Bombay Technologist

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A Review on Immune-Boosting Activity of Functional Foods- Prebiotics and Probiotics

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Panoramic research on the correlation of gut microbial health and the immune system led to an increase in awareness regarding the importance of functional foods like prebiotics and probiotics. Probiotics and prebiotics possess the capability to alter the gut microflora and thereby regulating the immune system. They can positively influence gut microbiota and correspondingly improve host immunity. Prebiotics are indigestible fibres that are metabolised by gut microorganisms to produce metabolites that are beneficial to human health. Probiotics are 'good bacteria' which play a pivotal role in maintaining the gut microbiome. This review paper aims to present an overview of the human gut, immune system, and their correlation. This review also assesses the immune-boosting mechanism of probiotics and prebiotics and provides a comprehensive view of the potential of functional food- prebiotics and probiotics for boosting immunity. The review has also provided a glimpse of the potential application of prebiotics and probiotics in boosting immunity against SARS-COVID-19 and the mechanism behind it. It discusses the future scope in the formulation and application of functional prebiotic and probiotic foods. It is suggested to further the research for improving the acceptability, efficiency, and to aid in the treatment of various diseases.

1. Introduction

There is a growing demand for functional foods that can help in the management of weight, sleep and even chronic diseases like diabetes, hypertension, cardiovascular diseases, etc [1]. In the wake of the ongoing COVID-19 pandemic, there is an increased awareness regarding the importance of immunity [2]. Hence nowadays, functional foods aimed to boost immunity are also gaining popularity. Functional foods such as prebiotics and probiotics can alter the gut microflora and thereby positively affect the immune system of the host. Prebiotics and probiotics are commonly consumed to deal with diseases like obesity, diabetes, poor digestion, and to boost immunity [1].

Several kinds of research have purported the correlation between gut health and the immune system. Numerous mechanisms have been theorised to elucidate the action of probiotics and prebiotics in altering gut microbiota and enhancing immunity [3]. Prebiotics are the source of energy for 'good bacteria' and increase *Bifidobacteria* and *Lactobacilli* [4].

The gut has an important role to play in immune cell activity which is based on probiotic and prebiotic interventions [2]. This review paper is aimed to give a synopsis of the gut immune system and its correlation with probiotics and prebiotics. This paper will also review definitions, criteria for probiotics and prebiotics, mechanism of boosting immunity and future aspects of probiotics and prebiotics as functional foods.

2. Relationship between gut health and the immune system

2.1 Overview on gut and gut microbiota

The gastrointestinal tract which is often called as 'gut' can be divided into two regions. The mouth, pharynx, oesophagus, stomach and duodenum come into the upper region, whereas, small and large intestine comes into the lower region [1]. The gastrointestinal tract consists of diverse microflora with 100 trillion microorganism and 150000 microbial genomes [4]. The diversity of microflora which is individual-specific is responsible for host metabolism and immunity regulation [5]. The gut microbiota is influenced by several reasons such as genetics, water, medical, farming, etc. Most of this microbial population is in the colon region of GI and certain sections of the upper region. Thus, an organism with specific metabolic constraint favours different section of GI tract [6]. Most of the microbial species belonged from one of the four following phyla *Firmicutes (*31.1%), *Actinobacteria* (25.9%), *Proteobacteria* (29.5%) and *Bacteroidetes* (7.1%) [7]. Lactic acid bacteria (LAB) and *Bifidobacteria* have enormous potential and hence widely studied. They are commonly called probiotics. This microbiota plays a critical role in metabolism as well as in the immune response system of the body. It has been found that there is a significant association between various diseases in other body's major system and gut microbiota. It has been studied those certain chronic diseases related to digestion and immunity have a direct correlation with the make-up of microbiota and their end-products [5]. The function of gut microbiota is to regulate and produce metabolites like short-chain fatty acids (SCFA), bile acids, indoles, etc [3]. These molecules which are either produced or transformed by micro-organism play a crucial role in communication with immune cells [8].

2.2 Overview on Immune system

The immune system can be characterised as non-specific (innate) or specific (adaptive) [1]. The stimulation of the immune system involves a change in the expression of large numbers of genes and can also involve the production of cytokines, lipid mediators, and tissue remodelling enzymes, etc. Immune cells generate energy using metabolic pathways such as TCA cycle, pentose phosphate cycle, FA oxidation, amino acid metabolism and FA synthesis. There are two types of immunity cells T-type and B-cells. B cells produce immunoglobin (Ig) which is linked to specific immunity in reaction to antigenic attacks such as allergens and pathogens. Ig is further categorised as Ig A, IgG and IgE classes. The manuscript will be dealing with IgA in relevance to this review paper, as they can be present in mucosa secretion, breast milk, tears, etc. Based on surface expression, T cells can be further classified as CD4 or CD8 cells [9]. The helper T cells (Th) which are CD4+ T cells assist other immune cells in their functions. They aid in the creation of diverse cytokines such as interleukin-4 (IL-4), interleukin-7 (IL-17) and interferon γ (IFN-γ) [1]. These helper Th cells maintain the integrity of the mucosal barrier and at the same time produce anti-microbial peptides [3].

Although the study of microbiota and its make-up and its correlation with immunity is complex; several studies have shown the significant relationship between gut health and the onset of other diseases. The fact that diet, exercise, supplements can alter the microflora implies that functional foods like pre-biotic and probiotic can be a dietary way to enhance gut health and thereby boost immunity [10].

3. Functional foods

Functional foods are distinct from regular food owing to their specific physiological advantages. However, functional food cannot be described by one definition and hence a wide variety of food can be categorized as functional foods. Functional foods are separate and unique category different from nutraceuticals, medi-food, or dietary supplement [11]. Although functional foods have therapeutic effects, they are not regarded as drugs. [12]. The purpose of functional food is to reduce the risk of disease and not avoiding it or curing it. Functional foods are routine food products consumed in a standard diet. They are comprised of either natural or unnatural compounds present in food that would not normally supply similar benefits. Functional foods have an optimistic effect in addition to their basic nutritive value. They may enhance health or help in reducing the risk of developing diseases. They have authorised and scientific-based claims. According to European consensus, functional food is defined as "A food can be regarded as functional if it is satisfactorily demonstrated to affect beneficially one or more target functions in the body, beyond adequate nutritional effects, in a way that is relevant to either improved stage of health and well-being and/or reduction of risk of disease. A functional food must remain food and it must demonstrate its effects in amounts that can normally be expected to be consumed in the diet; it is not a pill or a capsule, but part of the normal food pattern." However, it should be noted that this type of food might not be equally beneficial to the entire population since every individual has unique biochemical needs [11].

