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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 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

being the host faces high impact by these ecosystems continuously. The microbiome genetic material is formed by five components, including bacteria (bacteriome), archaea (archeome), fungi (mycobiome), viruses (virome), and parasites (parasitome), which influence digestion, development of immunity, and production of vitamins (Spacova *et al*., 2020). Basically, microbiome is alive microbes that can be used to develop into various types of products such as foods, drugs, and dietary supplements (Song *et al*., 2012). A previous study carries the presumption that enough numbers of microorganisms will remain useful and survives during their transport through the gastrointestinal tract (GIT); even after being put up against the various toxins such as bile salts and the acids that exist in the GIT. To obtain optimum clinical efficacy, the number of required viable microorganisms is contemplated to be 10^6 cfu\ ml in the small intestine and 10^8 cfu\ ml in the colon. A significant difference (*i.e*., in log units) in the microbe's survival over vulnerability that is caused by the bile salts and gastric juices; largely banks on the formulation, freeze-drying, and product's storage. According to the accepted definition of probiotics, the benefits are not solely transmitted by microorganisms; rather, a variety of mechanisms are recognized other than influencing the colonizing microbiota, which opens up a bigger diversity of the probiotic prospects and advances the research in this area (Sanders *et al*., 2019)

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). Inopportunely, 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)

Parts of the GIT	Probiotic species
Gut	Verrucomicrobia, Bacteroidetes, Proteobacteria, Actinobacteria, and Firmicutes
Small intestine	*Enterobacteriaceae*, *Erysiopelotrichaceae*, and *Lactobacillaceae*
Colon	*Ruminococcaceae*, *Bacteroidaceae*, *Rikenellaceae*, *Lachnospiraceae*, and *Prevotellaceae*
Cross-section of the colon shows the digesta	*Bacteroidaceae*, *Rikenellaceae*, and *Prevotellaceae*
The inter-fold regions of the lumen	*Ruminococcaceae* and *Lachnospiraceae*

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

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

Produce enzymes	• Production of lactase and bile salt hydrolase.
Manufacture small molecules with systemic effects	• Repletion of hormones along with neuro transmitters, such as tryptophan and serotonin; • Gamma-aminobutyric acid (GABA), cortisol, and several histamine derivatives; • Stimulates linoleic acid.

potential, and induction of cancer cell apoptosis. 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

Protein name	Characteristic (Location)	Role	References
Mucus-binding proteins (MUBs)	A characteristic c terminal LP×TG signal peptide (Surface of the cell)	Brings about covalent binding with the bacterial cell was leading to mucus binding	(Muscariello *et al*., 2020)
Fibronectin-Binding Proteins	Belongs to the family of adhesins, a type of microbial surface components recognizing adhesive matrix molecules (MSCRAMM) (anchored on the bacterial surface)	Bind to the exposed fibronectin and anchor the IECs	(Bisht *et al*., 2018)
Surface-Layers Proteins	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)	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	(do Carmo *et al*., 2018)
Teichoic Acids	composed of alditol phosphate repeating units, two types: lipotheicoic acid (LTA) and wall teichoic acid (WTA) (bacterial cell wall)	Inhibit adhesion to Caco-2 cells	(Kim *et al*., 2017)
Exopolysaccharides (EPS)	excreted as tightly bound capsule or loosely attached slime layer in microorganisms (surface carbohydrate polymers)	Facilitate probiotic adhesion, role in the interaction of probiotics with the host.	(Angelin and Kavitha, 2020)
Aggregation-Promoting Factors	secreted proteins which induce self-aggregation and facilitates the maintaining of cell shape (Cell wall)	Adhesion factor which participates in the interaction with the host mucus layer and IECs	(Nishiyama *et al*., 2015)

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 (Cytoplasm)	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

Disorder	Effect	Reference
Hospitalized patients suffering from *Clostridium difficile colitis* infection (CDI)	The activity of *Clostridium difficile* toxin is inhibited by the lytic peptides produced by the probiotics. *i.e*., Protease generated from *Saccharomyces boulardii* inhibits the toxin.	(Rodriguez and Miller, 2019)
Prevention of postoperative infections	*Clostridium difficile* has to two types of toxins; namely A and B, and both these toxins can be efficiently inhibited by certain probiotics.	(Franko *et al*., 2019)
Low birth weight new-borns	Various GI disorders such as abdominal surgeries, and necrotizing enterocolitis surgery can be treated with *Lactobacillus acidophilus*\ *Bifidobacterium infantis*	(Oncel *et al*., 2014)
Carcinogenesis	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.	(Das *et al*., 2016)
Acute pancreatitis and the enteral nutrition	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	(van Baal *et al*., 2014)
Preterm infants with late-onset sepsis	A significant lowering of late-onset sepsis (LOS) has been observed by administration of probiotic mixture of *Bifidobacterium infantis*, *Streptococcus thermophils*, and *Bifidobacterium lactis*	(Tewari *et al*., 2015)
Necrotizing enterocolitis in preterm infants	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.	(Goncalves *et al*., 2015)
Ventilator associated pneumonia	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.	Tegegne and Kebede, (2022); Li *et al*., (2022)

