Different formulation approaches to improve the survivability of probiotics in the digestive tract

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Abstract

This review aimed to present the various approaches that have been used to protect the probiotics from the toxic effects of the gastrointestinal tract (GIT) during oral delivery. A microbiota is a collection of trillions of microorganisms that live within the human body and form an ecosystem that is complex, adaptive, and unique to each organ. The probiotics have varied applications and exhibit side effects; as a result, they have been employed as food supplements with both therapeutic and prophylactic effects on disorders of the gastric and non-gastric regions. The significant contribution of probiotics to the maintenance of health has expanded the scope of their use in advanced research areas. A significant number of microorganisms will survive and thrive in the gastrointestinal tract (GIT); even after being exposed to several toxic substances such as bile salts and acids. Prevention of probiotics from the harmful effects of gastric acid and other enzymatic barriers, in addition to their delivery to the intestinal region, has been a great challenge. The new approaches designed for probiotic therapies work on stabilizing the probiotics through several mechanisms; leading to a significant number of probiotics that are available after passing through the various GI barriers, in addition to showing the desired therapeutic and/or prophylactic effects. A brief critique of the health effects of probiotics, issues associated with their delivery, and the various formulation strategies employed to improve the probiotic transport are provided in the current review.

Keywords: Probiotics, Microencapsulation, Bifidobacteria, Health, Survival, Lactobacillus spp.

1. Introduction

The human microbiota is formed of trillions of microorganisms living in the human body, which create ecosystems that are complex, adaptive in nature, and are specific to each organ. The human body as
Probiotics have intricate, heterogeneous, recurrently compounds, and strain specific process of actions. Though many roles have been classified; however, there is still a need for in depth knowledge; specifically pertaining the area of structure-function clarifications of their long-term effects and implications on health. Using the molecular effectors found on the cell structure or that are formed as metabolic by-products; the probiotics commune with the host and the microbiome. Metabolites from the probiotics can induce the microbiota via interactions with the other microorganisms; modifications of the gastrointestinal microenvironment such as lowering the pH, competence for nutrients and binding sites, and inhibition of microbial growth by generating strain-specific antibacterial compounds like the bacteriocins (Cunningham et al., 2021). The microbiota-directed effects tend to induce the health benefits of probiotics under the diseased conditions such as vaginal and oral dysbiosis; where there are overgrowths of the pathogens. The intestinal mucosal epithelium; entero-endocrine, immunological and vagal afferent fibers in the host cells have receptors with which the probiotic effector molecules can directly interact. These interactions result in systemic effects via the host immunological, endocrine, and neurological system mediators, in addition to the local gut effects, including improvement of the gut barrier stability and inflammation (e.g., via Toll-like receptors). Additionally, the probiotics are capable of enzymatically metabolising the host substances such as bile salts and the consumed xenobiotics (Cunningham et al., 2021). The pili; lipoteichoic acids, exopolysaccharides, and the different surface-layer proteins are specialized probiotic surface-associated effector molecules; many of which are strain-specific to facilitate the delivery of strain-specific effects (Cunningham et al., 2021). Hence, the study of new probiotic taxa is possible. Novel species of bacteria have been isolated and characterized from the human microbiomes; with potential health advantages and also they have the potential to be created as next-generation probiotics (O’Toole et al., 2017).

Probiotics have varied applications and rare side effects; accordingly, they have been employed as food supplements with both therapeutic and prophylactic effects on the various disorders of the gastric and non-gastric regions (Wadher et al., 2010). Inopportuneley, when probiotics are administered orally, they face several thought-provoking conditions, when they are supposed to show their actions in vivo. Low pH of the gastric fluids, the digestive enzymes, and the bile salts in the intestinal regions can cause lethal effects to the probiotics leading to their restricted colonization (Luo et al., 2022). According to Wilkinson, (2018), the term bacterial viability refers to the capability of the
bacterial cells to support and generate colony of cells under distinct environmental conditions. Few studies have stated that the functional properties of probiotics are the implication of viable cells (Terpou et al., 2019). The effectiveness of probiotics is not only dependent on the cell viability but it is also a dose and metabolic stability dependent (Rajam and Subramanian, 2022). Hence, it is imperative that the probiotics are protected from the harsh GI conditions so that they can travel further and the viable cells can possibly colonise the GI; thus imparting numerous beneficial health effects. The future will present diverse potential revolutionary detection methods, as our current comprehension of the human's microbiome functions and depths has grown (Veiga et al., 2020). The objectives of this review were to study the various reasons for poor survivability of the probiotics in the gastrointestinal regions, and investigate the main approaches used to enhance the viability of these probiotics after ingestion.

2. Gastrointestinal tract and the probiotic habitat

The gastrointestinal tract (GIT) is the storehouse of many probiotics that are termed as the gut microflora. Table (1) demonstrates the families or species of probiotics that are found throughout the different regions of the GIT (Donaldson et al., 2015).

3. Mechanisms by which probiotics benefit the GIT

The probiotics aid the human host health and are expected to be driven by a variety of processes. These mechanisms are driven directly by the interactions of probiotics with the resident microbiota in some situations, including the synthesis of antimicrobial compounds and cross-feeding on the other resident microorganisms. In other circumstances, such as direct engagement with the immune cells; the probiotics effects may be indirect via host cell interaction. In addition, the metabolites produced by the probiotics are able to interact with the brain-gut axis and they play an important role in the behavioral disorders (Plaza-Diaz et al., 2019). Overall, the probiotics' therapeutic effects may be derived from the combined activity of the various pathways (Sanders et al., 2019). Table (2) demonstrates the possible health effects of probiotics along with the proposed mechanisms of action.

