

## The contribution of horticultural products to create innovative functional food benefitting from prebiotics, probiotics and postbiotics: A review

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Fruits and vegetables have an essential impact to improve human health as, in addition to their nutritional properties, they contain dietary fibers and prebiotic compounds showing an important role in modulating human gut microbiota. Beneficial microbes enhance diet by helping to digest food efficiently and also play a tremendous role in boosting immune system. These bacteria are added in various vegetables and animal products to enhance their nutritive properties and commercial impact. The mentioned bacteria in different species of genera *Lactobacillus*, *Bifidobacterium*, *Streptococcus*, are termed probiotics and defined as live microorganism contributing health benefits to the host when supplied in adequate amount. On the other hand, prebiotics are defined as 'a substrate that is selectively utilized by host microorganisms conferring a health benefit'. In addition to prebiotics and probiotics, postbiotics are represented by microbial fractions or cell lysates of good bacteria. This review provides an overview of the importance of vegetables and fruits to trigger the circuit involving prebiotics, probiotics and postbiotic with the aim to produce functional food showing a significant impact on human health.

**Keywords:** Functional Food, Vegetables, Fruits, Oligosaccharides, *Lactobacillus*, *Bifidobacterium*, *Streptococcus*

### INTRODUCTION

Diet can have a positive but also a negative effect upon human health, and, indeed, in developed countries, a fat excess may cause diseases, such as hypertension and obesity. Conversely, in developing countries, a poor diet causes serious nutrient deficiencies. Therefore, in recent years, there has been an increasing interest in healthy food nutrition, and consumers have been increasingly demanding health-promoting foods, such as functional foods. Indeed, the importance of foods is not only referred to the nutritional value but also to their contribution to consumers' health and, in this respect, to the properties of

non-digestible oligosaccharides as prebiotic compounds<sup>1</sup>, which show benefits due to their "bioactive" or "functional" action in intestinal health. The consumption of natural sources of non-digestible oligosaccharides, such as fruits and vegetables, has economic advantages for consumers compared to the industrial products and, in addition, also contains other nutrients such as bioactive compounds, vitamins, and minerals<sup>2</sup>. The industrial by-products of fruits and vegetables also present a considerable amount of dietary fibre and prebiotics with important biological activities, which makes the incorporation of their extracts or powders to produce functional food<sup>3</sup>.

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Dietary and prebiotic compounds, present in fruits and vegetables, considerably improve human health through mechanisms such as microbiota modulation, reduction of post-prandial glycemic response, normalising the cholesterol level, preventing constipation, and transporting the phenolic compounds<sup>4</sup>. The fruit and vegetable wastes by-product can be valorised by extracting valuable compounds that can be further used in functional foods or in nutraceuticals<sup>5</sup>. The beneficial effects of prebiotics and probiotics on human health are being increasingly investigated worldwide. Current Scientific work regarding the properties and functionality of living microorganisms in food has suggested that probiotics play a noteworthy role in immunological, digestive and respiratory functions and that they could have a remarkable effect on the prevention of infectious diseases in children and other high-risk groups. Research relevant to beneficial bacteria/probiotic bacteria came into existence with the announcement made by a Nobel laureate and father of innate immunity, Elie Metchnikoff. He reported that there were 'good' bacteria that could be cultivated in the gut by consuming Bulgarian yoghurt and other forms of Curdled milk. He believed that, once in the intestines, these good bacteria would prevent putrefactive bacteria from forming on undigested food<sup>6</sup>. His theory of good bacteria re-emerged in the mid-1990s with the name probiotics. These probiotics are not only the subject of intense medical research but also the source of a multi-billion-dollar global industry<sup>4</sup>. In the past decade, the scientists emphasised prebiotics parallel to the probiotics, and a recent new term postbiotics, has been created upon the study of prebiotics and probiotics. The Food and Agriculture Organisation of the United Nations World Health Organisation, defines probiotics as "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host." The same organisation defines prebiotics as a non-viable food component that confers a health benefit on the host associated with modulation of the microbiota<sup>7</sup>. However, new terms such as para-probiotic or postbiotic have emerged to denote that non-viable microbial cells, microbial fractions, or cell lysates might also offer physiological benefits to the host by providing additional bioactivity<sup>8</sup>. Thus, the rationale of this paper is to review the current credentials and the possible beneficial effects of probiotics, prebiotics and the recently evolved term "postbiotics" on human and animal health. These good bacteria could be an answer against a current viral threat, as they help rejuvenate immunity or act as an immunity booster<sup>11</sup>.

