sup>60</sup> It has also been observed that the levels of HDL increased with a reduction in the levels of Total Cholesterol (TC), Low Density Lipid (LDL), Very Low Density Lipoprotein (VLDL), and Total Glycerides (TG).<sup>59</sup> According to Huang *et al.*, an *in vitro* study conducted on insulin-resistant HepG2 cells, the bioactive EPs component from *Lactobacillus plantarum* H31-2 showed a decline in the levels of glucose concentration.<sup>42</sup> Additionally, inhibition of pancreatic-amylase was also observed, followed by the expression of GLUT-4, Akt-2, and AMPK being upregulated due to the effect of postbiotic component treatment on the model system.

In addition to complications related to DM, a report based on the role of EPs from *Bacillus licheniformis* in counteracting oxidative stress and preventing diabetic complications by key tissue protection was found to be effective. Tackling diabetes with alterations in GM composition and activity is quite challenging.<sup>53</sup> It is very clear that a suitable GM and its postbiotic association with intestinal function would serve as an important factor for the maintenance of proper health and prevention of diabetes in individuals.

### **Postbiotics' role in Reactive Oxygen Species (ROS)**

Living cells produce reactive oxygen species (ROS) at the molecular level, which are involved in cell signalling by acting as secondary messengers for various metabolic processes.<sup>60</sup> Cell and tissue damage occurs because of an imbalance between ROS generation and defenses by anti-oxidant actions (oxidative stress). This could be a result of an increase in ROS production, environmental stressors (UV-B radiation), and xenobiotic compounds (mycotoxins).<sup>61</sup> Guerrero Encinas *et al.* found that rats with Aflatoxin B1-induced oxidative stress had better health when they received intracellular probiotics, such as *Lactobacillus casei* CRL 431 [IC-431].<sup>61</sup> The 2,2'-azino-bis (3-ethylbenzothiazoline)-6-sulfonic acid (ABTS) assay and the oxygen radical absorbance capacity (ORAC) assay revealed that IC-431 had antioxidant capacity values of 477 and 282 mol/L, respectively.

Studies suggested that the important anti-oxidant metabolites present in the intracellular fractions of *Lactobacillus casei* CRL 431<sup>62</sup> were saturated and mono-unsaturated fatty acids, as well as glutathione (GSH) and cysteine. Thus, the antioxidant properties of IC-431 were significantly proven by free fatty acids and GSH, as their bioavailability is directly utilized by the cells, which estimates about 10 % of the metabolites from the gut microbiota and is found to be present in the mammalian blood.

Disruptions of bacterial and metabolic by-products undergo physiochemical and physiological processes that have a significant impact on their structure and properties.<sup>63</sup> For instance, antioxidant function is affected by the partial oxidation of GSH and cysteine (thiol groups). This reaction is influenced by the pH and partial hydrolysis of GSH molecules that are associated with the disulfide groups of GS-SG and Glu-Cys.<sup>64</sup> Lipid damage in cells, tissues, and organs is promoted by uncontrolled biological production of ROS (superoxide anion and hydrogen peroxide).<sup>61</sup>

Additionally, the mutagenic product of lipid peroxidation is attributed to being malondialdehyde (MDA).<sup>61</sup> Mutagenesis and cell death are caused by hydroxide radical reactions. As there is no enzyme specificity against the destruction of hydroxide ions in biological systems, the most effective defense is possible through the reduction of intracellular precursor concentration. Catalases and peroxidases are involved.<sup>61</sup> Further dismutation of superoxide radicals into oxygen and hydrogen peroxide is performed by superoxide dismutase (SOD), and this hydrogen peroxide is converted into oxygen and water by the action of catalase (CAT). The final antioxidant activity is expressed in terms of M Trolox equivalents (TE).<sup>61</sup>

It was observed that the antioxidant activity was higher and lower malondialdehyde (MDA) levels were observed (TE-dIC-431-Antioxidant capacity of intracellular components of *Lactobacillus casei* CRL 431 after the digestive process) in the intestinal tissue. This turns out to be a protective mechanism stimulated by IC-431 against oxidative stress.<sup>61</sup> This oxidative stress is attributed to mechanical stress caused by the surgical manipulation of the intestine, thereby inducing ROS generation. In rats, Aflatoxin B1

(AFB1) is induced to generate oxidative stress, which is reduced by the intracellular components of *Lactobacillus casei* (CRL 431).<sup>62</sup>