4. Health benefits of the probiotics

The human health and microbiome have a broad connotation; as it’s been stated that alterations in the diversity of microflora are linked to various acute and chronic health conditions (Cremon et al., 2018). Most of the infectious diseases and other disorders, including food allergies, IBD, constipation and diarrhea, diabetes, colon cancer and obesity, can be efficiently treated through administration of the probiotics (Matouskova et al., 2021).

A previous study reported by Khaneghah et al. (2020) that activation of the antimicrobial peptides, lactic acid stimulation, hydrogen peroxide, inhibition of bacterial adhesion, and accumulation of bacteriocin-like inhibitory substances (BLIS) are all interlinked to the antimicrobial effects of probiotics. Probiotics have their roles in the development of the immune cells through generation of metabolites that activate the proper functioning of the immune cells. They are engaged in maintaining the intestinal and bone health; generation of antioxidants and anti-hypertensive components, and promoting lactose digestion (Khaneghah et al., 2020; Matouskova et al., 2021). Probiotics have the ability to synthesize several vitamins that can supply the host with the essential vitamins; namely vitamin K and several vitamin B derivatives, including nicotinic acid, pyridoxine, folate, thiamine, riboflavin, biotin, pantothenic acid and cobalamin (James and Wang, 2019). Eventually, probiotics express cancer preventive activities through several mechanisms, including decreased levels of nephrotoxic and immunosuppressive mycotoxins; binding and degradation of mutagens, inhibition of tumor cell proliferation, diminished activity of the enzymes that play a role in cancer formation, generation of components that have anti-cancer
Table 1. Probiotics distribution in the different regions of the Gastro-intestinal tract (Donaldson et al., 2015)

<table>
<thead>
<tr>
<th>Parts of the GIT</th>
<th>Probiotic species</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gut</strong></td>
<td>Verrucomicrobia, Bacteroidetes, Proteobacteria, Actinobacteria, and Firmicutes</td>
</tr>
<tr>
<td>Small intestine</td>
<td><em>Enterobacteriaceae, Erysiplorellaceae</em>, and <em>Lactobacillaceae</em></td>
</tr>
<tr>
<td>Colon</td>
<td><em>Ruminococcaceae, Bacteroidaceae, Rikenellaceae, Lachnospiraceae</em>, and <em>Prevotellaceae</em></td>
</tr>
<tr>
<td>Cross-section of the colon shows the digesta</td>
<td><em>Bacteroidaceae, Rikenellaceae, and Prevotellaceae</em></td>
</tr>
<tr>
<td>The inter-fold regions of the lumen</td>
<td><em>Ruminococcaceae and Lachnospiraceae</em></td>
</tr>
</tbody>
</table>

Table 2. The possible health effects of probiotics along with the proposed mechanisms of action (Sanders et al., 2019)

<table>
<thead>
<tr>
<th>Health effects of the probiotics</th>
<th>Mechanisms of action</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Modulate the immune system</strong></td>
<td>The process of phagocytosis is activated;</td>
</tr>
<tr>
<td></td>
<td>Reduced inflammatory response;</td>
</tr>
<tr>
<td></td>
<td>Elevation in antibody response.</td>
</tr>
<tr>
<td><strong>Interact with the gut microbiota</strong></td>
<td>Stabilization of microbiome;</td>
</tr>
<tr>
<td></td>
<td>Generation of antimicrobial elements;</td>
</tr>
<tr>
<td></td>
<td>Alteration in substrate and cross feeding;</td>
</tr>
<tr>
<td></td>
<td>Support the stability of microbiota.</td>
</tr>
<tr>
<td><strong>Produce organic acids</strong></td>
<td>Cross feeding caused by increased butyrate;</td>
</tr>
<tr>
<td></td>
<td>Lowered colon pH;</td>
</tr>
<tr>
<td></td>
<td>Activation of acetate, lactate, and propionate.</td>
</tr>
<tr>
<td><strong>Colonization resistance</strong></td>
<td>Strive for the colonization sites and nutrients.</td>
</tr>
<tr>
<td><strong>Improve the barrier functions</strong></td>
<td>Maintain health of the epithelial cells;</td>
</tr>
<tr>
<td></td>
<td>Increased levels of mucin production.</td>
</tr>
<tr>
<td><strong>Probiotic–host interactions mediated by cell surface structures</strong></td>
<td>Activation of the exo-polysaccharides; toll like receptor ligands, pili, surface associated proteins, lipoteichoic acid, and mucin binding protein.</td>
</tr>
</tbody>
</table>
Produce enzymes

- Production of lactase and bile salt hydrolase.
- Repletion of hormones along with neurotransmitters, such as tryptophan and serotonin;
- Gamma-aminobutyric acid (GABA), cortisol, and several histamine derivatives;
- Stimulates linoleic acid.

Manufacture small molecules with systemic effects

Moreover, the probiotics generate components that have anti-cancer activity; enhance the production of anti-inflammatory cytokines and immunoglobulins, and cause phagocytosis of the foreign bacteria, which lead to immunomodulation effects (Azad et al., 2018; Galdeano et al., 2019). According to Hill et al., (2014), only specific strains of probiotics have the tendency to produce anti-cancer, immunological bioactive and functional components. The advantageous effects of few particular microorganisms are apparently visible in the concept of probiotics, because their roles in clinical practice are broadening very fast (Usman, 2018).

5. Transit of probiotics through the GIT

Probiotics move through the GIT after oral delivery; from the mouth to the stomach, and then to the small intestine and colon. This path is known as the transit of probiotics through the GIT, which explains how the different parts of GIT compete with the probiotics.