## OVERVIEW OF PROBIOTICS, PREBIOTICS AND POSTBIOTICS

### Prebiotics

Gibson et al. 2017 defined prebiotics as 'a nondigestible

food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health'. However, the definition of prebiotics was subsequently modified to "selectively fermented ingredients that allow specific changes, both in the composition and/or activity in the gastrointestinal microflora that confer benefits upon host well-being and health"<sup>9</sup>. The Food and Agriculture Organisation (FAO) of the United Nations (UN) in 2008 redefined the prebiotic as "a non-viable food component that confers a health benefit on the host associated with modulations of the microbiota". Gibson *et al.*, (2010) redefined the 'dietary prebiotics' as "a selectively fermented ingredient that results in specific changes in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health"<sup>10</sup>. Bindal proposed that specificity requirements should be removed based on reports showing that multiple taxa, rather than particular species, were enriched by prebiotics<sup>12</sup>. This proposal led to another definition of a prebiotic as "a non-digestible compound that, through its metabolism by microorganisms in the gut, modulates the composition and/or activity of the gut microbiota, thus conferring a beneficial physiological effect on the host". In December 2016, the International Scientific Association for Probiotics and Prebiotics (ISAPP) amended the definition and the current definition of prebiotics is defined as 'a substrate that is selectively utilised by host microorganisms conferring a health benefit'<sup>9</sup>. Probiotic substances are not hydrolysed nor absorbed in the gastrointestinal tract but are available as substrates for probiotics, and the most commonly used ones at present are non-digestible fructo-oligosaccharides. According to the final definition of prebiotics, some compounds of the carbohydrate group, such as short and long chain fructans, e.g. fructooligosaccharide (FOS): inulin, lactulose, galactooligosaccharides (GOS) and human milk oligosaccharide (HMOs) can be classified as prebiotics. Low molecular weight carbohydrates, including sugar alcohols and mono, di- and oligosaccharides, in particular fructooligosaccharides and raffinose-family oligosaccharides, are significantly present in vegetables and fruits. Particularly, blueberry, pear, nectarine, garlic, leek, white onion, scallion and watermelon have been found rich in oligosaccharides, with the highest content of fructooligosaccharides in nectarine, scallion and white onion, while Jerusalem artichoke, chicory root, and garlic show the highest fructan levels<sup>13</sup>. The mentioned products are potential sources of prebiotics useful as ingredients for functional food. Biopolymer-based edible films containing probiotic-loaded edible films with common polysaccharides (alginate, gellan gum, and starch): proteins (zein, gelatin, and whey protein isolate): and prebiotics (oligofructose, inulin, and fructooligosaccharides) are a promising approach to preserve minimally processed fruits and vegetables. Indeed, they exhibit barrier function

against water vapor, gas, and aroma, as well as antioxidant and antimicrobial function while protecting the viability of probiotics, which is essential to maintain their nutritional value and quality<sup>14</sup>.

The following criteria are used to classify a compound as a prebiotic<sup>10</sup>

It should be tolerant of stomach acidity.

It cannot be hydrolysed by the digestive enzymes of humans and animals.

Should not be absorbed in the gastrointestinal tract.

It can be fermented by intestinal microbiota.

The growth and activity of the intestinal bacteria can be selectively stimulated by this compound, and this process improves the host's.

## Probiotics

The term probiotics means “for life”, and this term currently designates the bacteria associated with humans and animal gut. The first observation of the positive role played by some selected bacteria is attributed to Eli Metchnikoff, a Russian Nobel Prize winner working at the Pasteur Institute at the beginning of the 20th century, who hypothesised in 1907 that replacing the number of ‘putrefactive’ bacteria in the gut with lactic acid bacteria could normalise bowel health and prolong life<sup>15</sup>. He made a landmark observation that the habitual consumption of lactic acid bacteria in fermented dairy products, such as yoghurt, was associated with improved health and longevity in Bulgarian peasant populations. He linked this to the ‘Bulgarian bacillus’, which was discovered by Bulgarian doctor Stamen Grigorov, who demonstrated how healthy bacteria in yoghurt helped digestion and improved the immune system. The scientific justification for the health benefits of lactic acid bacteria was provided in his book “The Prolongation of Life”. He asserted that some of the bacterial organisms present in the large intestine were a source of ‘toxigants’ that contributed to illness and ageing. He suggested that ‘The dependence of the intestinal microbes on the food makes it possible to adopt measures to modify the flora in our bodies and to replace the harmful microbes with useful microbes’. To test the hypothesis on the health benefit of consuming lactic acid bacteria, Metchnikoff drank sour milk every day until his demise<sup>5</sup>. After more than half a century, the term probiotics was coined to reflect Metchnikoff’s idea. Now Elie Metchnikoff is regarded as the grandfather of modern probiotics. “At the same time, the French paediatrician Henry Tissier observed that children with diarrhoea had in their stools a low number of bacteria characterised by a peculiar Y-shaped morphology. These ‘bifid’ bacteria were, on the contrary, abundant in healthy children. He suggested that these bacteria could be administered to patients with diarrhoea to help restore a healthy gut flora<sup>16</sup>. The observations of Metchnikoff and Tissier were

the earliest to make scientific recommendations about the use of good bacteria. The term probiotics did not exist until 1965, when Lilly and Stillwell used the term probiotics in a different context to represent substances secreted by one organism that stimulate the growth of another<sup>17</sup>. Subsequently, probiotics were considered as live supplemental organisms or their metabolites, which contribute to intestinal microbial balance with beneficial effects or, according to a viable mono- or mixed culture of bacteria that beneficially affects the host by improving the properties of the indigenous flora<sup>18</sup>. This was followed by Salminen *et al.* who defined probiotics as foods containing live bacteria that are beneficial to health<sup>19</sup>. Guarner and Schaafsma gave a more recent definition as “live microorganisms, which when consumed in an adequate amount, confer a health effect on the host”<sup>20</sup>. As research in probiotics became more noticeable, the United Nations Food and Agriculture Organisation and the World Health Organisation (FAO/WHO) in<sup>7</sup> (FAO-WHO, 2001) sponsored an Expert Consultation following a request from the Argentinian government. During the consultation, which was chaired by Dr Gregor Reid, the Director of the Canadian Research and Development Centre for Probiotics, a consensus definition of probiotics was adopted as “live microorganism which, when administered in inadequate amounts, confers a health benefit to the host<sup>7</sup>. This is now the widely used and accepted definition as it embraces all applications of live microbes, not just those for intestinal benefits<sup>5,21</sup>. Following the FAO/WHO definition, the International Life Sciences Institute ILSI and the European Food and Feed Cultures Association launched similar definitions for a probiotic, namely a live microbial food ingredient that, when taken up in adequate amounts, confers health benefits on the consumers” and “live microorganisms which when ingested or locally applied in sufficient numbers, provide the consumer with one or more proven health benefits”, respectively. The FAO/WHO expert consultation restricted its scope to the discussion of probiotics “as part, of the food” and excluded reference to the term biotherapeutic agents and beneficial microorganisms not used in food. However, it is recognised that the definition is sufficiently broad to encompass a range of probiotic preparations and intended uses. In this respect, a probiotic can be a food or a dietary supplement, can be used in a drug application (also named a live biotherapeutic) or microbial feed or can be used as a genetically modified microorganism (GMM) or live vaccine if administered orally. For use in food, probiotics should be capable of surviving the digestive tract, by revealing acid and bile tolerance and withstanding digestive enzymes. They should be capable of proliferating in the gut and of exerting their beneficial effects on the host through growth and/or activity in the human body. Therefore, the potential to remain viable at the target site and to be effective should be confirmed for each strain.

