INTRODUCTION: “Let food be thy medicine and Medicine be thy food” – Hippocrates. Probiotics are live microorganisms; those can be introduced orally in the gastrointestinal tract or GI tract to improve intestinal microflora and positively contribute to the health of the host. Live bacterial cells are gaining large attention for contributing in treatment of several diseases including kidney failure uremia, cancer, inflammatory bowel disease, cholesterolemia, and others. It has been reported that probiotics can suppress the growth of undesirable microorganism in colon and intestine.

ABSTRACT: Probiotics are live microorganisms that when introduced orally positively contribute to the activity of the intestinal microflora and the health of its host. Various studies showed that probiotics are potentially able to contribute to treatments of many diseases. They may not cure any disease, but can significantly improve the patient’s condition and also can prevent many infections. Recent studies confirmed that gut microbiota has a direct connection to the behavior and development of the brain. This review is done in two parts. The first part discusses how micro-encapsulation can improve targeted delivery of probiotics where second part discusses the effects of probiotics on various diseases. On the first half of this review, we will see micro-encapsulation allows core ingredients or probiotics to be separated from the environment by a protective coating which can enhance shelf-life of probiotics. The objective of this review is to study the efficiency of different encapsulation materials and methods to understand and control the delivery of probiotic by the conventional method to improve drug uptake, release and absorption by making it resistant from the harsh environment (Gastric juice and bile salt). On the second half of this review, we will encounter how probiotics are an essential part of the gut-brain axis and gut-brain signaling, how they influence neural development and help in the treatment of various neurodegenerative diseases like Autism, Alzheimer’s disease, and Parkinson’s disease and how psychobiotics can improve mental health. Probiotics are very helpful in the treatment of various gastroenteritis disorders and also in cases of HIV and Cancer.
mention few contributions of probiotics. Probiotics regulate immunophysiology by strengthening indigenous micro flora in the gut that acts as a barrier against pathogens. Probiotics eliminate uropathogens and reduce the risk of urogenital infection. Probiotics also lower pH level in the colon, which is helpful in constipation, they help to better absorb protein, minerals, also helpful in lactose intolerance, and IBS (Inflammatory bowel syndrome), colitis, ulcer thus helps to improve the overall digestion process. Probiotics like *Lactobacillus* can alkalinize urine by reducing oxalate as a result decreases chances of nephrolithiasis. Evidence shows that probiotics reduce the duration of symptoms, decrease the severity of the infection and also reduce the frequency of infections that largely contribute to reducing the use of antibiotics thus lower antibiotic resistance. Recent studies show that probiotics are very much efficient in controlling obesity. Probiotics like *L. rhamnosus, L. planterum* had an anti-obesity effect on human and animals respectively. Probiotics are capable of preventing eczema in children. Probiotics also found to be helpful in the treatment of Autism, Parkinson’s disease, cancer that is discussed later.

**TABLE 1: SOME PROBIOTIC STRAINS** ³, ⁴, ⁵

<table>
<thead>
<tr>
<th>Name of the microorganisms</th>
<th>About the microorganisms</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bifidobacterium bifidium</strong></td>
<td>It is the most dominant beneficial microorganism in infants and the large intestine. It improves the production of vitamin in the gut, immune response, inhibits harmful bacteria and prevent diarrhea</td>
</tr>
<tr>
<td><strong>Bifidobacterium infantis</strong></td>
<td>Helps in reducing IBS symptoms, diarrhea and constipation</td>
</tr>
<tr>
<td><strong>Bifidobacterium breve</strong></td>
<td>Colonizes healthy gut community and crowd out bad bacteria</td>
</tr>
<tr>
<td><strong>Bacillus subtilis</strong></td>
<td>An endospore-forming probiotic elicits a potent immune response, supports GALT and suppresses the growth of harmful bacteria like <em>Salmonella</em> sp</td>
</tr>
<tr>
<td><strong>Lactobacillus acidophilus</strong></td>
<td>Improves lactose intolerance, lowers cholesterol levels and improves the production of vitamin K. Important in GALT immune strengthening. Also inhibits <em>E. coli</em></td>
</tr>
<tr>
<td><strong>Lactobacillus casei</strong></td>
<td>Boosts immunity, inhibits <em>H. pylori</em> and fights infection</td>
</tr>
<tr>
<td><strong>Lactobacillus brevis</strong></td>
<td>It can survive the G.I. tract environment, boosts cellular immunity, enhances natural killer cells and kills <em>H. pylori</em></td>
</tr>
<tr>
<td><strong>Lactobacillus bulgaricus</strong></td>
<td>A powerful probiotic that fights harmful bacteria those invade our digestive system and is stable enough to withstand acidic gastric juice, it also neutralizes toxins and can produce antibiotics naturally</td>
</tr>
<tr>
<td><strong>Lactobacillus rhamnosus</strong></td>
<td>Can survive the G.I. tract. Helps in balancing bacterial flora and keeps skin healthy. Fights urinary tract infection, respiratory tract infection and reduces anxiety by reducing stress hormone and GABA neurotransmitter receptors</td>
</tr>
<tr>
<td><strong>Saccharomyces boulardii</strong></td>
<td>It is a strain of yeast that restores the natural microflora in large and small intestine improving intestinal cell growth, used in the treatment of inflammatory bowel disease, reduces inflammation also shows the antimicrobial effect</td>
</tr>
</tbody>
</table>