It is assumed that part of the antioxidant intracellular components of *Lactobacillus casei* CRL-431 have a permeability of action through the duodenum intestinal tissue followed by passage into the blood stream to the liver, which is a potential site for participation in different biological processes.<sup>61</sup> Based on the *ex vivo* and *in vivo* assays conducted by Guerrero-Encinas *et al.*, it was hypothesized that IC-431 showed adaptive responses by modulating mitochondrial function in AFB1-induced oxidative stress conditions in rats.<sup>61</sup> According to Guerrero-Encinas *et al.* the mitochondria are protected naturally by antioxidants and scavengers in an intricate system.<sup>61</sup> In this regard, after the observation of lower hydrogen peroxide levels in rats after treating the samples with AFB1 and IC-431, it was revealed that AFB1, which induces mitochondrial damage, was protected after subjecting the samples to IC-431. As IC-431 maintains low levels of hydrogen peroxide, it promotes the expression of antioxidant enzymes in the hepatic mitochondria.<sup>61</sup>

Further connecting the modulation mechanism of H<sub>2</sub>O<sub>2</sub> with transcription factors in mammalian cells, it was found that low levels of H<sub>2</sub>O<sub>2</sub> act as molecular sensors in the activation and accumulation of the Nrf2 transcription factor in the nucleus, which in turn is responsible for antioxidant element (ARE)-mediated expression.<sup>65</sup> Oxygen consumption and isolated AFB1-treated rat livers showed higher mitochondrial membrane potential. This, in turn, shows higher impact of energy capacity on the inner mitochondrial membrane, thereby producing higher ATP synthesis.<sup>66</sup>

The conclusions drawn from (Guerrero-Encinas *et al.* states that AFB1 + IC-431 treated mitochondria are responsible for the activation of the physiological uncoupling mechanism for the mitigation of ROS production in the cells occurring through proton leakage induction.<sup>61,67</sup> This proton leakage does not affect energy production, as ATP production does not consume all oxygen. Proton leakage induction is involved in controlling oxidative stress via Nrf2 activation.<sup>68</sup>

## The role of postbiotics in the treatment of Cardiovascular Disease (CVD)

*Lactobacillus paracasei* lives in humans' mouths and intestines.<sup>69</sup> Marra and Svegliati-Baroni reported that a traditional fermented dairy product was the source of this bacterium, which could be credited as a "good" bacterium based on its high lipolytic activity to counteract metabolic syndrome with a bioactive status.<sup>70</sup> The lipolytic action of postbiotics limits the formation of complex lipids by influencing the activity of peroxisome proliferator-activated receptors (PPAR $\alpha$ ), which play a significant role in lipid metabolism control.<sup>70</sup>

The spectrum of microorganisms producing compounds constitutes postbiotics that act on health through the gut-microbiota-cardiovascular disease (CVD) axis. This occurs by modulation of gut microbiota, elevation of innate immunity, and enrichment of intestinal cells.<sup>52</sup> Microbiota-accessible carbohydrates are metabolized by gut microbes to produce SCFAs, which could be involved in host interactions for essential health benefits, especially in the treatment of CVD.<sup>71</sup> Endothelial G-protein-coupled receptor 41 is involved in the reduction of blood pressure by an acute microbial SCFA bolus.<sup>72</sup>

To fight against CVD through modulation of gut microbiota and their metabolites, certain foods and herbs act as postbiotics, such as propionate, which on induction in mice (200 mmol/L) resulted in important findings of alleviated cardiac hypertrophy, fibrosis, vascular dysfunction, and a decrease in cardiac ventricular arrhythmias, aortic atherosclerotic lesion area, and systemic inflammation. Subsequently, magnesium acetate supplementation in mice (200 mmol/L) caused a reduction in systolic and diastolic blood pressures, as well as a decline in cardiac and renal fibrosis, respectively.<sup>73</sup> Thus, it can be inferred from the work of Panyod *et al.* about the kinds of dietary nutrients as CVD risk factor precursors and the preference of food and herb choices to be made as a preventive medicine.<sup>73</sup> In addition studies revealed that self-health management tools aimed to reduce CVD-related risks and thereby attain good health and well-being.<sup>73</sup>

### Postbiotics' role in the treatment of Irritable Bowel Syndrome (IBS)

IBS is a complicated G.I disorder, especially observed among people under 50. This chronic condition is characterized by abdominal pain and bowel dysfunction, presenting as constipation, diarrhea, or alternating periods of both.<sup>74</sup> The symptomatic indications for IBS seem to be multifactorial and may include development, persistence following diagnosis, and treatment.<sup>74</sup> The features pertaining to The pathophysiology of IBS is based on an increase in intestinal permeability and micro-inflammation, followed by the association of visceral hypersensitivity with gut dysbiosis. In order to promote intestinal homeostasis, postbiotics have been suggested, especially in GI tract inflammation, which involves a mimicking mechanism of probiotics by postbiotics, thereby facilitating the beneficial effects and avoiding the risks of administered live bacteria.