### Syntactic modification of the definition of probiotics

The FAO/WHO definition has been widely adopted by researchers, regulators and consumers. Organisations and agencies such as Codex (which comes under the FAO/WHO umbrella): Health Canada, the World Gastroenterology Organisation, the European Food Safety Authority (EFSA) and the Institute of Food Technologists use the FAO/WHO definition when referring to probiotics. The International Scientific Association for Probiotics and Prebiotics, in October 2013, recommended a more grammatically correct definition for probiotics: “live microorganisms that, when administered in adequate amounts, confer a health benefit on the host” and supports the use of this wording going forward<sup>22</sup>.

The minimum requirements needed for probiotic status include (FAO)<sup>7</sup>.

- The valuation of strain identity (genus, species, strain level).
- *In vitro* tests to screen potential probiotic attributes.
- Safety assessments.
- *In vivo* studies for substantiation of health effects in the target host section may be divided into subheadings. It should provide a concise and precise description of the experimental results, their interpretation, and the experimental conclusions that can be drawn.

### Postbiotics

Postbiotics is the most recent term in the ‘-biotics’ field. The reports on postbiotic products are emerging in the literature with each passing day. In 2019, the International Scientific Association of Probiotics and Prebiotics (ISAPP) convened a panel of experts to review the definition and scope of postbiotics<sup>23</sup>. A panel described postbiotics as a preparation of inanimate microorganisms and/or their components that confer health benefits on the host. Postbiotics include microbial cells, constituent metabolites, short-chain fatty acids (SCFAs): microbial cell fractions, functional proteins, extracellular polysaccharides (EPS): cell lysates, teichoic acid, peptidoglycan-derived muropeptides and pili-type structures. Research to date indicates that postbiotics can have direct immunomodulatory and clinically relevant effects, and evidence can be found for the use of postbiotics in healthy individuals to improve overall health and to relieve symptoms in a range of diseases, such as infant colic and adult atopic dermatitis and different causes of diarrhoea<sup>24</sup>. Postbiotics can be used in combination with nutritional components to promote health. Two commonly mentioned types of postbiotics are paraprobiotics and fermented infant formula (FIFs). Paraprobiotics, or 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’<sup>25</sup>. Where FIFs are infant or follow-on formulas that have been fermented with lactic acid producing or other bacteria and in most cases do not contain viable bacteria<sup>2,26</sup>. The postbiotics concept may be a blessing for human life.

## GENERAL CHARACTERISTICS OF PROBIOTICS, PREBIOTICS AND POSTBIOTICS

### Characteristics of probiotics<sup>9</sup>

- Stability under acidic conditions of the stomach and colon.
- Resistance to enzymatic digestion.
- Hydrolysis and fermentation by colonic bacteria.
- Selective stimulation of the growth of limited numbers of beneficial colonic bacteria.

### Probiotics<sup>9,10</sup>

- Non-pathogenic and non-toxic.
- Acid- and bile-tolerant.
- Human origin/ Food grade.
- Potential to produce an antimicrobial substance.
- Adherence to human intestinal cells.
- Persistence in the human intestinal tract.
- Antagonism against enteric pathogens.
- Susceptible to antibiotics.
- Safety in food and clinical use.
- Able to maintain good viability.
- Clinically validated and documented health effect.

### Characteristics of postbiotics<sup>27</sup>

These are the metabolic end products of probiotics metabolism. They must have many health- regulating functions in the body. Some of their more well-known functions include the regulation of digestion, absorption of nutrients, detoxification, regulation of the immune system, gut- brain communication and much more.

These are various other benefits of prebiotics, probiotics and postbiotics are described in Fig. 1.