5.1. Mouth

As reported by Han et al., (2021), the orally delivered probiotics are initially exposed to saliva in the oral cavity after ingestion. When several strains of Bifidobacteria, Lactobacillus, and Pediococcus are exposed to saliva in vitro, there is no objectionable loss of the cell count when compared with the control group. The exposure of probiotics to saliva after oral administration is momentary; however, the dominance of saliva on the viability and survival rate of the probiotics is minimal.

5.2. Stomach

After oral ingestion, the probiotics enter the stomach after passing via the esophagus, and then they are directly exposed to the acidic gastric fluids. The probiotics are highly susceptible to low pH and hence the acidic environment has proved to cause mortality of the bacteria; especially those which are not acid resistant. The activity of the glycolytic enzymes decreases due to the hydrogen ion influx; thus causing effects on the F1F0-ATPase proton pumps. Accordingly, the gastric contents are one of the most significant factors that affect the transit of probiotics through the stomach (Yao et al., 2020).

5.3. Small intestine

The probiotics enter the small intestine through pylorus and then come in contact with ample amounts of bile and pancreatic juices. The intestinal fluid has neutralizing effects, accordingly the pH of intestine is 6.0-7.0, which is much preferred than the gastric pH (Cook et al., 2012). Disruption of the probiotics cell membrane and damage of DNA due to the action of several digestive enzymes; namely lipases, proteases, amylases, and bile acids may affect the viability of these probiotics (Han et al., 2021).

5.4. Colon

The human colon is a store house of huge amounts of bacterial count (10^{11} to 10^{12} cfu/ml), and represents the ultimate location where the probiotics will confront commensal colonization resistance. The probiotics compete with the host microflora for nutrients and adhesion sites, in order to colonise and multiply in the colonic mucosa. As a consequence of colonization resistance; most probiotics are expelled out from the colon with stools following oral delivery and their activities cease soon after consumption; thus making...
the probiotics undetectable (Han et al., 2021). The probable mechanisms that cause the probiotics colonization resistance are described in details in the section below.

6. Probiotics colonization of the intestinal mucosa

The probiotics must be fortunately being able to colonise the gastric regions for establishing an adequate host interaction, in order to provide health benefits (Alp and Kuleasan, 2019). Bacterial colonization of the digestive tract through adhesion between the bacterial surface components and the complementary structure of the host cell surface is one of the pivotal and imperative paths, which will lead to host benefits. The numerous components that assist in the mucosal and epithelial surface binding and lead to colonization and/or formation of vegetation are called adhesion factors (Du et al., 2022). The critical step in the probiotic colonization is the mucosal adhesion; however, the mechanism of such adhesions is still not completely explored (Han et al., 2021). Table (3) describes the several types of proteins and their characteristic roles in mucosal adhesion for probiotic colonization.

7. Gastrointestinal conditions destroying the probiotics

Apprehending the clusters of unavoidable circumstances that are experienced by foods before and after ingestion is essential in order to design a delivery system for the probiotics. The mechanical stressors; oxygen, light, digestive enzymes, bile salts, and gastric acids are only few physicochemical variables that affect the probiotics viability during food manufacturing; transport, storage, and gastrointestinal transit. The contents below describe the roles of the interaction of food and the gastrointestinal tract with the probiotics (Yao et al., 2020).

7.1. Interaction of probiotics with the food contents

Probiotics are susceptible to various environmental factors that can possibly cause changes in their viability during food production, transport, and storage. The stress causing environmental factors include pH; relative humidity, osmotic stress, and presence of oxygen. The elevated levels of these factors are deleterious to most of the probiotics species (Yao et al., 2020). Considering oxygen as a stress factor, the microflora in the gut is primarily anaerobic or micro-aerophilic, and hence oxygen may cause undesirable effects on the viability of probiotics (Donaldson et al., 2015). Moisture and humidity play important roles in the activation of the degradation processes of the probiotic bacteria. The degradation is supposed to start after ingestion; however due to the presence of moisture, the probiotics may start degrading before usage (Zhao et al., 2018). Hence, it’s essential to maintain the probiotics safe from the various deleterious effects, which can be encountered during the production, storage, and transportation of these probiotics.

7.2. The gastrointestinal tract (GIT)

When probiotics are consumed, saliva that is a mixture of several components such as amylase, mucin, and other mineral ions are the first chemicals that the probiotics encounter within the mouth. Within a fraction of seconds, they are swallowed through the esophagus, where they travel to the stomach. The GIT is a store house of various chemicals and has different conditions that can be altered. When probiotics are consumed, they come in direct contact with several incompatible conditions; specifically in the stomach, the small intestine, and the upper GIT. The pH at which the probiotics are stable is pH 6–7 (Yeung et al., 2016). However, in the stomach, the probiotics are exposed to low pH (less than 3) and high levels of pepsin (Derrien and van HylckamaVlieg, 2015). Accordingly, the possibility of cell inactivation and death occurs. Several factors are influenced by food consumption, including pH; the gastric juice volume, buffering capacity, and the transit time.
Table 3. List of proteins in the intestinal mucosa that are involved in probiotics surface adhesion