**Properties of Ideal Probiotic:**

There are various effects of probiotics on biological system like stimulation of the immune system by altering cytokine release, enhanced antibody production and neutral killer cell activity, modulation of dendritic cell, NF-kB and AP-1 pathway, induction of PPAR-γ and regulatory T-cells, regulation of apoptosis by improving strict protein phosphorylation, inhibition of proteasomal degradation of proteins, increase in mucus production, sIgA production, enhancement of epithelial cell glycosylation, competition with pathogen for mucosal binding site, production of acids, peroxidase or bacteriocins to prevent bacterial infection, exhibition antimicrobial effect by secretion of antimicrobial peptides like defensin, nitric oxide, inhibition of pathogenic bacterial invasion protecting the epithelial barrier, blockage
of bacterial adhesion to epithelial cells, alleviation of lactose intolerance, cholesterol reduction, tumor targeting $^{9,10,11}$.

**Targeted Delivery of Probiotics:** Many mechanisms are available for delivery of probiotics and other drugs to the intestine. Both conventional pharmaceutical process and non-conventional food products are involved. The non-conventional probiotic formulation includes cheese, yogurt, creams, chocolates, milk, meat, etc. These are good delivery systems and can be of great benefit for patients. One drawback is, very few products are able to deliver these good bacteria to the intestine. But conventional pharmaceutical formulations differ greatly. The survivability depends on various factors like the formulation process, the viability of dosed bacteria and variation in stability of different species of bacteria in different physiological conditions and ability to adhere to the intestinal wall. Conventional pharmaceutical products tend to be more effective in this regard and much more characterized compared to the food based carrier system. Conventional pharmaceutical formulations include beads, capsules, tablets, etc.

Each type of formulation possesses advantages with each having varying amounts of the viable probiotic bacterial cell. The effectiveness of any formulation depends on their ability to deliver the correct amount of viable bacteria at the time of administration, delivery of a correct strain of probiotic bacteria and protection from simultaneously administrated antibiotics. *Lactobacillus* sp. shows greater resistance to gastric acid than other species such as *Bifidobacterium*, where *Enterococci* are more resistant at gastric pH (2.0-3.0) than other bacteria tested for 60 min.

Bile tolerance is another important factor, because probiotic bacteria interact with bile on entry into the small intestine. This issue is not seen in case of naturally occurring probiotic bacteria. Because of being commonly exposed to bile salt in the intestine, they develop bile tolerance. Food delays the absorption of various medicines and probiotics and leads to the improper availability of given dosage. So, conventional methods like microsphere or microparticle can be taken under consideration to study the increase or decrease in the rate of the viability of probiotics. To be therapeutically active sufficient number of live probiotic must arrive at the intestine which is 106-107 cfu $^{12,13,14}$. Here, we are going to discuss the microencapsulation of probiotic using microsphere and microparticle.

**Micro-encapsulation:** It allows core ingredient or probiotic to be separated from the environment by a protective coating. Encapsulation is the most widely applied technique in research and industrial practices to improve the survival of probiotics, owing to its universal efficacy and little influence on the embedded microorganisms. Within the context of the present work, the encapsulation material is expected to be a food grade agent. The basic principle is that the probiotic bacteria are immobilized in such material and thus protected from the harsh conditions $^{15}$.

**Parameter for Encapsulation of Probiotics:**
- Easy techniques and accuracy of particle size;
- Encapsulation materials should have mechanical strength as required;
- Ability to release the cells at the target site is important;
- The material used should be able to withstand the adverse environment in vivo and should protect the cells;
- Biocompatible & biodegradable encapsulation materials are highly favored;
- It should be non-toxic $^{16-19}$.