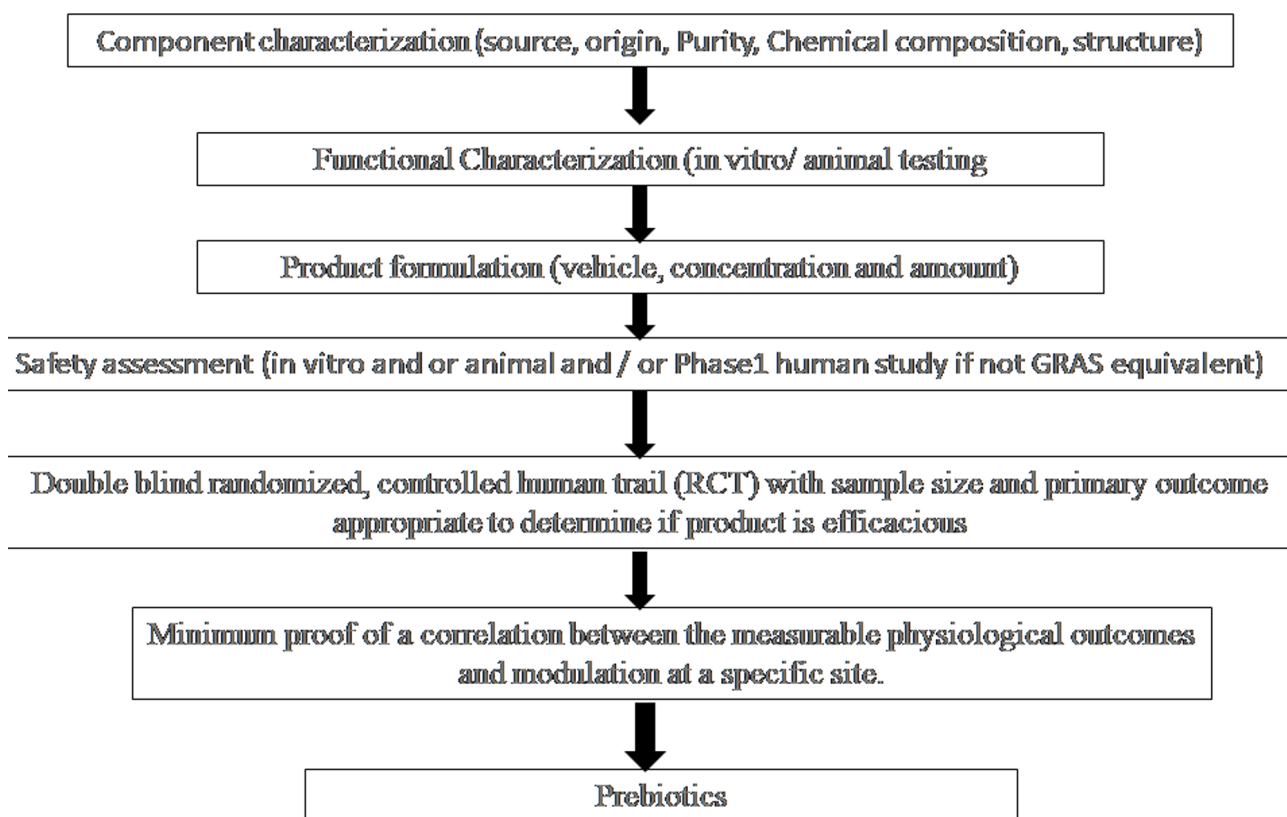
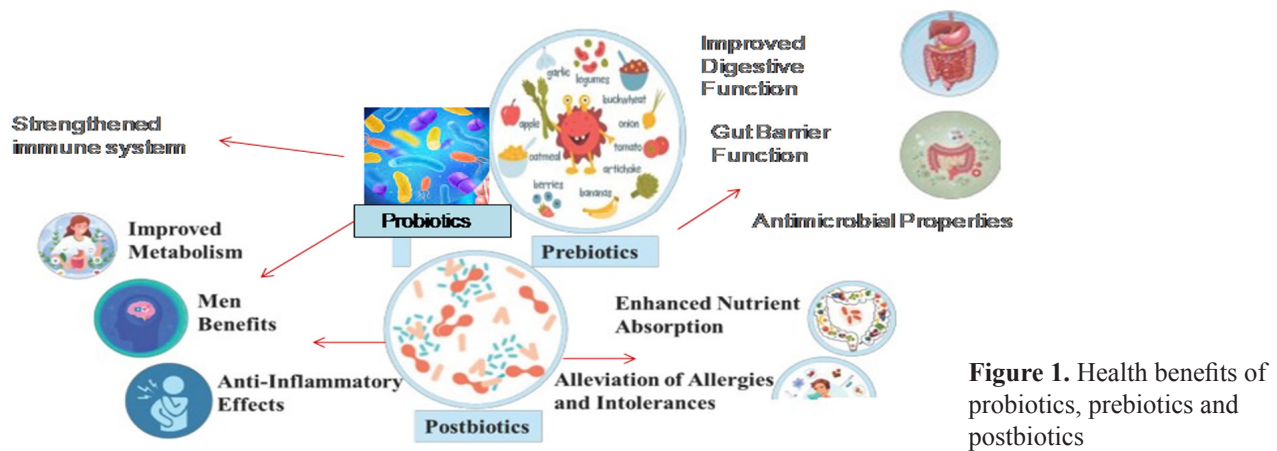
## HOW TO GET RECOMMENDATIONS FOR PROBIOTICS AND PREBIOTICS<sup>7</sup>

### Prebiotics

FAO, the technical committee provided guidelines for the evaluation and authentication of prebiotics as shown in Fig. 2.