Functional foods are categorised based on the active functional compound present in them like fibres, vitamins, flavonoids, electrolytes, etc [13]. They can also be categorised based on their intent of use such as enhancing immunity, sleep and stress management, energy-boosting, improving stamina, etc. Probiotics and prebiotics are functional foods that are used for weight management, improve digestion, reduce the risk of hypertension, boost immunity and other diseases. They are prepared from dairy and non-dairy sources. It is often said that "Let food thy medicine and medicine be thy food". Many functional foods earlier incorporated several nutrients and phytochemicals to improve immunity such as vitamin C, vitamin D, zinc, iodine, *Andrographis paniculate*, *A. purpura*, and *Astragalus radix*. However, with an increase in research and its enormous potential, probiotics and prebiotics are gaining popularity in the consumer market [14].

4. Probiotics

Earlier probiotics were defined as 'growth-promoting factors produced by microorganism." A robust definition was later adopted by United Nations World Health Organisation and Food and Agricultural Organization (FAO) which is as follows, "live microorganism which, when administered in adequate amounts, confer a health benefit on the host" The probiotics were historically used for fermentation and preservation of milk. Later, it was found out that it had good sensory properties. Many microorganisms can be classified as potential prebiotics but generally, probiotics largely fall under the genera of *Lactobacillus* and *Bifidobacterium*. Other micro-organisms that have been considered as potential probiotics include some strains of yeast like *Saccharomyces cervisiae*, and *S. boulardii* and bacteria like *Bacillus pumilus*, *B. clausii*, *B. cereus*, *B. subtilis* and *Escherichia coli*. (Table 1) [15]. Non-pathogenic yeasts are considered as probiotic and research interest in probiotic yeasts has increased for product development of functional foods [16]. Yeast strains like *S. cerevisiae* are used as probiotics in beer, cheese, and wine because of their nutritional benefits like proteins, high contents of vitamin B etc. In an investigation of interaction between yeast and other probiotic bacteria by V. Zoumpourtikoudi showed that yeast supplements enhance the hydrophobicity of probiotics, reduce the pH by increasing the lactic acid level and as a result improve immune action against infections [17].

Due to increasing awareness of the importance of improving immunity, probiotics have become a popular choice in diet. Milk and fermented milk beverages are popular choices among consumers. Probiotics are also recommended in the diet to improve immunity against SARS COVID-19 [20].

5. Prebiotics

In the 20th century, prebiotics was known as "a non-digestible 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." The definition was further evolved and in 2016 International Scientific Association of Probiotics and Prebiotics (ISAPP) describes prebiotics as "a substrate that is selectively utilized by host microorganism conferring a health benefit." [21] Carbohydrate based prebiotics include insulin, galactooligosaccharides (GOS), fructooligosaccharide (FOS), xylooligosaccharides (XOS), (Figure 1), and some other non-carbohydrate prebiotics candidates include minerals, polyphenols, and polyunsaturated fatty acids [22].

Mainly, the role of gut microbiota is to metabolise branched chain fatty acids (BCFA), short chain fatty acids (SCFA), biogenic amines, branched chain amino acids (BCAAs), bile acids (BAs), vitamins, and xenobiotics, as well as to produce gases [10, 23]. The metabolised compounds take part in the preservation of barrier function, immune regulation and [5, 8, 10]. Although, the interplay of overall health and the gut microbiota is crucial and is diverse; several factors can result in defects of regulator signals that are linked to the onset of diseases. Prebiotics can thus have an important

Lactobacilus	*Bifidobacterium*	Yeast
• *L. johnsonii*	• *B. animalis*	• *S. cerevisiae*
• *L. amylovorus*	• *B. bifidum*	• *S. boulardii*
• *L. crispatus*	• *B. breve*	• *S. bayanus*
• *L. delbrueckil*	• *B. infantis*	• *S. pastorianus*
• *L. fermentum*	• *B. coagulans*	• *S. sensu*
• *L. acidophilus*	• *B. lactis*	• *P. kudriavzevii*
• *L. gallinarum*	• *B. adolescentis*	
• *L. paracasei*	• *B. longum*	
• *L. kefir*		
• *L. plantarum*		
• *L. gasseri*		
• *L. reuteri*		
• *L. sakei*		
• *L. helveticus*		
• *L. rhamnosus*		
• *L. salivarius*		

Table. 1 Most reported Probiotics Microbial Strains for Human Consumption: [17, 18, 19]

effect in maintaining the overall microbiota diversity [5]. The beneficial bacteria in the gut breakdown and metabolise the dietary prebiotics [4]. These metabolites (SCFA, BCFA, BA, etc) that are produced by probiotics are useful for primary degraders as they provide substrate for other bacterial groups which are not capable of metabolizing parent compounds like long chained fatty acids. Thus, it can be safely said that it is viable to alter/ modulate particular gut bacteria that targets to produce specific metabolites or regulate the bacterial population size. This can be very beneficial to maintain and restore host health. Fermentable fibres, fatty acids, and micronutrient share the boundary of definition with prebiotics. In vivo studies show them as a potential candidate to impart health benefits upon the host [15].

Before selecting a prebiotic, it must satisfy some standard conditions which are as follows. It must [1, 23]:

1. Be vulnerable to gut fermentation.
2. Be advantageous to consumer's health.
3. Be a selective stimulus for probiotic growth.
4. Remain stable in the food matrix during processing, preservation, and storage.
5. Encourage probiotic growth.
6. Resist breakdown in the upper section of alimentary canal.

The viability and growth of probiotics are improved when prebiotics like maltodextrin, pectin and FOS are supplemented through functional beverages and infant formulations. In a study, fermented milk was augmented by addition of 2 probiotic strains of *Bifidobacterium lactis* Bi-07 and *Lactobacillus acidophilus* along with a prebiotic (isomaltooligosaccharide). A dose of 480g/ day for 2 weeks was given to 100 healthy people. It was found that all the subjects showed an increased faecal population of *Lactobacilli* and *Bifidobacteria* in comparison to the controlled group. Also, there was a substantial decrease in the population of *Enterobacilli*. Thus, such formulations have the potential to influence the gut flora and influence the immune system of the host [24].