<table>
<thead>
<tr>
<th>Protein name</th>
<th>Characteristic</th>
<th>Role</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mucus-binding proteins (MUBs)</strong></td>
<td>A characteristic c terminal LP×TG signal peptide (Surface of the cell)</td>
<td>Brings about covalent binding with the bacterial cell was leading to mucus binding</td>
<td>(Musciariello <em>et al.</em>, 2020)</td>
</tr>
<tr>
<td><strong>Fibronectin-Binding Proteins</strong></td>
<td>Belongs to the family of adhesins, a type of microbial surface components recognizing adhesive matrix molecules (MSCRAMM) (anchored on the bacterial surface)</td>
<td>Bind to the exposed fibronectin and anchor the IECs</td>
<td>(Bisht <em>et al.</em>, 2018)</td>
</tr>
<tr>
<td><strong>Surface-Layers Proteins</strong></td>
<td>Surface (S-) layers, non-covalently bonded semi-porous crystal arrays comprised of self-assembling proteinaceous subunits called S-layer proteins (SLPs) (outermost strata of the bacterial cell wall)</td>
<td>S-layers have been shown to be involved in a number of processes including maintaining cell shape, protecting the murein sacculus from lysozyme attack, acting as molecular sieves and antifouling coating, serving as binding sites, and promoting bacterial adhesion</td>
<td>(do Carmo <em>et al.</em>, 2018)</td>
</tr>
<tr>
<td><strong>Teichoic Acids</strong></td>
<td>composed of alditol phosphate repeating units, two types: lipotheicoic acid (LTA) and wall teichoic acid (WTA) (bacterial cell wall)</td>
<td>Inhibit adhesion to Caco-2 cells</td>
<td>(Kim <em>et al.</em>, 2017)</td>
</tr>
<tr>
<td><strong>Exopolysaccharides (EPS)</strong></td>
<td>excreted as tightly bound capsule or loosely attached slime layer in microorganisms (surface carbohydrate polymers)</td>
<td>Facilitate probiotic adhesion, role in the interaction of probiotics with the host.</td>
<td>(Angelin and Kavitha, 2020)</td>
</tr>
<tr>
<td><strong>Aggregation-Promoting Factors</strong></td>
<td>secreted proteins which induce self-aggregation and facilitates the maintaining of cell shape (Cell wall)</td>
<td>Adhesion factor which participates in the interaction with the host mucus layer and IECs</td>
<td>(Nishiyama <em>et al.</em>, 2015)</td>
</tr>
</tbody>
</table>
Enolase: multifunctional protein (cell surface) facilitating the adhesion of bacterial cells to the host (Wei et al., 2016)

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH): cytoplasmic protein adhesion to mucin (Deng et al., 2020)

Elongation factor Tu (EF-Tu): has domains I, II, and III, forming different sites for binding of guanosine triphosphate (GTP) and aminoacyl-tRNA (intracellular protein) bacterial adhesion to host cells, invasion, and immune evasion (Lopez-Ochoa et al., 2017)

Molecular Chaperones: stacked double-ring structure (Cytosol) binding activity to mucins and IECs (Bascos and Landry, 2019)

Other factors related to the probiotics formulation design include the probiotics species and the type of the dosage form, which affect the stability of these probiotics throughout GIT (Fredua-Agyeman and Gaisford, 2015). Bile-juice as a component of the gastric fluids is responsible for causing harmful effects that are more potent than low pH; as the bile juice has a detergent property that can disrupt the probiotics membrane (Ilango et al., 2016). Bile juice exposure alters the lipids in the probiotics cell membrane; potentially influencing both the cell permeability and the interactions between the membrane and its surroundings (Taranto et al., 2003).

8. Prophylaxis and therapeutic uses of probiotics

In a healthy gastrointestinal tract; with proper mucus production and suitable bacterial colonization, the expansion of harmful bacteria is prevented, disease processes are modulated, and the broad inflammatory diseases are prevented. The probiotics' roles in maintaining the health and their significance in avoiding diseases are better understood, which benefit the patients' overall well-being. In the future, the probiotics may be widely used since the importance of the helpful bacteria for maintaining good health and preventing disease is becoming well recognized (Tegegne and Kebede, 2022). The prophylactic uses of probiotics with their produced beneficial effects and mechanisms are presented in Table (4). The probiotics' most significant therapeutic effects are determined by how they affect the digestive system (GIT); either directly or indirectly. This is attributed to the fact that the probiotics are able to interact and come in contact with the mucous membrane microflora of the host cells; in addition of being consumed orally. The benefits of a single probiotic species cannot apply to the others, because not all the probiotics microorganisms are common in the human gut flora (Tegegne and Kebede, 2022). The therapeutic uses of probiotics in addition to their produced effects and mechanisms are depicted in Table (5).

9. Approaches to protect the probiotics throughout the GIT

The ascendancy of oral drug delivery is notorious (Enck et al., 2020). The conclusive effects of most therapies are to deliver the therapeutic agents safely till
Table 4. Prophylactic uses of the probiotics with their produced beneficial effects and mechanisms of action