### Probiotics

- Potential probiotic strains must be identified by methods including internationally accepted molecular techniques and named according to the International Code of Nomenclature, and strains should preferably



- be deposited in a reputable, internationally recognised culture collection.
- To be termed a probiotic, the probiotic micro-organism must be able to confer defined health benefits on the host.
- There is a need for refinement of *in vitro* and *in vivo* tests to better predict the ability of probiotic microorganisms to function in humans.
- There is a need for more statistically significant efficacy data in humans.
- Good manufacturing practices must be applied with

- quality assurance, and shelf-life conditions must be established, and labelling must be made clear to include minimum dosage and verifiable health claims.
- The regulatory status of probiotics as a component in food has to be established at an international level.
- The Consultation recommends that a regulatory framework be established to better address issues related to probiotics, including efficacy, safety, labelling, fraud and claims.
- Probiotic products shown to confer defined health benefits on the host should be permitted to describe

- these specific health benefits.
- Surveillance system includes track-back and post-marketing surveillance, which should be put in place to record and analyse any adverse events associated with probiotics in food. Such a system could also be used to monitor the long- term health benefits of a probiotic strain<sup>28</sup>.
- Postbiotics is an upcoming field and, therefore, no information regarding related recommendations has been published in the literature.

## EXAMPLES OF PROBIOTICS, PREBIOTICS AND POSTBIOTICS

### Prebiotics

Most common prebiotics are carbohydrate-based, but other substances, such as polyphenols and polyunsaturated fatty acids converted to their respective conjugated fatty acids, might be used as prebiotics. Most of the first prebiotics evaluated in humans and used commercially were shown to stimulate *Lactobacillus* and *Bifidobacterium*. Significant prebiotics and their reported functions have been listed in Table 1.

### Probiotics

The seven genera of microbial organisms most often used in probiotics are *Bifidobacterium*, *Lactococcus*, *Streptococcus*, *Saccharomyces*, *Bacillus* and *Escherichia*. Table 2 shows the functions and therapeutic effects of different genera and their species reported as probiotics.

### The world's most popular probiotics: Drohhira's probiotics (<http://choosetobehealthy>)

The world's most popular premium probiotic supplement

has been prepared by Dr Ichiroh Ohhira. This probiotic is a fermented food product that is produced utilising a multi-year fermentation process. Dozens of different Kinds of organically grown food (vegetables, fruits, seaweeds and mushrooms) are chopped and shredded, and then added to the fermentation vats along with 12 strains of live prebiotic bacteria. His formula J:B: is over 12 strains of *Lactobacillus* and *Bifidobacteria*.

### The names of 12 probiotic strains used in Dr. Ohhira's formula

1. *Enterococcus faecalis*TH-10
2. *Bifidobacterium breve* ss. Breve
3. *Bifidobacterium infantis* ss. Infantis
4. *Bifidobacterium longum*
5. *Lactobacillus acidophilus*
6. *Lactobacillus brevis*
7. *Lactobacillus bulgaricus*
8. *Lactobacillus fermentum*
9. *Lactobacillus casei* ss. Casie
10. *Lactobacillus helveticus* ss. Jagurit
11. *Lactobacillus plantarum*
12. *Streptococcus thermophilus*

### Postbiotics

In general, the postbiotics can be differentiated either by their elemental composition, i.e., lipids( e.g. butyrate, propionate, dimethyl acetyl- derived plasmalogen): proteins (e.g. lactocepin, p40 molecules): carbohydrates (e.g. galactose-rich polysaccharides and teichoic acid): vitamins /co-factors( e.g. B- group vitamins): organic acids (e.g. propionic and 3-phenyllactic acid) and complex molecules such as peptidoglycan- derived muropeptides, lipoteichoic acids or by their physiological

**Table 1.** Significant Prebiotics and their reported functions

| Name of prebiotics                                                                                          | Reported functions                                                                                                                                                                                          | References                 |
|-------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------|
| Inulin                                                                                                      | Stimulate <i>Lactobacillus</i> and/ or <i>Bifidobacterium</i> spp.<br>Antitumorigenic activity                                                                                                              | 28,29                      |
| Fructo-oligosaccharides (FOS)                                                                               | Enrichment of <i>Lactobacillus</i> and/ or <i>Bifidobacterium</i> spp.                                                                                                                                      | 9, 29                      |
| Galacto-oligosaccharides (GOS)                                                                              | Encourage <i>Bifidobacteria</i> , <i>Lactobacilli</i> , <i>Enterobacteria</i> ,<br>Bacteroidetes, and <i>Firmicutes</i>                                                                                     | 9, 29, 30,<br>31           |
| Soy oligosaccharides, Starch                                                                                | Stimulate <i>Ruminococcus bromii</i> , and <i>Bifidobacterium adolescent</i><br>is at greater extent, and Eubacterium rectale and Bacteroides to a<br>lower extent                                          | 32, 33                     |
| Xylo-oligosaccharides, Pyrodextrin,<br>Isomalte- oligosaccharides,<br>lactosucrose, polydextrose, lactulose | Enhance the growth of Bifidobacteria and Lactobacillus, and<br>decrease the number of Bacteroids                                                                                                            | 31                         |
| Human milk oligo-saccharides<br>(HMOs)                                                                      | Increase the proportion of Bifidobacteriaceae and Bacteroidaceae.<br>These are important for the development of the newborn baby's<br>intestinal microbiota and metabolic and immunological systems,<br>HMO | 11, 20, 34<br>35 36,<br>37 |