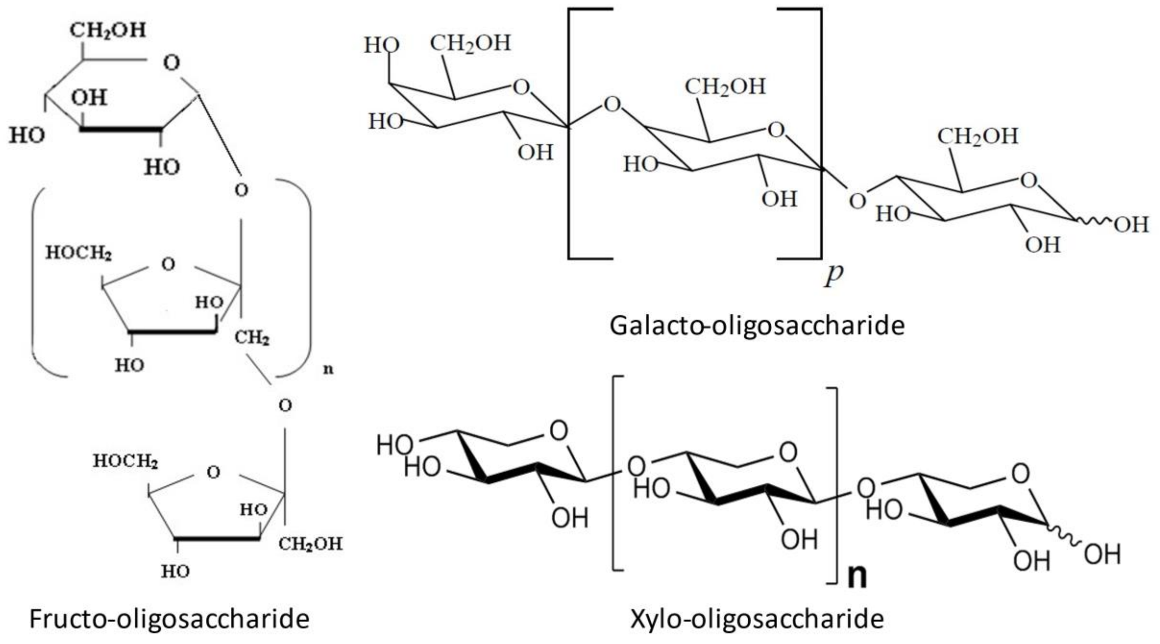

Fig. 1 Structure of major carbohydrate-based prebiotics [25].

6. Immunity-boosting action activity and mechanism of probiotics

The mechanisms behind immunity boosting activity of probiotics involve interaction with gut epithelium layer, production of immune cells, and regulating immune system. Several in vivo and in vitro studies have been carried out to conceptualize

the mechanism of probiotics. However, few mechanisms are yet to be confirmed for every probiotic cell and on human. It is a complex task because of the microbial diversity in humans and the convoluted interaction within the gut. The immune-

boosting activity may vary for different probiotic strain. It may also vary according to the inherent makeup of gut microbiota for every individual. The review summarises substantiated action mechanism of probiotics. The mechanism by which probiotics enhance the immunity include competitive exclusion for binding site and nutrients, maintaining and enhancing the barrier function, secreting anti-microbial compounds, and immune regulation [1].
Regarding the mechanism, the cell surface macromolecules including proteins and non-protein compounds mediate the interaction between probiotics and host tissue cells [26]. For an instance, *L. rhamnousus* GR-1 has a unique cluster of exopolysaccharides that assist in vaginal activity [27]. Similarly, *L. rhamnousus* GG uses pili for intestinal interaction. Several probiotic species of *Lactobacillus* and *Bifidobacterium* increases the expression of tight junction proteins [1, 4, 27]. Probiotic *L. rhamnousus* GG improves the barrier function by counter damaging the tight junction zonula occludens 1 and occludin caused by IFNγ56 [27]. A study of samples taken from patients of inflammatory bowel diseases showed that the probiotic yeast strain *S. boulardii* aids in restoring the tight junction through E-cadherin and p120 catenin expression in colon [28]. Probiotic strains might reduce pathogen binding to epithelial cell by upregulating the expression of mucus-secretion genes [9, 21, 27]. Another mechanism for boosting immunity involves cell-surface bodies like capsules and fimbriae [1, 5, 25]. Since the pathogens and LAB both have binding sites resembling the host receptors, they compete for host pattern recognition receptors (PRRs) like tool-like receptors (TLRs) [3, 4, 24]. Probiotics can also inhibit the adhesion of pathogen through receptor alterations brought out by probiotic enzymes, receptor analogue production, and biosurfactant secretion [29].

6.1 Most commonly encountered foodborne pathogens in food systems and action of probiotics on them.

6.1.1 *Escherichia coli*

E. coli is a common foodborne pathogen causing several diseases like diarrhoea, cholangitis, urinary tract infection, etc in the human body. Even at a small concentration, it has an insidious effect on human health. Studies have shown that probiotics are effective against E. coli O15:H7 [29]. According to one of the proposed mechanisms, probiotic bacteria *Lactobacillus helveticus* R0052 inhibits attachment of E. coli to the epithelial cells because of certain components of the bacterial surface like surface-layer proteins from probiotic organism. A similar study shows that Lb. acidophilus reduces the E. coli production of extracellular autoinducer-2 molecules that are used for bacterial interspecies communication. This result in the decrease of expression of the location which is involved in carrying gene involved in the attachment of *E. coli* to host cell that leads to the haemorrhagic colitis syndrome [30].

6.1.2. *Salmonella*

Salmonella is another widely known bacterium for causing foodborne infections around the world. Several in vitro studies have shown that probiotics are effective against salmonella. Studies carried out in chicks and chicken demonstrated that probiotic species have antimicrobial activity against salmonella. Studies carried under non-stress conditions have demonstrated to reduce the pathogen interaction with epithelial cells; probiotics have also shown reduction in *Salmonella* adhesion when subjected to thermal stress [22]. Hence, it can be safely said that probiotic effect has been observed irrespective of stress conditions. An in-vitro gut fermentation cellular model has shown a positive effect of *Bifidobacterium thermophilum* on *Salmonella*. It has been observed that the probiotic cocktail of Bifidobacterium *infantis* and *B. breve* provides resistance against Salmonella by releasing a proteinaceous soluble factor that improves the epithelial integrity of T84 cell and thereby maintaining immunity against *Salmonella* [18, 29]. Another study showed that the lactic acid produced by *L. rhamnosus* GG had an anti-microbial effect on *Salmonella* [30]. According to the proposed mechanism behind the antimicrobial activity is lowering the pH Lactic acid acts as a permeabilizer for gram-negative bacterial outer membrane and allows other compounds to act symbiotically with lactic acid [30, 31]. Another microbial inhibition mechanism is that organic acids, like lactic acid, chelate with iron which is essential for growth and discourage the proliferation of *Salmonella* [32]. It has also been shown that the expression of the gene hilA, an important virulent gene of *Salmonella*, can be influenced by lactic acid produced by *Lactobacillus* species of probiotic [30].