These postbiotic or cell components have shown positive effects on the improvement of host health. Some postbiotic molecules, including acetate, polyP, ACh, and antimicrobial compounds (potentially bacteriocins) produced by probiotic strains, have been used to treat IBS-associated microorganisms.<sup>75</sup> The production of acetate relies on the carbon source treated with the strains of *L. Plantarum* and the efficient results have been observed in the highest acetate concentration production, thereby attributing it to arabinose as its carbon source,<sup>75</sup> followed by which large amounts of Ach were also obtained from the strains of *Lactobacillus plantarum* (12.5 mg/L for CECT7484 and 13.8 mg/L for CECT7485).<sup>75</sup> The findings showed that the mechanisms pertaining to the secretion of antimicrobial products from the influence of probiotic bacteria have been recommended for the treatment of diarrhea, lactose intolerance alleviation and reduction of irritable bowel symptoms. In addition, the effect is pronounced in inflammatory bowel diseases by increasing relapse time.

### Methods used for the study of postbiotics

Postbiotics can be qualitatively and quantitatively analyzed by several conventional and next-generation techniques, such as flow cytometry, polymerase chain reactions (PCR), enzyme linked immunosorbent assay, Fourier-

transform infrared (FT-IR) spectroscopy, NMR, and electron microscopy.<sup>1</sup>

### Potential applications of postbiotics in the food industry

The profound increase in the knowledge of functional foods has resulted in the evolution of novel health products, such as probiotics. The major issue related to probiotics is their antibiotic resistance genes in a few probiotic strains, which have the potential to be transmitted to pathogenic microorganisms through horizontal gene transfer.<sup>76</sup> On the other hand, the bacterial viability of food products during processing and storage is another concern for its activity, which may depend on various factors, including pH of the product, temperature, interaction with other microbial species, water activity, nutrient availability, inoculation level, fermentation time, dissolved oxygen level, and formulation techniques (spray drying, freeze drying, spray freeze drying, etc.).<sup>77</sup> Therefore, inadequacy of the required microbial load limits its anticipated health benefits and applications in food products.

Conversely, postbiotics, which are metabolic by-products of live bacteria, have been reported to be more stable than probiotics.<sup>78</sup> They are employed in different forms of product formulation, such as lyophilization or drying. However, the processing conditions influence its composition and potential activity in food products. Studies showed that freeze drying destroys the hydrogen peroxide level in the product formulation, which is responsible for antimicrobial activity as well as volatile metabolites. Furthermore, antagonistic effects of pH, storage time, and conditions on postbiotics have been documented in the literature.<sup>79</sup>

### Postbiotics as Class-I preservatives

Class-I preservatives refer to compounds obtained from natural sources that are used to enhance the quality and shelf life of food products. A bio-preservative is a Class-I preservative, as it is a remnant or bioactive metabolite derived from bacteria that inhibit foodborne pathogenic microbes.<sup>80</sup> The principal advantage of biopreservatives is that they are harmless and do not change the quality of food.<sup>81</sup> The antimicrobial properties of postbiotics are mediated by the

presence of bacteriocins, organic acids, fatty acids, peptides, vitamins, and hydrogen peroxide. Currently, preservation of perishable foods by the application of postbiotics is a novel technique in the food industry.

#### **Postbiotics as bio-preservatives in dairy products**

Milk is considered an excellent vehicle for supplementing consumers with probiotics; however, it is also supposed to be the best medium for microbial contamination, which will spoil dairy products. Furthermore, some external and internal factors negatively affect the viability of probiotics and shelf life of the products.<sup>82</sup> In contrast, postbiotics can act as biopreservatives in dairy products to enhance their safety and shelf life. Hamad *et al.* incorporated a freeze-dried postbiotic mixture obtained from *Lactobacillus acidophilus*, *Lactobacillus plantarum*, and *Bifidobacterium bifidum* into soft cheese.<sup>83</sup> Only 1% of postbiotic mixtures of all strains were found to be more effective against pathogenic microorganisms in the product than individual strains.