**Microspheres:** Microspheres are spherical shells those are usually made up of biodegradable or resorbable plastic polymers, those are of very small diameter, usually in the micrometer or nanometer range, and are often filled with substances (as a drug or antibody) for release as the shells are degraded. Hypromellose is considered suitable in the preparation of mucoadhesive microspheres due to its favorable mucoadhesive properties, and it is safe for oral consumption by a human.

Another suitable encapsulating agent is whey protein, a byproduct of the cheese industry that causes high environmental contamination. Different strategies for producing microcapsule with whey protein include spray drying, cold-induced gelation, complex coacervation and a
combination of different methods. EC (ethyl cellulose) is derivative of cellulose in which some of the hydroxyl groups of anhydrous glucose units are modified into ethyl ether groups, largely called as non-ionic ethyl ether of cellulose. EC is used for microencapsulation of various pharmaceuticals to stabilize them against interaction, hydrolysis oxidation. It is also used as a matrix forming or coating agent to achieve the controlled release of antibiotics and probiotics. An EC microparticle can be considered as a mini-osmotic pump.

It is possible to fine-tune the release kinetics of EC microparticles by altering osmolality of the dissolution medium or formulations and EC film’s mechanical characteristics can be altered by selecting proper EC molecular weight, EC substitution grades, and coating weight and size of pores. The release of a highly or sparingly soluble drug is considerably reduced when osmolality of dissolution medium is increased, causing a reduction in osmotic pressure across the release controlling membrane. Drug release reduces considerably with the increase in EC molecular weight. Higher molecular weight results in increase in the polymer chain and thus results in the stronger film, increased elasticity, and tensile strength. Stronger films may resist greater hydrostatic pressure and damage of the film due to channel formation or a stress fracture. Lower molecular weight, faster release where higher will be the molecular weight slower will be the release.

**TABLE 2: TECHNOLOGIES TO PROCESS MICROSPHERE**

<table>
<thead>
<tr>
<th>Technology</th>
<th>Principle feature</th>
<th>Advantage</th>
<th>Disadvantage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spray drying, freeze-drying &amp; fluidized bed drying techniques</td>
<td>Drying of the aqueous medium of probiotic and carrier material and getting into concentrated powder form</td>
<td>Less complicated probiotics in the dosage form and less viability</td>
<td>Release of entrapped probiotics in the dosage form and less viability</td>
</tr>
<tr>
<td>Extrusion</td>
<td>Gel bead formation by extrusion of hydrocolloidal probiotic mixture in a gel-forming solvent</td>
<td>Simple, able to retain a high number of cells, automated and uniform size beads</td>
<td>Size and shape of beads depending on the diameter of the nozzle and distance between nozzle and gelling solution. Beads have a greater size than capsules</td>
</tr>
<tr>
<td>Emulsification</td>
<td>Dispersing probiotic containing disperse phase in the continuous phase</td>
<td>Produces very similar size capsules compared to beads</td>
<td>Complicated, expensive process, use of oil, un-uniformed capsule size</td>
</tr>
</tbody>
</table>

**FIG. 2: STRUCTURE OF PROBIOTIC COATED MICROSPHERE**

**TABLE 3: EXCIPIENTS USED FOR MICROENCAPSULATION**

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Characteristics</th>
<th>Remark</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alginate</td>
<td>Insoluble in acidic media. 60-80 degree C. temperature is needed to dissolve it.</td>
<td>Protects the cells against acidic condition</td>
</tr>
<tr>
<td>Carrageenan</td>
<td>Kappa carrageenan and iota carrageenan has gelation property due to their structural conformation. Dissolves at 60-80 degree C. Solidifies after cooling</td>
<td>Forms a gel and entraps cell</td>
</tr>
<tr>
<td>Chitosan</td>
<td>Positively charged polysaccharide. Insoluble at higher pH than 5.4, forms a negatively charged semi-permeable membrane</td>
<td>Can be used in combination with other polymers like alginate, for providing stability</td>
</tr>
<tr>
<td>Cellulose acetate phthalate</td>
<td>pH-dependent cellulose derivative, insoluble at pH below 5 but soluble in pH greater than 6</td>
<td>CAP cannot form gel beads by gelation; the capsule is developed by emulsification. It’s a great coating agent</td>
</tr>
<tr>
<td>Locust bean &amp; starch</td>
<td>Specific interaction occurs during mixing with other polymers</td>
<td>Used by mixing with alginate or carrageenan to develop gel beads</td>
</tr>
</tbody>
</table>

International Journal of Pharmaceutical Sciences and Research
In non-conventional food product, cheddar cheese is good for delivering a variety of probiotic bacteria like *Lactobacillus* and *Bifidobacterium*. Increase in intestinal flora has been observed after ingestion of probiotic cheese. Milk has also been considered as a good delivery medium for probiotics. Delivery of *Lactobacillus* sp. showed an increase in colony numbers after administration through milk. The Casein micro peptide is a glycopeptide containing nitrogen and amino sugars; those works as growth factors for *Bifidobacterium*.