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hospitalized patients suffering from <em>Clostridium difficile</em> colitis (CDI)</td>
<td>The activity of <em>Clostridium difficile</em> toxin is inhibited by the lytic peptides produced by the probiotics. <em>i.e.</em>, Protease generated from <em>Saccharomyces boulardii</em> inhibits the toxin.</td>
<td>(Rodriguez and Miller, 2019)</td>
</tr>
<tr>
<td>Prevention of postoperative infections</td>
<td><em>Clostridium difficile</em> has to two types of toxins; namely A and B, and both these toxins can be efficiently inhibited by certain probiotics.</td>
<td>(Franko et al., 2019)</td>
</tr>
<tr>
<td>Low birth weight new-borns</td>
<td>Various GI disorders such as abdominal surgeries, and necrotizing enterocolitis surgery can be treated with <em>Lactobacillus acidophilus</em> and <em>Bifidobacterium infantis</em></td>
<td>(Oncel et al., 2014)</td>
</tr>
<tr>
<td>Carcinogenesis</td>
<td>The faecal enzymes that has a role in development of colon cancer, such as alpha-glucuronidase, azo-reductase, and nitro-reductase can be potentially inactivated by using the lactic acid probiotic cultures.</td>
<td>(Das et al., 2016)</td>
</tr>
<tr>
<td>Acute pancreatitis and the enteral nutrition</td>
<td>Intestinal barrier function is modified using a probiotic pre-treatment with acute and severe pancreatitis. Probiotics fails to cause a beneficial effect if administered after induction of pancreatitis</td>
<td>(van Baal et al., 2014)</td>
</tr>
<tr>
<td>Preterm infants with late-onset sepsis</td>
<td>A significant lowering of late-onset sepsis (LOS) has been observed by administration of probiotic mixture of <em>Bifidobacterium infantis</em>, <em>Streptococcus thermophiles</em>, and <em>Bifidobacterium lactis</em></td>
<td>(Tewari et al., 2015)</td>
</tr>
<tr>
<td>Necrotizing enterocolitis in preterm infants</td>
<td>Boosting the host’s immune system; eradication of harmful bacteria, and significant reduction in the bacterial movement across the mucosa, are several proposed mechanisms by which living microbial supplements assist in lowering the necrotizing enterocolitis and its associated consequences.</td>
<td>(Goncalves et al., 2015)</td>
</tr>
<tr>
<td>Ventilator associated pneumonia</td>
<td>Probiotics can either locally or systematically help in treating the ventilator associated pneumonia (VAP) through several mechanisms, including remobilization of bacteria; stimulation and elevation in levels of host cell antimicrobial peptides, rehabilitation of the intestinal microbiome, boosting intestinal barrier function, and cause reduction in growth of the pathogenic bacteria.</td>
<td>Tegegne and Kebede, (2022); Li et al., (2022)</td>
</tr>
</tbody>
</table>
Table 5. Therapeutic uses of probiotics with their produced beneficial effects and mechanisms of action

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment of <em>Helicobacter pylori</em> infections</td>
<td>Multiple mechanisms are proposed through which probiotics help in eradicating <em>H. pylori</em> infections. Probiotics can act as bacteriostatic or bactericidal agents; decrease the levels of cytokines, transact with the hosts innate immune system, and strengthens the defence systems of the gastric regions. Thus, several immunological functions are regulated, and this serves as an antidote to the microorganism.</td>
<td>(Jung et al., 2018)</td>
</tr>
<tr>
<td>Inflammatory and autoimmune diseases</td>
<td>The mechanisms of the probiotic and immune system interaction can be outlined as:</td>
<td>(Liu et al., 2018)</td>
</tr>
<tr>
<td>- Stimulation of the nucleoside signalling in the gut regions;</td>
<td></td>
<td></td>
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<tr>
<td>- Secretion and transmission of short chain fatty acids (SCFA);</td>
<td></td>
<td></td>
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<tr>
<td>- Increased activity of intestinal histamine-2 receptor;</td>
<td></td>
<td></td>
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<tr>
<td>- Activation of aryl hydrocarbon receptor and increased tryptophan metabolism.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elevated blood pressure</td>
<td>The angiotensin-converting enzyme (ACE) inhibitory peptides are released as a result of the fermentation process.</td>
<td>(Noce et al., 2019)</td>
</tr>
<tr>
<td>Inflammatory bowel syndrome</td>
<td>Different microorganisms show their actions independently, which can be as follows:</td>
<td>(de Souza et al., 2015)</td>
</tr>
<tr>
<td>- <em>Lactiplantibacillus plantarum</em> Lp91 intensifies the Interleukine-10 expression and decreases the levels of COX-2 and tumour necrosis factor (TNF);</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- <em>L. paracasei</em> L74 CBA helps in inhibiting the pro-inflammatory cytokines and also stops the expression of the NF-Kb;</td>
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<td></td>
</tr>
<tr>
<td>- <em>Streptococcus salivarius</em> has inflammation lowering properties.</td>
<td></td>
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</tr>
<tr>
<td>Prevention of candidiasis infection in denture wearers</td>
<td>Probiotics such as <em>Limosilactobacillus reuteri</em>, <em>Lactobacillus</em> strains and <em>Lactocaseibacillus rhamnosus</em> lower the colonization of <em>Candida</em> spp. in the oral cavity</td>
<td>(Hu et al., 2019)</td>
</tr>
<tr>
<td>Autism spectrum disorder</td>
<td>- Neurotransmission of signalling peptides and the emotional state of patient are influenced by the vagus nerve-mediated Gut-Brain-Axis (GBA).</td>
<td>(El Khatib et al., 2020)</td>
</tr>
<tr>
<td></td>
<td>- Bacterial strains involved in this process, include <em>Lactobacillus</em> sp. (<em>L. acidophilus, L. helveticus, L. rhamnosus, and L. plantarum</em>) and <em>Bifidobacterium</em> sp. (<em>Bifidobacterium longum, Bifidobacterium breve, Bifidobacterium infantis, and Bifidobacterium bifidum</em>)</td>
<td></td>
</tr>
<tr>
<td>Migraine</td>
<td>Studies have revealed the potentials of the intestinal bacteria to stimulate the levels of serotonin synthesis. Eventually this effect can be helpful in modifying the gut function, which helps in migraine treatment.</td>
<td>(Xie et al., 2019)</td>
</tr>
</tbody>
</table>
Probiotics have been shown to be effective in treating both Type-I and Type-II diabetes. Combination of *L. plantarum* and *L. genus* helps in preventing or delaying the autoimmune diabetes. On the other hand, several probiotic species such as Lactobacilli (*L. acidophilus*, *L. casei*, *L. delbrueckii* subsp. *L. bulgaricus*, and *Lactiplantibacillus plantarum*), and Bifidobacteria (*Bifidobacterium longum*, *Bifidobacterium infantis*, and *Bifidobacterium breve*) have been proven to be of anti-diabetic potentials.