6.1.3. *Listeria monocytogenes*

Listeria monocytogenes infects human and causes severe human listeriosis. It can be circulated from the mesenteric lymph nodes to the spleen and the liver by crossing the intestinal barrier. Research has substantiated protective effect against *L. monocytogenes* infection by probiotic species of *Lactobacillus delbrueckii*. It is found that probiotics trigger the production of TNF-α and IFN-γ cytokines. Nitric oxide, which is secreted by these cytokines (TNF-α and IFN-γ), activate the macrophages to abolish the intracellular bacteria and hence inhibit the bacterial infection [29]. One research on mice that are challenged by *L. monocytogenes* showed that the probiotic *Lactobacillus plantarum* diminished the pro-inflammatory interleukin secretion. The inhibition mechanism of *Bifidobacterium* was associated with the extracellular proteinaceous secreted compound. *Lactobacillus'* acid production and secretion of an unidentified protein displayed an antibacterial activity against *L. monocytogenes*. Likewise, probiotics have shown antibacterial activity against *Shigella* species and *Campylobacter jejuni* as well [29].

6.1.4 Viruses

Furthermore, foodborne viruses can multiply and grow in the intestinal epithelium and cause illness. The mechanism for the antagonist capacity of probiotics against the viral pathogen is hypothesised to be like those against microbial pathogens:
The proposed mechanism of anti-viral action involves the following:

1. *Enhancement of intestinal barrier function*: probiotics can prevent pathogenic translocation, enhance the gene expression of genes that can enhance the integrity of epithelial barrier, improve intestinal mucin production, confides water and chloride secretion that are produced by pathogenic bacteria.
2. *Modulation in the immune system*: probiotics are capable of stimulating IgA, modulate cytokine production, enhance NK cell activity, and increase the phagocytic capacity of macrophages.
3. *Competitive exclusion for adhesion sites and nutritional resources*: probiotic creates hostile environment by lowering the pH, the physical block the receptor site, and compete for nutrients.
4. *Production of antimicrobial substances*: probiotics produce carbon dioxide, organic acids, lantibiotics, baceriocins, bacteriolysins which have anti-microbial activity.
5. *Inhibiting quorum sensing signalling molecules:* probiotics are able to produce compounds that inhibit the quorum sensing signalling of pathogens and thereby limiting the toxicity by bacteria
6. *Increased adhesion to intestinal epithelial cells:* The strong adhesion and colonization by probiotic microorganism in gut boost the immune system in the host body [29].

6.2. Immune action of probiotics against respiratory tract infections (RTI)

In respiratory tract infections (RTI) the most common pathogens are rhinovirus, influenza virus, parainfluenza, adenovirus, coronavirus, etc. RTI is caused because of the imbalance in microbiota in GI and respiratory tract (RT) by affecting the lung mucosa. Probiotics have shown positive effect in treatment in inhibition of gastric coronavirus, rotavirus, H1N1 influenza virus in vivo and in vitro case studies [2]. Probiotics positively affect the specific as well as the non-specific immune system.

Moreover, it has been found that the severity of infection also decreases in the upper respiratory tract as well as in GI. In a study on mice, it was observed that immune responses are improved because of *Lactobacillus plantarum* in comparison to controlled subject [33]. In another study, *Bifidobacterium animalis* diminished the danger of upper RTI [3, 33].
Anti-viral effects of probiotics against COVID-19 and some other forms of coronavirus have been shown by few probiotic strains like *Lactobacillus rhamnosus* (LGG), *L. plantarum* DSM 32244, *Lb. acidophilus* DSM 32241, *L. helveticus* DSM 32242, *L. paracasei* DSM 32243, *Streptococcus thermophilus* DSM322245, *Bifidobacterium lactis* DSM 32247, and *B. lactis* DSM 32246, [2, 14, 20]. Emerging studies demonstrate a correlation between gut health and microbiome responses to SARS-CoV-2. Some of the infected patients have been diagnosed with GI disorders such as diarrhoea and vomiting. In several faecal samples, it has come under observation that the virus nucleocapsid protein and the viral entry receptor angiotensin-converting enzyme 2 (ACE2) are present in epithelial cells of GI tract. These conditions are also allied with amplified gut permeability, abridged gut microbiome diversity and colon inflammation. Some studies have reported that few lactobacilli have the potential to interact with ACE2 and release peptides with a high affinity for ACE during milk fermentation. One of the most extensively researched human antimicrobial peptide cathelicidin LL-37 with a wide range of antimicrobial activity against several bacteria and viruses can be delivered by food grade probiotics [2]. The probiotic cathelicidin-expressing *Lactococcus lactis* have an immunomodulating effect that can be used for combating SARS-CoV-2. However, it is yet unknown whether these or some other immunomodulating effect will be efficient in SARS-CoV-2 treatment [14]. Although, clinical trials have proved that certain probiotics can lessen the chances and residence time in the upper respiratory tract in infected children, adults, and long-term nursing individuals [2]. To understand the optimum stain of probiotic, dosing regimens and their interaction with SARS-CoV-2 more medical trials are necessary.

6.3. Modes of immunity boosting mechanism of probiotics (Figure 2 and Table 2)

6.3.1. Production of antimicrobial agents

Probiotics produce antimicrobial agents and metabolites which include organic acid, bacteriocins, hydrogen peroxide [10]. These peptides produced by probiotic species can directly interact with pathogens in the colon. In a study conducted on mice, it was shown that milk supplemented with probiotic *L. lactis* can reduce the concentration of *Enterococci* [7]. Similarly, a recent study showed that *S. boulardii* secretes anti-microbial peptides to inhibit the growth of *Bacillus cereus* [28].

6.3.2. Competitive exclusion

Probiotics can inhibit pathogen's growth by competitive exclusion mechanism [1, 34]. This involves adhesion to site and nutrients and thereby competing with pathogenic microorganism for the same; since gut pathogens and LAB microorganism have similar binding site receptors. For an instance, HIV can be precluded from binding to the host cell by the recombinants (2-domain CD4 protein) secreted by *L. jensenii*. Mucin secretions are regarded to have immune-boosting activity [8,18]. These secretions are boosted by probiotics as a result of improved intestinal barrier to restrict the adhesion of pathogens.