**Table 2.** Therapeutic effect of probiotics genera and their species

| Name of genera        | Reported function/ Therapeutic effect                                    | Species                   | References |
|-----------------------|--------------------------------------------------------------------------|---------------------------|------------|
| <i>Bifidobacteria</i> | Support the immune system                                                | <i>B. animalis</i>        | 21,37      |
|                       | Limit the growth of harmful bacteria in the intestine                    | <i>B. adolescentis</i>    | 38         |
|                       | Prevention as well as treatment of necrotising enterocolitis in newborns | <i>B. bifidum</i>         | 29,39      |
|                       | Help in breaking down lactose into nutrients the body can use            | <i>B. breve</i>           |            |
|                       | Cholesterol- lowering capacities                                         | <i>B. infantis</i>        |            |
|                       | Reduce risk for eczema, food allergies                                   | <i>B. lactis</i>          |            |
| <i>Lactobacillus</i>  | Lactose digestion                                                        | <i>B. longum</i>          |            |
|                       | Inhibit pathogenic bacteria                                              | <i>L.acidophilus</i>      | 39,5, 21   |
|                       | Help to increase the body's absorption of minerals                       | <i>L. brevis</i>          |            |
|                       |                                                                          | <i>L. bulgaricus</i>      |            |
|                       |                                                                          | <i>L. casei</i>           |            |
|                       |                                                                          | <i>L. delbrueckii</i>     |            |
|                       |                                                                          | subsp. <i>bulgaricus</i>  |            |
|                       |                                                                          | <i>L. fermentum</i>       |            |
|                       |                                                                          | <i>L. johnsonii</i>       |            |
|                       |                                                                          | <i>L.leuteri</i>          |            |
|                       |                                                                          | <i>L. paracasei</i>       |            |
|                       |                                                                          | <i>L. plantarum</i>       |            |
|                       |                                                                          | <i>L. rhamnosus</i>       |            |
| <i>Lactococcus</i>    | Treatment of antibiotic- associated diarrhoea                            | <i>L. sakei</i>           |            |
|                       | Modulation of brain activity                                             | <i>L. salivarius</i>      |            |
|                       | Antimicrobial activity against <i>C. difficile</i>                       | <i>Lactococcus lactis</i> | 40,41      |
| <i>Streptococcus</i>  |                                                                          | subsp. <i>lacti</i>       | 39         |
|                       | Dental caries management                                                 | <i>S. salivarius</i>      | 42         |
|                       | Reduction of irritable bowel syndrome symptoms                           | <i>S. pyogenes</i>        |            |
|                       | Reduction of necrotising enterocolitis in preterm infants                | <i>S. thermophilus</i>    | 78         |
| <i>Bacillus</i>       |                                                                          | <i>S. pneumonia</i>       | 39         |
|                       | Treatment of antibiotic- associated diarrhoea                            | <i>B. subtilis</i>        | 43         |
|                       | Efficient against diarrhoea                                              | <i>B. coagulans</i>       |            |
|                       | Treatment of bacterial vaginosis with immunological support              | <i>B. cerus</i>           |            |
|                       | Prevention of caries in children                                         | <i>H. pylori</i>          |            |
| <i>Escherichia</i>    | Efficient animal probiotics                                              |                           |            |
|                       | Treatment of functional constipation in adults                           | <i>Escherichia coli</i>   | 44         |
|                       | Treatment of inflammatory bowel disease                                  | (Nissle, 1917-EcN)        |            |
|                       | Treatment of gastrointestinal disorder                                   |                           | 45         |
| <i>Saccharomyces</i>  | Typhimurium intestinal colonisation by iron competition                  |                           |            |
|                       | Treatment of diarrhoea                                                   | <i>S. boulardii</i>       | 26         |
|                       | Treatment of irritable bowel syndrome                                    | <i>S. cerevisiae</i>      | 39         |
|                       | Treatment of moderate ulcerative colitis                                 |                           | 46         |
|                       | Treatment and reduction of infection caused by <i>C.difficile</i>        |                           | 47         |
|                       | Treatment of acute gastroenteritis                                       |                           |            |
|                       | Effective against Crohn's disease                                        |                           |            |
|                       | Anticancer                                                               |                           |            |

which include immunomodulation, anti-inflammatory, hypocholesterolemia, anti- obesogenic, anti- hypertensive, anti-proliferative and antioxidant effects<sup>48</sup>. A review article published in 2002 reported that over 22,000 postbiotic metabolites have been identified in the scientific literature.

## MECHANISM OF ACTION OF PROBIOTICS, PREBIOTICS AND POSTBIOTICS

### Prebiotics<sup>63,64</sup>

Prebiotics indirectly exert a beneficial effect on the host by

following the mode of action:

- They promote the growth of beneficial microorganisms residing in the host's gut, as prebiotics are a substrate/energy source for gut microflora (Fig. 3).
- The gut microbes are fermenting prebiotics, thus releasing fermentation by-products and reducing the pH of the gut. By this pH diminution, prebiotics inhibit certain strains of potentially pathogenic bacteria, viz. *Campylobacter jejuni*, *E. coli*, and *Salmonella enteritidis*. In this way, prebiotics help reduce the risk of intestinal infections.
- Prebiotics contribute to mineral absorption and immunity regulation in the host as the expression of the binding proteins or active carriers enhances and, at the same time, the level of harmful metabolites, i.e. nitrogenous end-products, reductive enzymes decreases.

### Probiotics<sup>64,65</sup>

The probiotic exerts its positive effect on the host through multiple modes of action.

- They colonise and adhere to the colon and release gut-protective metabolites, viz. arginine, glutamine, short-chain fatty acids, conjugated linoleic acids, thus reinforcing the barrier function of the intestinal mucosa, helping in the management of intestinal infection and food allergies (Fig. 2).
- Probiotics compete with pathogens for available nutrients and other growth factors.
- Probiotics also antagonise pathogens through the production of antimicrobial compounds such as lactic acid, acetic acid, butyric acid, cytokines, bacteriocins, H<sub>2</sub>O<sub>2</sub>, etc.
- They secrete proteolytic enzymes that digest the bacterial toxins.
- Probiotics activate the immunologic mechanisms, e.g. increase the level of circulating immunoglobulins, especially immunoglobulin A in infants infested with rotavirus. They enhance the nonspecific immune-phagocytic activity of circulating blood granulocytes. They increase the frequency of interferon-producing peripheral blood monocytes.
- They alter the initiation and promotional events of the chemically induced tumours by binding to the chemical carcinogens.