In type-II diabetes, the weight gain in humans is inhibited along with the fat tissue mass adiposity, which eventually helps to treat the diabetes using the probiotics such as combination of *Bifidobacterium lactis*, *Lacticaseibacillus rhamnosus*, and *L. gasseri*.

The toxic components like mercury, beryllium, and cadmium that are present in hair can be eliminated using several probiotics. The levels of cadmium can be lowered as *Limosilactobacillus reuteri* and *L. gasseri* bind to this element. Whereas the levels of mercury and beryllium can be lowered with consuming the probiotics for a duration of at least 6 months.

**10. Micro-encapsulation as an approach for producing GIT stable probiotics**

Microencapsulation has been regarded as the most effective method for protecting the probiotics and ensuring their stability without affecting the native strains characteristics. It is a known technology that is used for packing solids, liquids, or gaseous substances in small sealed capsules, which has the ability to deliver the active ingredients at the controlled and specific site (Rajam and Subramanian, 2022). Today, many probiotics that are utilized as commercial goods belong to the genera of *Lactobacillus* or *Bifidobacterium*, which are especially vulnerable when meet the unendurable environments encountered mostly in the meals along with the human gut (Sarao and Arora, 2017). Further, the new probiotics genera, including *Faecalibacterium prausnitzii* and *Akkermansia muciniphila*, are found highly responsive to several factors, including bile salts, the stomach...
acid, and oxygen; thus limiting their effective use in the dietary treatments to improve the human health. (Cani and de Vos, 2017). As a result, effective methods for increasing the viability of probiotics in meals and throughout their transit from the human gut are urgently needed. Microencapsulation has been put forward as a viable way to protect the probiotics from intestinal degradation (Cook et al., 2012).

Encapsulation is the most effective method for protecting the probiotics and ensuring their stability without affecting the native strains characteristics. During encapsulation of the probiotics, the live bacteria are enclosed in an encapsulating material that increases the probiotics' stress resistance during manufacturing, storage, and at the site of action. Accordingly, the encapsulation procedure also provides tailored distribution (Douillard and de Vos, 2019).

The encapsulation process enhances the viability of probiotics, where their survival depends on the physiochemical properties of the microcapsules. It’s the polymer chosen for encapsulation that will govern the stability of whole product. Probiotics are frequently microencapsulated using a variety of biopolymers, including plant by-products (i.e., arabic and acacia gums), marine extracts (i.e., alginate, carrageenan), proteins (i.e., milk or whey protein, soy protein, gelatin, and gluten), and dietary fibers (i.e., resistant starch, maize starch), in addition to several microbial and animal polysaccharides such as chitosan and xanthan. The compositions of the capsule wall materials have a significant impact on the encapsulation efficiency, physiochemical properties, and stability of the microcapsules (Fu and Chen, 2011).

Encapsulation of the food components in coating materials can be accomplished using a variety of approaches (Yoha et al., 2021). The main elements that influence the selected technique include the average size of the food particle; the applications of the encapsulating substance, the physical and chemical properties of the carrier material, and the required release mechanism, in addition to the prices (Rajam and Subramanian, 2022). The three primary stages of the probiotics encapsulation are: microcapsule manufacturing; the integration of the bioactive components in a matrix, and microcapsule stabilization using chemical, physicochemical, and/or physical techniques (Eshaq Safi et al., 2021). The main methods used for encapsulation of the probiotics include:

10.1. Fluidized bed drying

Fluidized bed dryer (FBD) is amongst the most versatile equipment used in pharmaceuticals. Microencapsulation either being single coated or multi-coated can be designed using FBD. The combination of four polymers, including shellac, sodium alginate, arabic gum, and chitosan have been used to coat L. reuteri probiotics, which improved their stability and they become resistant to the unfavorable conditions (Calinoiu et al., 2019; Zaghari et al., 2020).

10.2. Spray drying

Modifying the storage of probiotics can be easily performed using a spray drying technique, which has fast drying rate; provides controlled particle size, and produces a free flowing powder (Fazilah et al., 2019). Spray drying is used to dry numerous strains of Lactobacillus and Bifidobacterium. The microencapsulated L. rhamnosus cells are more stable than the non-encapsulated cells. The probiotics have been treated with hydrogen peroxide, sodium chloride, and heat before encapsulation, which resulted in stable microcapsules in the gastric region (Farahmandi et al., 2021).

10.3. Extrusion

The probiotics microparticles can be prepared using an extrusion technique. The process of encapsulation using extrusion is a two-step method, which firstly involves the extrusion of probiotics with hydrocolloid solution through nozzle dripping into a gelling mixture, followed by the formation of a membrane,
which yield porous hydrogel beads (Baral et al., 2021). The extruded microcapsules have several limitations such as scalability and particle size limitation, which can be improved through using a vibrating nozzle technique; spinning disc atomization, electro spraying, and jet cutting (Sultana et al., 2022). Applying an extrusion technique for encapsulating L. acidophilus with various polymers including inulin; alginates, rice bran, and hi-maize, has helped in the production of probiotics with different encapsulation efficiencies; with a recorded higher efficiency on using inulin (Poletto et al., 2019). A type of an extrusion method under vibrations has been previously employed by Eckert et al., (2018); using whey permeate-alginate-pectin (WPAP) and whey-alginate-pectin (WAP) for encapsulating L. plantarum ATCC8014, Lacticaseibacillus paracasei ML33, and Lactiplantibacillus pentosus ML82), and has shown minimum loss of cell viability. Hence, using an extrusion technique for encapsulation can produce stable and highly viable encapsulated probiotics.