6.3.3. Production of antibodies

Probiotics like LAB and *Bifidobacteria* enhance antibodies like IgA and IgG which aid in preventing the invasion of the mucosal barrier [1]. This improves the defence against invasion of pathogen and hence acts as an adjuvant vaccine. Some probiotics also serve as adjuvant vaccines by increasing the production of antibodies which improves the defence against the pathogen. Administration of probiotics can aid in increasing vaccine's longevity. For an instance, reported literature, which studied the prevalence and interval of cold and flu-like symptoms ensuing influenza vaccinations, have shown a reduction in infectious occasions in those who were administered probiotic supplementations [24]. They also act with lymphocytes and monocytes which are an important part of innate and adaptive immunity [35].

6.3.4. Production of cytokines

Anti-inflammatory cytokines like IL-10 and TGF-beta can be induced by certain probiotics [1]. They can down-regulate the expression of proinflammatory cytokines like TNF and IFN-gamma and thereby enhance mucosal immune responses [3]. Probiotic bacteria when activated the helper cells Th0 are differentiated into Tregs by DCs [29]. This inhibits responsive actions of Th1, TH2 and TH17 that can cause inflammation [9]. Research conducted in patients having inflammatory bowel disease by Abbas et al demonstrated that administration of *S. boulardii* led to substantial decrease in serum level of pro-inflammatory cytokines like TNF-α and IL-8 and simultaneously boost the concentration of anti-inflammatory cytokines like IL-10 [28].

In adaptive immune system, probiotics have a positive effect against diseases which they owe to their aptitude to enhance the Treg cells. *B. longum* can upregulate the proportion of Treg cells in rat and thereby IL-10/IL-12 ratio increases. And at the same time, the pro-inflammatory cytokines like IL-12, IL-17 and IL-23 are down regulated [5]. The metabolites of probiotics, mainly SCFA can induce IL-10; whereas, the other metabolites like butyrate up-regulate Treg cells [36].

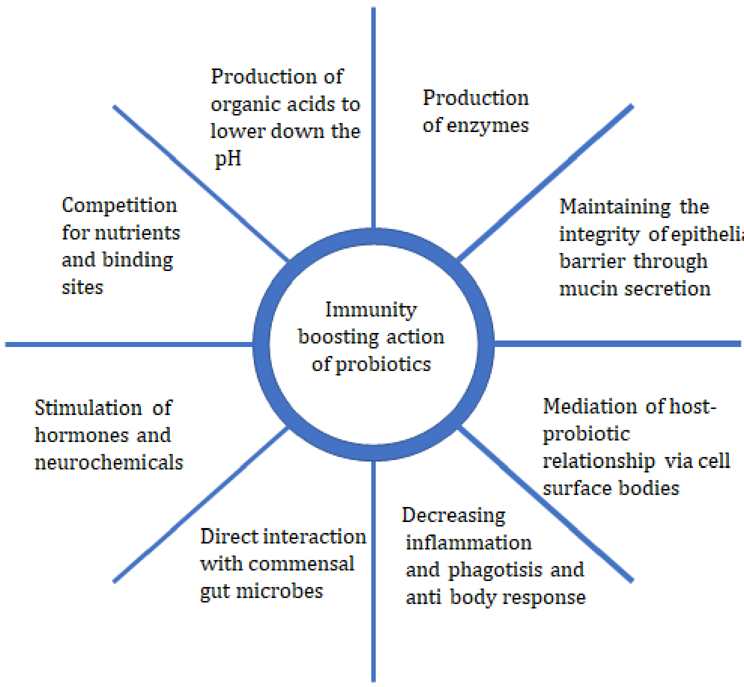

Fig. 2 Modes of action mechanism of probiotics [1]

6.3.5. Probiotic relationship with cell-surface receptors

Dendritic cells (DC) and intestinal epithelial cells play a crucial role in immune regulation by communicating with probiotics and other gut microorganism [35]. The gut-associated lymphoid tissues (GALT) are occupied by intestinal dendritic cells or they are scattered over intestinal lamina [5]. They use PPRs like Toll-like receptors (TLRs) and c-type lectin receptors for communication [32]. Probiotics are positioned within the endocytic vesicular membrane and other intracellular membranes [1]. The TLRs' cytoplasm has traditional Toll/interleukin-1 (IL-1) receptor (TIR)

[32]. This receptor is accountable for numerous adaptor molecules interactions which are important in cytokines and other immune cell fabrication [32]. For an instance, the activation of mitogen-activated protein kinase (MAPK) and NF-κB are caused by myeloid differentiation protein (MyD88) [7, 8, 22]. *B. longum* and *B. infantis* augments IL-10 production. Besides, probiotic *Lactobacillus rhamnosus* induces T regulatory (T reg) cells within GALT. Moreover, intestinal inflammation

because of pathogen *Citrobacter rodentium* can be protected by bacterial exopolysaccharide which is derived from *Bacillus subtilis* [8, 35].

6.3.6. Maintenance of epithelial barrier integrity

One of the most important functions of the epithelial cell is its absorptive function [35]. Epithelial cells that are stimulated by probiotics can produce defensins. Defensins are small peptides and antimicrobial proteins (AMPs) which act against viruses, bacteria, and fungi [29]. They form a protective mucosal barrier and thereby protect the host from pathogenic infection and toxic agents. These AMPs act like an enzyme to degrade cell wall structure or non-enzymatic rupturing of pathogen's cell membrane. Therefore, the pathogen dies by the defensins pores incorporated in its membrane [1]. For an instance, through stimulating antimicrobial peptides such as n β- defensin-2 (hBD-2), the probiotics can strengthen the mucosal barrier defences [35]. Another mechanism supports that the probiotics contest with pathogens for nutrients and binding sites. Additionally, probiotic species of *Bifidobacterium* can help in the maintenance of epithelial barrier by upregulating the autophagy responses. Moreover, probiotic enhance the mucin production which serves as the first line of defence [35].

Probiotic lipid antigens are influenced by natural killer (NK) cells. They increase the level of NK cells [2]. For example, *Bifidobacterium lactus* probiotic supplements have shown to improve the NK activity in the elderly [37]. Likewise, *B. polyfermenticus* augment CD56+ NK cells [9, 5]. Furthermore, *Lactobacillus casei* has been found to influence the surface expression of CD69 and CD25, and thereby activating NK cell activation [35]. Regular consumption of probiotic functional food has shown to improvise the NK cell functioning. Moreover, a study on patients undertaking HIV-1 treatment demonstrated that S. boulardii aids in restoring the structure of intestinal epithelium and as a result reduces the inflammation as well as microbial translocation across the epithelium [28]

6.3.7. Production of enzyme

Probiotics secrete microcin which binds to iron siderophore receptors that are for cell entry or synthesize insidious compounds after cell entry. This leads to inhibition of intracellular enzymes like ATP-synthase, DNA gyrase and RNA polymerase, and their functions like mRNA translation, which then causes pathogen cell death [1].