Garnier *et al.* demonstrated the antifungal activity of postbiotics in semi-hard cheese and sour cream prepared using three probiotic strains in milk.<sup>84</sup> Their results showed a reduction in fungal growth in dairy products without significantly altering the sensory quality. In another study, three different probiotic yoghurts were fortified with postbiotics (0.05–0.15%) prepared from *Lactarius volemus*.<sup>85</sup> Incorporation of postbiotics into the yoghurt samples has presented a significantly higher viable count and essential amino acid content with the best sensory characteristics (at 10% polysaccharide (postbiotics) addition).<sup>85</sup> Moradi *et al.* stated that the postbiotics prepared in MRS media may substantially alter the sensory properties of dairy products, thereby affecting their acceptability in terms of color and appearance.<sup>86</sup> Instead, in situ production of postbiotics by co-culturing *Lactobacillus* species with other probiotic strains has been reported to be the best choice.<sup>79</sup>

#### **Postbiotics as bio-preservatives in Fish and Meat Products**

Microbial contamination can have a significant impact on the nutritional value, taste, and safety of fish and meat, making them even more dangerous to eat.<sup>79</sup> *Clostridium perfringens*

and different species of *Enterobacteriaceae* are the most common microbial contaminants in these commodities.<sup>87</sup> Therefore, the application of postbiotics directly to fish, meat, and related products by coating or spraying methods is a superior option for their preservation.

For instance, coating with postbiotic extract is preferred for meat fillets, while spraying techniques are best suited for minced meat.<sup>45</sup> Mokhtar *et al.* demonstrated an extended shelf life of minced meat of up to 3 months when applied with *Bifidobacterium lactis* Bb-12 and stored at 4 °C.<sup>88</sup> Similarly, the application of postbiotics from *Lactobacillus rhamnosus* EMCC 1105 on minced meat at a concentration of 100 mg/g destroyed *Clostridium perfringens* during storage at 6°C.<sup>89</sup>

Furthermore, Moradi *et al.* reported that the incorporation of postbiotic compounds from *Lactobacillus salivarius* (35 mg/mL) into minced meat stored at 4 °C minimized psychrotrophic spoilage bacteria.<sup>79</sup> Moradi *et al.* tested the antimicrobial activity of cell supernatants from *Lactobacillus salivarius* (Ls-BU2) against *E. coli* in ground beef stored under refrigeration.<sup>79</sup> Their results confirmed that cell-free supernatants of *Lactobacillus salivarius* are effective in controlling the microbial growth and oxidative spoilage of ground beef samples in a dose-dependent manner. Consequently, reports in the literature have confirmed the antimicrobial activity of postbiotics and suggested their role as biopreservatives in meat, fish, and their products.<sup>86</sup>

#### **Postbiotics as bio-preservatives in fruits and vegetables**

The role of postbiotics as antimicrobial agents in fruits and vegetables has been tested in a few studies.<sup>90</sup> Postbiotics were applied in a solution form to cut vegetables in the industry. For instance, Lee *et al.* demonstrated a > 1.5 log reduction in coliforms, aerobic mesophilic bacteria, and mold count in RTE (ready-to-eat) baby leafy vegetables upon the addition of postbiotics (5%) from *Leuconostoc mesenteroides* WK32 along with 0.1 % grape seed extract.<sup>27</sup> In a similar study, a considerably higher reduction (approximately 2.9 log CFU/g) of pathogenic microorganisms, such as *Escherichia coli* O157:H7 and *Listeria monocytogenes* was observed in vegetables treated with postbiotics in combination

with grape seed extract.

In a recent study, home-processed tomato paste was treated with postbiotics prepared from *Lactobacillus plantarum* and *Lactobacillus acidophilus* ATCC 314, and a reduction in the microbial load of *Staphylococcus aureus*, *Aspergillus niger*, *Escherichia coli*, and *Aspergillus flavus*. This resulted in an enhanced shelf life of tomato paste of up to 25 days at room temperature.<sup>91</sup> Another study found that cell-free supernatant (15 mg/mL) of a new *Lactobacillus* sp. RM1, which is found in traditional Egyptian milk, has antifungal properties against *Aspergillus parasiticus* in wheat grains.<sup>92</sup>

### Postbiotics in active food packaging systems

Excessive application of preservatives in the food matrix is not recommended, as the spoilage of food starts from the surface due to microbial invasion, particularly fungi.<sup>93</sup> The application of postbiotic compounds could be a promising technology to address spoilage and food safety issues in packaging. Despite the effective antimicrobial activity of postbiotic components, their direct incorporation into the food matrix with the aim of enhancing shelf life has few limitations.