Table 5. Therapeutic uses of probiotics with their produced beneficial effects and mechanisms of action

Disorder	Effect	Reference
Treatment of *Helicobacter pylori* infections	Multiple mechanisms are proposed through which probiotics help in eradicating *H. pylori* 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.	(Jung *et al*., 2018)
Inflammatory and auto immune diseases	The mechanisms of the probiotic and immune system interaction can be outlined as: -Stimulation of the nucleoside signalling in the gut regions; -Secretion and transmission of short chain fatty acids (SCFA); -Increased activity of intestinal histamine-2 receptor; -Activation of aryl hydrocarbon receptor and increased tryptophan metabolism.	(Liu *et al*., 2018)
Elevated blood pressure	The angiotensin-converting enzyme (ACE) inhibitory peptides are released as a result of the fermentation process.	(Noce et al., 2019)
Inflammatory bowel syndrome	Different microorganisms show their actions independently, which can be as follows: -*Lactiplantibacillus plantarum* Lp91 intensifies the Interleukine-10 expression and decreases the levels of COX-2 and tumour necrosis factor (TNF); -*L. paracasei* L74 CBA helps in inhibiting the pro-inflammatory cytokines and also stops the expression of the NF-Kb; -*Streptococcus salivarius* has inflammation lowering properties.	(de Souza et al., 2015)
Prevention of candidiasis infection in denture wearers	Probiotics such as *Limosilactobacillus reuteri, Lactobacillus* strains and *Lacticaseibacillus rhamnosus* lower the colonization of *Candida* spp. in the oral cavity	(Hu et al., 2019)
Autism spectrum disorder	-Neurotransmission of signalling peptides and the emotional state of patient are influenced by the vagus nerve-mediated Gut- Brain-Axis (GBA). -Bacterial strains involved in this process, include *Lactobacillus* sp. (*L. acidophilus*, *L. helveticus*, *L. rhamnosus*, and *L. plantarum*) and *Bifidobacterium* sp. (*Bifidobacterium longum*, *Bifidobacterium breve*, *Bifidobacterium infantis*, and *Bifidobacterium bifidum*)	(El Khatib et al., 2020)
Migraine	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.	(Xie *et al*., 2019)

Type-I and type-II diabetes	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*	Belizario *et al*., (2018); Kang and Cai, (2018)
Probiotics on hair toxic element levels	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.	(Fang *et al*., 2018)

the gut, followed by their enhanced bioavailability due to absorption (Sharpe *et al*., 2014). However, the contents of the gastric regions have competency to debase the therapeutics such as probiotics, which are highly susceptible to the gastric contents. The protection of probiotics from the gastric acid, the other enzymatic barriers, and their delivery to the intestinal region has been a great challenge. Several new approaches have been designed in the probiotics therapies that work on stabilizing the probiotics through several mechanisms; leading ultimately to a significant number of available probiotics after passing through the various GI barriers, and thus they become available for showing their desired therapeutic or prophylaxis effects. Furthermore, the pharmaceutical organizations will benefit from the increased probiotics efficacy in the use of their drugs, and will be much interested in such a delivery vehicle (Enck *et al*., 2020).

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 un-favorable 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 animal*is 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 *Kluyeromyces 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-disperbible 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 Ghibaudo *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 (Fe_3O_4 and Fe_2O_3) 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 (WP), and maltodextrin (MD) have imparted good probiotics viability.

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 %.

13.3. Microfluidics

The microfluidic technology while working simultaneously with several molecular, tissue engineering, microbiological and cell identification fields, has contributed in addressing the various difficulties in the different phases of the microbiota research (Tan and Toh, 2020). Amongst the several methods in microfluidics; preparation of a microfluidic double water-in-oil-in-water emulsion is also known to be a deep functional profiling method, which continues to offer the ability to empower the functional characterization of the single cells of microbiomes (Terekhov *et al*., 2018). In a previous study conducted by Chen *et al*., (2018), a lactic acid bacterial strain improvement has been made using droplet-based microfluidics. Several factors such as the distribution pattern of the applied cell concentration in the disperse medium can determine the formation of a droplet; as a result, the ratio of cells in a droplet helps to determine the density of the cells. Employing this droplet technique, yields microbeads that are mono-dispersed and highly stable (Chen *et al*., 2018).

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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Conflict of interest

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