6.3.8. Production of neurochemicals

It has been found that neurochemicals are directly or indirectly produced by the gut microbiota. Neurochemicals such as serotonin, dopamine, cortisol, gamma-aminobutyric acid, and acetylcholine are modulated by host microbiota [5, 10]. With probiotics, it is possible to relieve part of symptoms linked with the diseases, by improving the global scores and reducing cortisol levels [25, 27].

Probiotic Microorganism	Immune boosting role	Mechanism
Lactobacillus plantarum MON03	Detoxify toxins	Binding via surface structures
Lactobacillus kefiri KFLM3	Detoxify toxins	Adsorption and biotransformation

Saccharomyces cerevisiae KFGY7 *Acetobacter syzygii* KFGM1		
Bacillus licheniformis CFR1	Detoxify toxins	Enzymatic degradation
Lactobacillus helveticus ATCC 12046	Detoxify toxins	Binding via surface structures
Saccharomyces cerevisiae HR 125a	Detoxify toxins	Binding via surface structures
Lactococcus lactis JF 3102, *Lactobacillus plantarum* NRRL B-4496	Detoxify toxins	Binding via surface structures
Streptomyces cacaoi subsp. Asoensis K234, *Streptomyces luteogriseus* K144, *Streptomyces rimosus* K145	Detoxify toxins	Enzymatic degradation
Several other *Lactobacillus* strains	Produce IgA	Stimulate dendritic cells to produce TGF- β
	Secrete IgA	Stimulate dendritic cells to produce IL-6
	Improve natural killer NK cell activity	Stimulate secretion of IL-12
	Inhibit Th2 activity	Stimulate secretion of IL-12
	Improve oral tolerance	Induce Tregs
	Inhibit Th2 activity	Stimulate secretion of IL-12
	Reduce inflammation	Weaken pro- inflammatory cytokines and chemokines by down-regulation of TLR-signals
	Detoxify toxin	Binding via surface structures
	Reduce inflammation	Induce of IFN-β
	Produce IgA	Up-regulate expression of pIgR
Several other *Bifidobacterium* strains.	Protect from enteropathogenic infection	Produce acetate and improve intestinal defence with epithelial cells
	Reduce Inflammation	Inhibit allergy
	Inhibit allergy	Suppress Th2 chemokines

S. cerevisiae 45	Feed to prebiotics and improve digestive activity	Production of short-chain fatty acids (SCFAs)
	reduction of intestinal pH	Moderate gut pH and creates hostile environment for growth of unwanted microorganisms
	Discourage proliferation of unwanted intestinal pathogens	Competition for nutrients with pathogenic bacteria
	Reduce inflammation	Produce cytokines TNK- α and IL-8

Table. 2 Immune boosting activity of certain probiotic microorganism and its mechanism [1, 38]

7. The Immunity-Boosting activity of prebiotics

Prebiotics are a group of dietary fibres (DF) that selectively ferment complex carbohydrates and change the structure and function of GI microbiome, thus promoting health benefits to the host [22]. For fulfilling the prebiotic criteria, it must be a non-digestible oligosaccharide and bifidogenic in nature. It has been observed that when type-2 diabetes patients were given a diet rich in fibre and prebiotics there was a positive effect development of intestinal bacteria [13, 23]. Autolysed yeast and its component can also serve as prebiotic and enhance growth of healthy bacteria in gut [38]. In studies, it was found that

Eubacterium hallii can cross-feed on lactate, which is produced by *Bifidobacterium adolescentis* that were developing on FOS [36]. Similarly, in a mutual cross-feeding case where *B. longum* degraded bionoxylan oligosaccharide (AXOS) which concurrently improved acetate-converting butyrate-producer [21, 36].

Gentibiose-derived oligosaccharide is produced by glucansucrase E81 wherein gentibiose is the sugar acceptor and sucrose. In vitro, these oligosaccharides were found to have immune-modulatory functions which included the manufacture of cytokines (IL-4, IL-2 and TNF-α). They promoted the growth of probiotic species especially *Bifidobacteria* [31].

As discussed earlier there is a close correlation between colon health and diseases related to metabolism and immune regulation. It is evident that non-alcoholic fatty liver diseases (NAFLD) are also a result of metabolism syndrome; since several NAFLD patients are found to be obese or over-weight [32]. Excess storage of lipids in hepatocytes is one of the major characteristics of NAFLD. Much research has shown that probiotics and prebiotics have a positive effect on the treatment of NAFLD. Several mechanisms have been put forward which include improvement in insulin and leptin sensitivity, improvement of the intestinal barrier function due to enhanced integrity of the intestinal epithelium, modulation of intraluminal bile salt metabolism, conjugated linoleic acid, lower cholesterol production, reduced availability of calories from indigestible carbohydrates, and reduction of hepatic fatty acid oxidation, [22, 32].

It has been also reported that insulin resistance is caused by the immune response in peripheral tissues. Appetite regulating hormone-like leptin and insulin is also regulated by gut secreted hormones like peptide YY(PYY) and GYP-1. According to another study, enhanced leptin sensitivity is due to the conversion of non-digestible fibres into SCFA. The fermented end product of prebiotics like SCFA plays a major role in the epigenetic modulation of inflammatory pathways. Hence it can be safely said that prebiotic and probiotics are viable dietary supplements for the treatment of obesity and NAFLD [32].

7.1 Enhancement of short chain fatty acids production

It is well known that prebiotics enhances the SCFA production which influences epithelial and immune cells. SCFA are a significant source of energy for maintaining the intestinal mucosal structure and function and thereby stimulating the innate immune system. Additionally, SCFAs affect epithelial and immune cells by binding themselves to specific cellular receptors that are G-protein coupled receptor ((GPCR)41 and GPCR)43) [35]. These G-protein receptors play a crucial role in maintaining the colon mucosa and the immune system. It has been demonstrated that there is an improvision in epithelial integrity and the immune system because of the physiological effects of binding of SCFA to G-protein receptors and other similar fatty acid receptors. In a study on mice where it was seen that GPR43 downplayed the pro-inflammatory action of neutrophils by SCFA [35].

The most important function of prebiotics is to enhance the growth of probiotics and their functioning. Various prebiotic fibres like fructan, glucan and arabinoxylan are degraded by probiotics into short chain fatty acids (SCFAs) [36]. These SCFAs have an immune regulatory effect on the host cell through a different pathway. Glycans or polysaccharides are carbohydrate-based polymers which regulate various processes including regulation of immunity [34].