For instance, the impact of postbiotic interactions or sequestering with the food components or additives in the food reduces its potential.<sup>83</sup> Furthermore, elevated food processing temperatures and pressures adversely affect the stability and concentration of postbiotic components applied to food products. Another drawback is the poor miscibility of certain postbiotic components in the food systems. Hence, the valorization of postbiotics in packaging systems is an ideal choice to extend the shelf life of food. Active packaging is a technique in which the packaging material, food component, and environment positively interact to improve the shelf life and quality of food.<sup>94</sup> The advantages of using postbiotics in active packaging systems are as follows:<sup>45</sup>

1. The majority of postbiotics are generally regarded as safe (GRAS) for use in food.
2. No adverse effects on eukaryotic cells have been reported.
3. They did not affect the intestinal microflora.
4. Several postbiotic compounds are resistant to a variety of pH conditions.

5. Their antimicrobial efficiency has been proven against a wide range of foodborne pathogenic microorganisms, even at low concentrations.
6. Some postbiotics are stable at high temperatures and are possible to incorporate into food matrixes by extrusion and spray drying processes.

Postbiotics are used in packaging systems in different forms, such as:

1. Inclusion of a coating or adsorbing material in the packaging matrix
2. Ionic and covalent linkages are used to immobilise postbiotic components in the polymer matrix.
3. Direct incorporation into the packaging system.
4. Active material in the packaging systems, which improves the stability and migration.

Among the different forms of postbiotic application in packaging systems, they are directly incorporated into the packaging polymer matrix.

Active packaging films were developed by Beristain-Bauza *et al.* using whey protein isolate, calcium caseinate, and various concentrations of postbiotics (6, 12, or 18 mg/mL) from *Lactobacillus Rhamnosus* NRRL B-442.<sup>95</sup> Their results showed that a postbiotic concentration of 18 mg/mL presented greater antimicrobial efficiency against *Salmonella typhimurium*, *Escherichia coli*, *Listeria monocytogenes*, and *Staphylococcus aureus* without altering the properties of the film. Meira *et al.* prepared a nanocomposite film with corn starch-halloysite clay incorporated with nisin and pediocin.<sup>96</sup> The developed nanocomposite films were effective against *Listeria monocytogenes* and *Clostridium perfringens*.

The application of halloysite as a nanofiller increased the bacteriocin diffusion rate and enhanced anti-microbial retention in the packaging polymeric matrix. A bacteriocin like substance from *Lactobacillus curvatus* P99 was used in different concentrations (15.6 µL/mL and 62.5 µL/mL) as an active material in a starch-based edible film. The post-biotic-loaded casing exhibited potential antibacterial activity against *Listeria monocytogenes*. Furthermore, the storage of sliced cheese in the developed active film for 10 days reduced *Listeria monocytogenes* levels in packaged cheese without altering its sensory properties.<sup>97</sup> In one study, natural and

artificial casings were developed using ovine, collagen, porcine, cellulose, and bovine tissues as carriers of postbiotics from *L. curvatus* ACU-1. Casing was used to wrap sausage meat, and it was found that all postbiotic casings exhibited antimicrobial properties against *Listeria innocua* and *Listeria monocytogenes*.<sup>98</sup> In another study, a chitosan nanoparticle-based film developed by incorporating postbiotics produced by seven *Lactobacillus* species, including *Lactobacillus plantarum*, *Lactobacillus helveticus*, *Lactobacillus rhamnosus*, *Lactobacillus reuteri*, *Streptococcus thermophilus*, and *Enterococcus faecium*, showed strong antibacterial and antifungal efficacy in Egyptian cheese.<sup>99</sup>

### Postbiotics as anti-biofilm agents

A biofilm is a network of one or more microorganisms that can develop inside a polysaccharide or protein matrix.<sup>100</sup> They are produced by both gram-positive and gram-negative fungi.<sup>101</sup> Biofilms are still an important food quality and safety issue in the food industry that requires great attention as they exhibit bacterial resistance to antimicrobials.<sup>86</sup> Several studies have demonstrated that postbiotics can be used as antibiofilm agents.<sup>88</sup> Generally, two different approaches are used to employ postbiotics as antibiofilm agents. Primarily, postbiotics could be used to prevent biofilm formation and, secondly, to destroy the biofilm developed on the product or material surface. Bacteriocin, biosurfactants and exopolysaccharides are some of the postbiotic compounds with antibiofilm properties.