10.4. Freeze drying

Utilization of the conventional freeze-drying method for encapsulation is an emerging method for stabilizing the probiotics powder. The viability of the encapsulated probiotics can be efficiently increased through combination with nanoparticles (NPs), and the same results have been presented by Yao et al., (2018) by using magnesium oxide NPs for enhancing the encapsulation efficiency of Pediococcus pentosaceus (Li05). Compared with the other methods of encapsulation such as electro-spraying or spray drying, the freeze-dried L. rhamnosus (ATCC 7469) using whey protein isolate (WPI), persian gum, and inulin is more viable (Moayyedi et al., 2018). Similarly, (Bora et al., 2018) have used two probiotic species of L. acidophilus and L. casei for encapsulation using freeze-drying with WPI and Fructo-oligosaccharide (FOS) as polymers, which have increased the cell viability and encapsulation efficiency, respectively. Several previous studies have suggested that using a cryo-protectant can increase the probiotics cell viability, and this has been confirmed by Halim et al., (2017); through using calcium alginate and 10 % skim milk as crypto-protectants for P. acidilactici (ATCC 8042). The skim milk not only acts as a buffer in the system but also protects the encapsulated species from the harsh conditions.

11. Oral delivery of the probiotics

Nowadays, probiotics are available in a variety of oral medications, including capsules; tablets, lozenges, films, powder, chewing gum, etc. For prevention of the gingival and halitosis, lozenges; films, and chewing gum are efficient, while as dietary supplements and disease treatments; the tablets, capsules, and powders are highly recommended (Sreeja and Prajapati, 2013).

11.1. Oral films

Mouth dissolving films are a form of dosage that is used in the oral cavity to deliver the medicines and the probiotics bacteria. The type of polymer employed in its production influences the properties of this film. Oral films are classified into two types: Mucoadhesive and Non-Mucoadhesive (Oro-dispersible film) (Thakur and Singh, 2019). The oro-dispersible films are thin strips that are placed on the patient's tongue and immediately release the drug into the oro-mucosal area. Patients who have difficulty in swallowing will benefit greatly from these fast-dissolving films. Probiotics are commonly associated with natural protection that benefits the oral health. The orally disintegrating films have been developed by Heinemann et al., (2013) using Bifidobacterium animalis and L. acidophilus subsp. lactis in a starch-based matrix with carboxymethyl cellulose and gelatin. Similarly, Tomaro-Duchesneau et al., (2014) used carboxymethyl cellulose as a polymer to make oral thin films for treatment of the oral disorders. For the treatment of dental decay and oral candidiasis, L. fermentum NCM 5221 (6.751108 kill/film) has been added to the film Tomaro-Duchesneau et al., (2014). A previous study reported by Gagliarini et al. (2019) has looked into using whey protein and a polysaccharide kefiran to make probiotic microorganism-loaded films of Kluyveromyces marxianus CIDA 8154 and L.
paracasei CIDCA. During storage, the oral films have shown an increased viability of the microorganisms. A muco-adhesive wafer for the delivery of Bifidobacterium bifidum BB12 through an oral route has been developed; using a combination of two different polymers, namely Carbopol 974 P® and poloxamer 407, which significantly increased the probiotics cell viability (De Souza Ferreira et al., 2021).

11.2. Granules

Granules as a dosage form have offered several advantages. In order to develop controlled release from the probiotics, the granules can be coated with polymers, which also increase their stability in the acidic environment of the stomach. In the previous study reported by Pyar and Peh., (2014), L. acidophilus ATCC 4962 granules have been prepared through wet granulation followed by coating with an enteric polymer, in order to act in the acidic conditions (i.e., Eudragit L30D-55). The standardized extract granules of Olea europaea leaves (Phenolea® Active F) have been prepared by Aponte et al. (2018); using a wet granulation method along with L. plantarum 299v.

11.3. Tablets and capsules

The tablets and capsules are the most preferred earlier dosage forms. A pH sensitive phthalyl-inulin coated L. reuteri LRT18 tablets have been designed to protect the probiotics from the harsh gastric conditions (Kim et al., 2019). On the other hand, oro-disperible tablets have been developed by Hoffmann et al., (2020); using several mucoadhesive polymers, in order to protect the L. plantarum Lp299v. The enteric coated probiotics with hydroxy propyl methyl cellulose (HPMC) and mosapride have been used to fill the hard gelatin capsules, which ultimately increased the survival of these probiotics and contributed to their protection from the gastric environment (Kim et al., 2014).