### Postbiotics

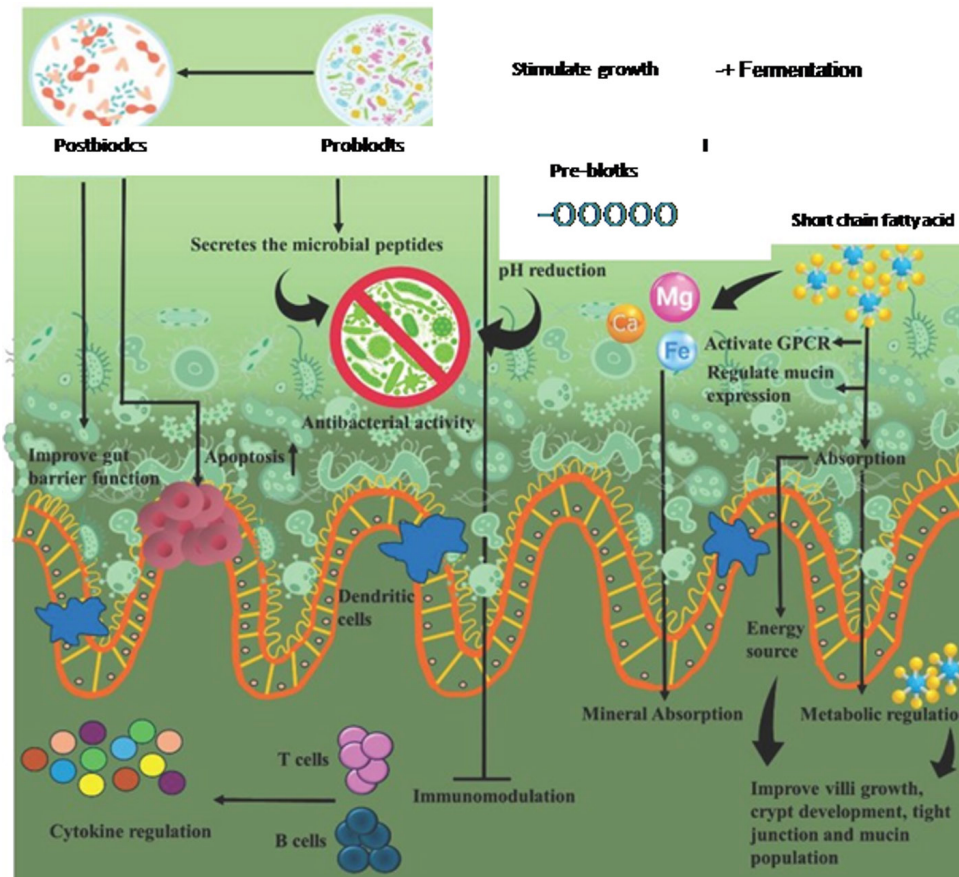
Probiotic bacteria break down dietary fibers and convert them into a wide range of compounds that are referred to as postbiotic metabolites. Some of these soluble metabolites may be secreted by live bacteria, and some are released after bacterial lysis, into the host environment, offering additional physiological benefits (Fig. 2). Postbiotic metabolites are the latest advancement in microbiome

science. Scientists are learning that postbiotic metabolites are the master health-regulating compounds in the body. They play a role in regulating every organ system, including our immune system and our brain. In addition, research conducted by Schendereov revealed that postbiotics have favourable absorption, metabolism, distribution, and excretion abilities, which could indicate a high capacity to signal different organs and tissues in the host, thus bringing out several biological responses<sup>66</sup>. Though the mechanisms of action are not fully understood. It has been suggested that the immunomodulatory and anti-inflammatory capacities of postbiotics are mediated by the inhibition and induction of the immune system in various animal models. It has been found that postbiotics regulate the production of the cytokine response and Th1 pathway inhibition<sup>67</sup>. The antimicrobial activity of postbiotics may be attributed to the presence of several known and unknown antimicrobial compounds, usually including but not limited to bacteriocins, enzymes, small molecules, and organic acids, which exhibit bacteriostatic or bactericidal properties against both gram-positive and gram-negative microorganisms<sup>58</sup>. All these properties suggest that postbiotics may contribute to the improvement of the host's health status by providing better and more specific physiological effects, although the exact mechanisms remain to be elucidated<sup>66</sup>.

As a result, the emphasis is gradually moving from viable probiotic bacteria to macromolecules derived from probiotics. Because they provide a variety of health-promoting qualities, postbiotics are emerging contenders among functional foods in this regard. Parabiotics are either crude cell extracts (i.e., with complicated chemical composition) or inactivated microbial cells of probiotics (intact or ruptured cells including peptidoglycans, teichoic acids, surface proteins, etc.). Better rheological and sensory qualities were observed in a yoghurt preparation containing killed strains of *L. acidophilus* ATCC SD5221 and *B. animalis subsp. lactis* BB-12<sup>68</sup>.