The antibiofilm property of postbiotics produced by *Lactobacillus acidophilus* LA5, *Lactobacillus casei* 431, and *Lactobacillus salivarius* on the biofilm shaped by *Listeria monocytogenes* on a polystyrene surface was tested by Moradi et al.<sup>79</sup> The results of this study showed that the application of natural acid-based postbiotics effectively damaged biofilm formation. Cui et al. demonstrated the antibiofilm activity of postbiotics derived from *Lactobacillus crustorum* ZHG 2-1 against biofilm produced by *Pseudomonas aeruginosa*.<sup>102</sup> This ability to hamper biofilm formation is ascribed to the binding ability and disruption of extracellular signal molecules, such as AHL and C4-HSL.

Furthermore, a ZHG 2-1 postbiotic

compound was proposed to suppress the regulation and expression of quorum sensing (QS) genes.<sup>102</sup> A biofilm developed by *Bacillus subtilis* BM19 was destroyed by bacteriocins produced by *Lactobacillus acidophilus* ATCC 4356.<sup>103</sup> Petrova et al. reported the antibiofilm potential of postbiotic compounds, L1p1 and L1p2, produced by *Lactobacillus rhamnosus* GG, against the biofilm matrix developed by *S. typhimurium* and *E. coli*.<sup>104</sup> This study demonstrated the interaction of postbiotics with pathogenic microbial constituents such as exopolysaccharides, proteins, DNA, and lipids. The target molecules for L1p1 and L1p2 are said to be cellulose and heteropolysaccharide residues of glucose, galactose, glucuronic acid, and fructose in the biofilm matrix, which destabilize the initial biofilm formation.<sup>104</sup>

Exopolysaccharides are high-molecular-weight polymers produced by bacteria that participate in biofilm development, including bacterial communication and superficial interactions leading to attachment and biofilm formation.<sup>105</sup> The exopolysaccharides of *Lactobacillus acidophilus* A4 suppress genes associated with curli and chemotaxis, which regulate the adhesion capacity of enterohemorrhagic *E. coli*. Exopolysaccharides from *Lactobacillus acidophilus* suppress the growth of both Gram positive and Gram negative pathogens and biofilm formation of *E. coli* O157:H7 with 87% to 94% efficiency depending on the surface.

### Postbiotics as decontaminant agents

Recent reports have suggested that postbiotics are a promising area for the decontamination of food from chemicals, such as pesticides, biogenic amines (BA), and mycotoxins. In this context, Mah et al. have focused on the impact of postbiotic compounds on BA levels.<sup>106</sup> BAs are a group of low-molecular-weight chemical constituents that have adverse health effects, including tryptamine, tyramine, histamine, cadaverine, and putrescine.<sup>107</sup> These compounds are produced in fermented products by the decarboxylation of amino acids, such as lysine, tyrosine, tryptophan, and histidine.<sup>106</sup>

The role of postbiotics on the destruction of BAs was postulated by Toy and Zogul.<sup>108</sup> The following hypothesis was proposed:

1. Inhibition of growth of *Lactobacillus* species

producing BAs

2. Suppression of BA production by altering the growth conditions such as pH
3. Direct destruction of BAs by postbiotics

Postbiotics of *Streptococcus thermophiles* and *Lactobacillus* strains at 25% and 50% concentrations efficiently reduced the tyramine production from *Staphylococcus aureus*. Postbiotics of *Lactobacillus acidophilus* suppressed the tyramine synthesis at a 50% concentration, as reported by Toy and Ozogul.<sup>108</sup> In a study by Xie *et al.*, the influence of postbiotics and heat treatment (100°C, 10 min) from *L. plantarum* on the growth of *Enterobacteria* species and diamine production was investigated.<sup>109</sup> The results showed a considerable reduction in the growth and diamine production of most *Enterobacteria* species.<sup>109</sup> In another study, Niu *et al.* had demonstrated the degradation efficiency (40%) of thermostable amine oxidase obtained from *L. plantarum* CAU 3823 for BAs such as tyramine, histamine, cadaverine, and putrescine.<sup>110</sup>