7.2 Role of prebiotics in cardio-vascular diseases:

There are several well-researched therapies based on prebiotics and probiotics for various associated to gut such as obesity, constipation, type-2 diabetes, GI tract related diseases and also non-gut related diseases such as osteoporosis, CVD, mental health-related, neurological, etc [39]. However less researched information is available on prebiotic therapy for respiratory illnesses [3]. An excess amount of prebiotic fibre in the diet can lead to a surplus amount of gas formation in the intestine [37]. As a result of an overdose of prebiotic fibre bloating and abdominal pain can occur [7, 25].

Prebiotics comprised of polysaccharides and oligosaccharides which are polymers of carbohydrates. These polysaccharides have a significant role in cellular immunity and eliminating viral infections. In the treatment of COVID-19, the Chinese yam polysaccharides are serving as adjuvant vaccines owing to their immunity-enhancing function and immunomodulatory functions [29].

It has been found that the COVID-19 virus and its important cellular target ACE2 are vastly glycosylated. In a research study, it was demonstrated that glycans are crucial for antibody functions and other immunity-related aspects [30]. Furthermore, it was observed that in vitro the GOS and arabinoxylan amplified the assembly of T helper cell INF-γ, whereas the T helper cell 1 cytokine TNF-α was declined. There is potential to find a strong interrelation between the gut microbiome and microbiota interventions of SARS-CoV-2.

In a study where 1099 COVID-19 cases were analysed in China, it was observed that lymphocytopenia is a key aspect; since patients with lymphocytopenia were shown to have low chances of survival [20]. The severity of illness was found to be in association with IL-6. Moreover, IL-6 and IL-8 were found to be in negative correlation with lymphocyte. For an instance, the proportion of CD8+ IEl increased when dietary sugar beet fibre was consumed [1]. Although, there is no robust evidence yet to prove that dietary probiotics and prebiotics can be efficient in COVID-19 management [20].

7.3 Prebiotic-xylo-oligosaccharide immune boosting mechanism

One of the promising sources of prebiotic is xylo-oligosaccharides (XOS) as they are obtained in abundance from residues of crops and economic. The molecular formula of XOS is C5nH8nþ2O4nþ1; where, n¼2 to 6 [40]. It is derived from natural resources such as honey, bamboo-shoot, and other crops. It is non-carcinogenic in nature and positively affects the host cell by regulating insulin-secretion, mineral absorption, and acts as a mild laxative. XOS in vitro was found to stimulate the growth of probiotic microbes like *Bifidobacterium bifidum, B. adolescentis, Lactobacillus plantarum, L. brevis,* etc. A study on rat revealed that XOS significantly 1,2-dimethyldrazine induced precancerous lesions. Moreover, it was more effective in stimulating the growth of *Bifidobacterium* species in comparison to fructo-oligosaccharide. Since XOS is an indigestible fibre, it retains its structural integrity once it reaches the hindgut. XOs selectively used by probiotics like *Bifidobacteria*, during their growth and multiplication, produce SCFAs which play an integral role in immune regulation [36, 40].

7.4 Prebiotic- galactooligosaccharides immune boosting mechanism

A group of researchers reported that when GOS was administrated to healthy elderly it was observed that there is a rise in beneficial bacteria *Biffidobacterium* spp., *Lactobacillus-Enterococcus spp., Clostridium coccoides–Eubacterium rectale*, and decrease in *Bacteroides* spp, *Clostridium hystolyticum* group and *E. coli*, and had affirmative immunomodulatory response effects. Similarly, a regular dose of FOS showed a higher percentage of T cells and faecal *Bifidobacteria*. However, it also decreased phagocytic activity and IL-6 mRNA expression [37].
Prebiotic oligosaccharide can directly regulate the host mucosal signalling. This results in hypo-responsive intestinal epithelial cell to a pathogen as well as induces mitogen-activated protein kinase and nuclear factor kappa B. It was found in studies on boilers that serum concentration of IgG, IgA and IgM increases because of consumption of oligochitosans. In a study, it was observed that when a mixture of oligosaccharide was administered to children under six-month of age, there was reduction in number of incidences of atopic dermatitis [41]. Non-digestible oligosaccharides like nigerooligosaccharide were found to activate NK cells in vitro condition through linking with specific lectin-type receptors [35].

7.5 Polyphenols as prebiotic

Large and complex polyphenols can also serve as prebiotics by promoting the growth of certain bacteria. They are non-digested by the enzymes that are passed into the colon where these polyphenols are broken into smaller metabolites. For an example, polyphenols that are derived from red wine augments the proportion of *Bifidobacterium* probiotic in rats and humans [23, 35]. Polyphenols are found not only to increase the population of good bacteria but also obstruct the growth of pathogens [13]. Some polyphenols extracted from cranberry extract have found to stimulate the growth *of Akkermansia muciniphila* and antimicrobial activity against pathogens [43]. In a study, it has been found that the growth of *Helicobacteria* can be prevented by polyphenols from tea and wine [5]. They can interact and block TLR4 and thus mediate inflammation by interfering with the manufacture of inflammatory mediators like IL-1β, IL-6 and TNFα [4].

7.6 Seaweed polysaccharide as prebiotic

Seaweeds are also a good source of polysaccharide. Moreover, they also contain phenolics, flavonoids, and other bioactive compounds. Recently there is a growing interest to study seaweeds owing to their splendid polysaccharide and bioactive compounds. A study on polysaccharide extract of seaweeds (PES) such as *Enteromorpha compressa* (green), *Sargassum wightii* (brown) and *Acanthophora spicifera* (red) to estimate their prebiotic effect was conducted. In this study, it was found that the prebiotic scores for all the PES were positive. PES is found to augment the growth of *L. plantarum* and decrease the proliferation of pathogen *S. typhimurium*. Polysaccharide extracted from *E. compressa* shows the utmost prebiotic activity and is followed by *S. wighitti* and *A. spicifera*. Thus, the PES are a potential candidate for developing functional food [42].
Lycium barbarum is a traditional Chinese medicinal plant commonly known as Goji. Recent research has shown that Lycium barbatum polysaccharide (LBP) contains a variety of biological properties including immune-modulating effects. It can also act as an adjuvant in vaccines. In vitro studies have supported that LBP has a prebiotic effect and can enhance the growth of beneficial probiotics such as *Lactobacillus* and *Bifidobacterium* spp. Moreover, it can also improve innate immune functions and its combination with probiotics may impart beneficial health effects on the host cell [33].