12. Nano drug delivery systems

Electro spinning is one of the common methods that are used to develop nano-fibers by applying strong electric field to the polymeric solution. L. paracasei KS-199 has been encapsulated using alginate base nano-fibers, which ultimately protected the probiotics from the harsh conditions in the GI region (Yilmaz et al., 2020). Additionally, chitosan-based NPs are now in demand due to their ability to stabilize the probiotics in GIT. They have been used to encapsulate Escherichia coli nissle 1917, and have shown resistance to high temperature, varied concentrations of bile salts, and also to the low pH of the gastric regions (Mawad et al., 2018). In another study, Zhang et al., (2018) have used sodium alginate and 2,2,6,6-tetramethyl-1-piperidinyloxy radical (TEMPO) oxidized cellulose nano-fibers to design a microsphere, which possesses pH responsive properties. Due to the acidic conditions, hydrogen bonds have been developed that stabilize the gel-based microspheres, which have provided protection to the probiotics. NPs are small particles that consist of any active substance that diffuses cages, encloses and/or attaches a drug to a nanoparticle matrix. The irritable bowel syndrome (IBS) disease, which includes colitis and Crohn's diseases, is treated with NPs in the colon (Hua et al., 2015). Embedding the probiotics bacteria in microgels that are doped with inorganic NPs have enhanced their viability (Yao et al., 2018). Microgels that are formulated using alginate and gelatin with and without magnesium oxide NPs are used to grow the bacterium Pediococcus pentosaceus Li05. The use of NPs has been shown to improve the probiotics viability during the long-term storage; heat treatment, and GI transit (Yao et al., 2018). A previous study conducted by Ghiaudino et al., (2019) has investigated the biophysical stability of NPs of iron-pectin, and also the suitability of using them as a method of delivery system for a probiotic bacterium (i.e., L. plantarum). In the last 15 years, the use of NPs as delivery systems for the low-water-solubility nutrients has increased exponentially (Ghibaudo et al., 2019), which have been influenced by the different drug delivery strategies. The magnetic iron oxide (Fe₃O₄ and Fe₂O₃) NPs are especially suitable for several biomedical
applications, due to their non-toxicity, super-paramagnetic properties, and simple separation methodology (Ali et al., 2016). The fusion of magnetic iron oxide NPs and pectin appears to be a promising approach for delivering iron through the mouth. The manufacturing of many foods along with probiotics has been initiated by the lactic acid bacteria, and hence they have a major role in the food as well as in the pharmaceutical industries. As a result, the use of pectin-coated iron NPs as matrices for the dehydrating probiotics lactic acid bacteria may be a promising approach to protect the bacteria, delivering iron in a soluble form, ensuring their secure passage through the GI, and their final delivery in the gut (Golowczyc et al., 2011).

13. New techniques for developing probiotics with improved stability

13.1. Refractance window drying

Refractance window (RW) is a new generation drying process used for encapsulating the probiotics; along with its variation conductive hydro drying method. RW dryer is constructed so that it has a hot water reservoir with a top placed transparent polyester plate, which is used to dry the homogenized mixture of probiotics with the encapsulating material when the mixture is spread over this plate. The rate of drying is increased due to the combined effects of all modes of heat transfer, and hence it’s a quick process (Yoha et al., 2019). The RW drying technique has been used by Yoha et al. (2019) with varied combinations of probiotics to encapsulate L. plantarum (NCIM 2083); as it is known that the encapsulation efficiency is dependent upon the composition of the wall material and the drying temperature. Amongst all combinations; fructo-oligosaccharide (FOS), whey protein isolate (WPI) with sodium alginate (SA), and whey protein isolate (WPI) with fructo-oligosaccharide (FOS); for encapsulating L. plantarum (MTCC 5422), which have encapsulation efficiency around 94 %. Similarly, a study reported by Cao et al. (2019) has used different concentrations of skimmed milk powder as a wall material to encapsulate S. cerevisiae, which showed encapsulation efficiency up to 75 %.

13.2. Spray-freeze-drying

The spray-freeze-drying technique utilises the combined benefits of spray drying with those of freeze drying. Maltodextrin (MD) and trehalose have been utilized as encapsulating materials by Semyonov et al. (2010) for spray-freeze-drying of L. paracasei (LMG P-21380). The cell viability is not disturbed during the spraying stage; however during the freezing stage, the cell viability is affected due to the osmotic pressure of the solute concentration. A previous study conducted by Rajam and Anandharamakrishnan, (2015) has used different polymers and their varied combinations, including denatured whey protein isolate (DWPI) with fructo-oligosaccharide (FOS), whey protein isolate (WPI) with sodium alginate (SA), denatured whey protein isolate (DWPI) with sodium alginate (SA), and whey protein isolate (WPI) with fructo-oligosaccharide (FOS); for encapsulating L. plantarum (MTCC 5422), which have encapsulation efficiency around 94 %. Similarly, a study reported by Cao et al. (2019) has used different concentrations of skimmed milk powder as a wall material to encapsulate S. cerevisiae, which showed encapsulation efficiency up to 75 %.
Conclusion

As a result of the advent of novel and drug resistant pathogens, the medical community is searching for alternative treatments. Due to this, the probiotics are used in a variety of diseased and health conditions. It is found that on shelf storage and during gastrointestinal transit, most of the commercial products with probiotics undergo a significant deprivation of the bacterial viability. More researches should be conducted, and it is needed to ascertain the safety of the commercial probiotics. This review involves some of the approaches used for the development of strategies for protection of the probiotic bacterial cells and maintaining their viability throughout the GI transit. There are many significant encapsulation technologies that have been established to keep the probiotics stable under the harsh conditions. When compared to the free or non-encapsulated bacteria, the encapsulated bacterial probiotic cells are recorded to being the most successful at shielding the bacteria under the simulated gastrointestinal conditions. As a result, the constructed probiotic formulations using the aforementioned methods have enormous potentials for the delivery and protection of the probiotics in foods, supplements, and pharmaceutical products. Particularly highlighted in this study is the widespread application of several embedding and coating technologies to enhance the probiotics efficacy. However, as discussed in the current study; the probiotics formulations that contain multispecies of bacteria have already proven to be effective during the various clinical trials and across the variety of health disorders.

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