### COMMERCIAL STATUS AND FUTURE PROSPECTS

The probiotics market is bifurcated based on its application, including food, beverage and dietary supplements. They are widely utilised in preparing supplements to treat digestive disorders and chronic and inflammatory skin diseases. The global probiotics market is merged, and key industries include DuPont Danisco, Chr. Hansen, Valio, Morinaga Milk Industry, Yakult Honsha, BioGaia, Kemin Industries, PROBI, Kerry Group, Protexin, Lal lemand, Cerbios - Pharma SA, Bifodan A/S, and Winclove have emerged in its production. Industries are developing various strains which have increased application usage in the food & beverage, dairy and dietary supplements industries. In April 2017, BioGaia signed an agreement with Prodentis to manufacture oral food probiotic supplements containing the natural lactic acid bacteria *Lactobacillus reuteri* Prodentis.



**Figure 3.** Mechanism of probiotics, prebiotics, and postbiotics in the human gut

The strain has shown positive effects on reducing the symptoms of gingivitis and periodontal disease in over 30 clinical studies. The agreement will help the company strengthen R&D facilities, diversify its product portfolio, and strengthen its presence in Canada, Vietnam, and the Philippines. In March 2018, Danone entered a strategic partnership with Yakult and completed the sale of 14% of Danone's stake in Yakult for USD 1.48 billion. This joint venture will promote activity and research towards manufacturing strains with low side effects and high performance efficacy, which are likely to augment the probiotics industry growth.

In June 2018, General Mills invested USD 12 million in NextFoods, the maker of Goodbelly products. This joint venture may bring further innovation within probiotics for brands such as Yoplait or the non-traditional probiotics industry. The company may diversify into probiotics-based snacks and high-performance energy bars to attract more customers. The probiotics market research report suggests that it is projected to grow at a CAGR of 6.8% and it will reach 73.8 billion USD by 2026<sup>69</sup>.

Probiotic survival and properties (adhesion to Caco-2 cells, cholesterol attachment, increase angiotensin-converting enzyme (ACE) inhibitory, antimicrobial activities, antioxidant, and decrease systolic blood pressure)

can be enhanced by applying emerging technologies to probiotic products. Moreover, it can enhance bioactivity (aglycones, vitamins, calcium): create or preserve molecules of interest (oligosaccharides, bacteriocin, phenolic compounds, peptides, flavonoids): and improve sensory qualities. Prebiotic degradation did not occur when new technologies were applied to prebiotic goods. Nevertheless, it led to increased levels of health benefits (inhibition of ACE,  $\alpha$ -glucosidase, and  $\alpha$ -amylase, and antioxidant activity) and of bioactive substances (citric and ascorbic acids, anthocyanins, polyphenols, and flavonoids). Postbiotics with greater health effects can also be obtained through emerging technologies<sup>68</sup>.

### Current challenges and limitations of probiotic, prebiotic and postbiotic-based functional food products

Significant contributions of horticultural-based products for the production of innovative functional food products but several challenges limit their successful incorporation. Major challenges associated is the viability during the processing, storage and passage through gastrointestinal tract with probiotic enriched food. Many products efficacy is altered due to exposure to air, moisture and alteration in temperature. Success of market depends on sensory appeal,

maintaining the balance and palatability of product.<sup>70</sup> In addition, probiotic are highly strain specific hence requires careful selection and validation.<sup>71</sup> Horticultural matrices often exhibit variable physicochemical properties, including pH, water activity, and phytochemical composition, which may negatively affect probiotic performance<sup>72</sup>.

Excessive intake of prebiotic compounds may lead to bloating and flatulence.<sup>73</sup> Although postbiotics offer improved stability and safety as compared to others, careful selection and validation are required.<sup>74,75</sup> Addressing these challenges through advanced formulation technologies, robust clinical studies, and harmonised regulatory frameworks is essential for enhancing the efficacy, safety, and market acceptance of next-generation functional foods.<sup>76,77</sup> Future research should focus on precision nutrition approaches, advanced encapsulation technologies, omics-based characterisation, and robust clinical validation to fully exploit the potential of horticultural products in next-generation functional foods<sup>78,79</sup>.

## CONCLUSIONS

The present review has illustrated the importance of vegetables and fruits to provide beneficial compounds, mainly referred to as oligosaccharides, defined as prebiotics, exerting crucial effects on human gut microflora, i.e., probiotics, and their metabolites. The functional food produced by the mentioned consortia is useful to combat various diseases like Diarrhoea, Cancer, Cardiovascular and Kidney imbalances. A wide range of consumers, including those who are lactose intolerant and vegetarians, can benefit from the probiotics, paraprobiotics, and prebiotics found in plant food. The food business and the health sector have benefited greatly from the study that has been done thus far on the potential benefits of using probiotics in human meals. However, there are still a lot of obstacles in the way of producing safe probiotic meals. First, understanding the connections between probiotic bacteria and human health helped to leverage research in this field and develop processing technologies that guarantee the safe and practical distribution of probiotics so that health benefits are realised. In conclusion, prebiotics, probiotics and postbiotics offer significant health benefits, thus stimulating future scientific studies in this research field.

## AUTHOR'S CONTRIBUTION

Conceptualization, NG, SS, GS; methodology, DT, SS; software, DT, NG, RP, MDS; validation, FL, OCM, SS, GS; formal analysis, DT, NG, RP, MDS; investigation, DT, NG, RP, MM; data curation, MM, MDS, SS, GS; writing -original draft preparation, DT, NG, MDS; writing review and editing, MM, MDS, SS, GS; visualization, NG, SS, GS; supervision, NG, SS, DT; project administration, SS.

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## DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article; further inquiries can be directed to the corresponding authors.

## CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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