### Challenges in postbiotics research

As of now, as per the literature survey, postbiotics are believed to have health-promoting activities against various metabolic disorders in animal models. However, there is no scientific evidence available to prove their efficacy against the concerned disease conditions. Furthermore, their metabolic signaling is not well established. Recent research on suckling rats found that a diet supplemented with scGOS and lcFOS resulted in softer feces, changes in microbiota composition, a different SCFA profile, and an increase in Toll-like receptor gene expression.<sup>8</sup> But when consuming the direct postbiotics, the healthiness of the vital organs is questionable, especially for children, pregnant women, and heart and kidney patients. Despite the fact that animal models demonstrated efficacy, human trials are required for FDA regulatory approval. So, the toxicity of these products should be well documented and scientifically proven, and clinical trials are mandatory. Also, the dead cells of many microbes have already been used in the form of vaccines. Whether postbiotics are taken as dietary supplements or are prescribed, the inanimate postbiotic molecules can eliminate pathogenicity. As a result, its toxicity can be

determined before it is used for human purposes. More research is needed on the viability and shelf life of postbiotic molecules, which must be tested both in vitro and in vivo.

### Future perspectives

Postbiotic compounds show promise as potential nutraceuticals for human and animal health enhancement. It can serve as an alternative to existing medications, and the antimicrobial active postbiotic molecules can be administered with available drugs to combat antimicrobial drug resistance problems. The microencapsulated postbiotic molecules can be administered to solve several metabolic syndromes, which can be screened through bioimaging systems. Screening tools can be developed to authenticate gut products. Future techniques must reveal the quantity and quality of gut microbes, as well as their liberation. A new methodology, in particular, must validate the microbiomes of diseased people. There is a live screening system to understand the activity of gut microbes. Imaging systems can be developed to live monitor the number of gut microbes and their secretions on molecular signaling pathways in various disease conditions. There is a model required to monitor the postbiotic molecule secretions during the dysbiosis condition. The beneficial microbes' interactions with beneficial or pathogenic organisms and their quorum sensing signals can be monitored for the production of postbiotic molecules. Biomarkers must be developed for inanimate products.

### CONCLUSION

Postbiotics are composed of inanimate cells or metabolic products. These secondary metabolites play crucial roles in maintaining metabolic homeostasis. Most of these products can be easily quantified in the fecal matter. In particular, the short-chain fatty acid concentration in the fecal matter helps to analyze the healthiness of an individual. Postbiotics help overcome metabolic syndromes, such as diabetes, irritable bowel disorders (IBD), and different types of cancers. These postbiotic molecules produce immune signals to establish defence systems at the cellular level. The anti-microbial peptide postbiotic establishes strong inhibitory action against various

entero-pathogens and neutralizes their toxins in the gut system. Therefore, postbiotics may reduce the incidence of anti-inflammatory and autoimmune disorders. Bioaccumulation of antibiotics and non-antibiotic molecules recommended for various disease conditions may have adverse effects on beneficial intestinal microbes and influence postbiotics liberation. Dietary substrates act as prebiotic sources and are responsible for the metabolic capacity of the gut microbes. These prebiotics are effective sources for aggravating the production of postbiotics. Postbiotic molecules can substitute antibiotics and anti-cancer drugs, which have side effects. To date, their roles in communicable and non-communicable diseases have not been thoroughly studied. New product development with postbiotics is a promising area of research and has an impact on the food and nutrition field. Postbiotic molecule applications in packaging systems in the food industry as Class-I preservatives, biopreservative agents, antimicrobial, and decontaminating agents are areas that need to be explored. Postbiotics molecules studies are still in infancy. It can be further studied for its molecular signalling and quorum activities. Such identified molecules must be transferred from lab to land scale through food industries to common public. In addition, advanced analytical techniques are required to detect postbiotics in the gut.

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#### CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

#### AUTHORS' CONTRIBUTION

ASB and PS conceptualized the study. ASB, PS, AGK, TS and NG wrote the manuscript. SAOA reviewed and edited the manuscript. All authors read and approved the final manuscript for publication.

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#### DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

#### ETHICS STATEMENT

Not Applicable.

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