A study on animals demonstrated that intake of dietary fibres leads to enhanced production of SCFAs which prevents the infection from pathogens by decreasing the pH of the colonic lumen. Hence, consumption of prebiotics can avoid the adhesion of pathogens, their proliferation and maintain the barrier in the host [1].

However, to advance the knowledge on the effects and limitations of prebiotics it is important to understand the location of action of prebiotics and the pathogenic bacteria. Though, conventionally it is believed that prebiotics passes through the small intestine undigested to the large intestine where it serves as food to beneficial bacteria and provides energy; some other hypotheses believe that prebiotics also contributes to increasing the growth of certain bacteria in the small intestine as well [8].

Table 3 enlists the probiotic and prebiotic providing natural foods.

Food Groups (and their associated nutrients)	**Specific food to consume**
Whole grains (fibres, oligosaccharide, minerals, and vitamins)	Whole wheat, brown rice, barley
Plant-based proteins (soluble fibres and amino acids)	Bean, tofu, nuts, lentils, chickpea
Fibre fortified food	Cereals, supplements, breads, fortified drinks/ beverages
Fermented beans or vegetables (*Lactobacillus*)	Miso, saukraut, sour pickles soyabeans
Vegetables and fruits (fibres, oligosaccharides, and vitamin A, B, C, D, E, K)	Most leafy vegetables, fruits, legumes, mushrooms
Fermented dairy (*Bifidobacterial* and *Lactobacillus*)	Yogurt, kefir, sweet acidophilus milk, buttermilk, cheese, gouda, cottage, cheddar, mozzarella.

Table.3 Natural foods containing high content of prebiotics and probiotics [20]

8. Future Trends

There is a growing research interest in the probiotic capability of species other than *Lactobacillus* and *Bifidobacterium* which are also called 'next-generation probiotics. They include various bacteria like *Faecalibacterium prausnitzii*, *Roseburia intestinalis, Bacteroides* spp., *Eubacterium* spp. and *Akkermansia muciniphila* which are retrieved from the human gut [25]. Similarly, prebiotics will include a wider range of candidates compounds/ substances such as mimic and animal-derived substrates, yeast-based substrates, and non-carbohydrate compounds (phenols, fatty acids, micro-nutrient) [39]. There is an increasing research interest in developing

genome engineering tools in yeast strain owing to its growing demand in production of value-added product [28]. Attempts are made to use yeast strains obtained from beer fermentation as probiotics [19]. However, to qualify them as probiotics or prebiotics and use them in formulation they must satisfy safety standards and guidelines [12, 34]. There is a possibility that the probiotic strain gets mutated, and many unsafe strains are produced [1].

Nevertheless, most of them are found to be safe. Thus, safety and efficiency will be a paramount factor for research.

Since every individual has unique gut microbiota makeup it can be used to predict diseases and the type of precautions to be taken. Large data sets can be generated from microbiome research and based on them targeted strategies and formulations can be tailored. To interpret the towering data tools like bioinformatics are necessary to enhance the clinical trials [39]. Probiotics and prebiotics have the potential to positively alter gut microbial signatures by using multiple mechanisms [25]. There are attempts to increase the efficiency of probiotic strains through genetical modifications. Genetically modified organisms (GMO) can improve the target towards specific pathogen or can be selectively target to toxin. But, due to reluctance from consumer, GMOs are yet to capture market [15, 44]. Scientists across various nations like Spain, Canada, China, Belgium are investigating the exact mechanism of action of probiotics on RTI [2]. The researchers are also trying to explore any possible therapeutic influence of probiotics and prebiotics in the treatment of SARS-CoV-19. Because probiotics and prebiotics are well known to regulate the immune system in various ways, they are a suitable candidate in preventing RTI and minimising the risk of ventilator-connected pneumonia [33]. However, there is not yet clear evidence to support their use for treatment and prevention they do hold the potential to serve as adjuvant therapy. More research is required to investigate the treatment, safety, and effectiveness.

There has been a significant change in incorporating probiotics in dairy and non-dairy based food products [13]. There is increased demand from consumers for simple and convenient options. When probiotics and prebiotics are delivered through dairy formulations like yoghurt, cheese, milk, etc they have immense potential to positively alter gut health, the immune system in children. Research across the globe is trying to improve the functionality and developing functional food that can improve digestion and immunity. Yet, it remains uncanny whether the immunomodulatory effects of probiotics sustained and regenerative or has short term effect [13]. In the coming future probiotics and prebiotics are imagined contributing to therapy for different conditions like obesity, HIV, antibiotic resistance, type-2 diabetes, etc.

Consumers having lactose intolerance demand vegan alternative to probiotic sources. Hence utilizing non-dairy matrices for delivering probiotics have become popular. Moreover, in vitro studies, vegan probiotics have hypocholesterolemic and anti-carcinogenic effect [12]. Although, in vitro studies, support the effectiveness of vegan probiotic products; in vivo studies are necessary to bolster the claims and to make them market-ready. However, it is a big challenge for biotechnology to deliver probiotics via a non-dairy matrix. Various factors such as the probiotic strain used, the matrix of choice, pH, its transactions with other compounds in the food matrix, etc challenge the viability of probiotics [13]. Moreover, processing and storage conditions are also important factors while developing vegan probiotic functional food.

Various technologies such as nano-formulation, micro-encapsulation, etc are developing to improve the efficiency of functional probiotic foods. In future, by using sonification, acid, enzyme and oxidation treatments, high pressure, etc the traditional prebiotics can be modified structurally or chemically [22]. There is a scope to use different combinations of prebiotics to create a unique formulation with increased benefits and efficiency. There is a pressing need to conduct research aiming to improve the efficiency of functional probiotics and prebiotics and to meet consumer acceptance [13].

9. Conclusion

In the field of functional foods, probiotics and prebiotics will be in demand. Probiotic intake may be regarded in various therapies as a dietary supplement or adjuvant vaccines. Moreover, probiotics owing to their various mechanism of action such as competition for exclusion, maintenance of intestinal barrier, production of antibodies, modulating and regulating immune system may offer medical aid in the prevention of diseases like depression, type-2 diabetes, obesity, etc. Likewise, prebiotics will be engendered in the processes of probiotics. A comprehensive study on humans need further understanding the mechanism of probiotics and prebiotics and thereby incorporating them in food formulation, which may aid in finding the excellent strains of probiotic and prebiotic and meet consumers need. There is a lot of scope for research in investigating, interpreting, and developing functional prebiotic and probiotic foods. Therefore, new research along with traditional understanding will broader the understanding and application of prebiotics and probiotics functional food.

10. References

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