*Lactobacillus rhamnosus* cell count increases when administrated through yogurt. The ability of yogurt to deliver probiotic was found to be low when compared to other products due to its acidity and oxygen content. It is especially not favorable for anaerobic *Bifidobacterium*. Due to higher fat content chocolate and ice-cream provide greater protection to probiotic against gastric juice and bile salt compared to yogurt.

**Microparticle Formulation:** Microparticles are small solid particles within the size range 1-200 µm. Depending on the type of drug is being encapsulated the method of microparticle formation is determined, and the drug is dissolved, entrapped and encapsulated in the microparticle matrix. Biodegradable polymers have been of long interest in controlled release technology for their ability to be reabsorbed by the body. These microparticles show bulk erosion hydrolysis. The release rate of both hydrophilic and hydrophobic drugs have changed significantly when incorporated through micro-particles. Pappas *et al.*, 1995 drugs were incorporated into non-degradable silicone or degradable gelatin. The drug released from the microparticle within the silicone didn’t exhibit high release in-vitro.

Microparticle size range from 1-100µm. Microparticle designed for parental drug delivery can be composed of a variety of different materials with different physical characteristics such as biodegradability, biocompatibility, injectability, sterility, compatibility and stability. To prepare micro particles several techniques are applied. Most commonly used are organic solvent evaporation and/or extraction method. Depending upon the solubility of drug simple or multiple emulsion technique, oil-in-water (o/w) and water-in-oil-in-water (w/o/w) methods are used. In o/w technique, lipophilic drug is dissolved into an organic phase, which is dispersed into the outer aqueous phase. Then, upon contact organic solvent diffuses into the external water phase and evaporates at its surface. The drug is precipitated and entrapped by the polymer.

If the drug is hydrophilic, then w/o/w technique is used where the direct contact of the drug-containing phase with an outer water phase is avoided. An aqueous solution of the drug is emulsified into an organic phase containing dissolved polymer. Water and organic solvent emulsion are dispersed into an outer aqueous phase. During solvent diffusion/evaporation, the polymer precipitates and the drug get incorporated. Albumin microparticles are hugely used because they are biodegradable, non-antigenic, and non-toxic, also can control physiochemical characteristics of the micro-particle produced and are readily available.

**A spectrum of Probiotic Application: Recent Studies and Promises:**

**Gut-Brain Connection** To understand how gut microorganisms are involved in gut-brain crosstalk, it is necessary to understand how the immune system is directly connected to our cognitive health as we all know gut microflora is an inseparable part of our immune system.

Psychoneurology pays very deep attention to stress and how it modulates the body’s defense against disease and thus sheds light on how resilience buffers stress elicited changes. Resilience is developing a positive mindset and adapting to adverse situations, trauma, loss, relationship related and financial stress to recover. The relationship between stress and immunity is bidirectional because immune mediators influence how the brain processes information and responds to it. It can potentially be modulated with the help of probiotics.

Exposure to acute stress increases levels of cortisol & glucocorticoid hormone. Those usually reduce inflammation by reducing the release of pro-inflammatory cytokines. Individuals exposed to acute stress first adapted to increased cortisol level by reducing the responsiveness of immune cells to
cortisol which leads to failure in regulation of immune response. Even sickness can lead to symptoms of depression. Sometimes due to polymorphism in cytokine gene inflammatory and anti-inflammatory cytokines get increased or decreased. Infection, leaky gut or stress results into systemic inflammation and activation of IDO1 (Indoleamine 2, 3 dioxygenases) which induces the release of inflammatory mediators resulting into somatic symptoms like reduced appetite, fatigue, sleep disorder, etc. It also leads to increased production of Kynurenine that passes through the blood-brain barrier and converts into neurotoxic metabolites of Kynureine. Which in turn produces cognitive/ affective symptoms of depression. After that brain macrophages & microglia get activated and CD8+ T cells recruited in the meninges and choroid plexus, and macrophages get deactivated, and recovery starts. The gut is considered a second brain because of the huge number of neurons in gut walls, but the connection between gut microorganisms and brain are being studied only recently. It is also considered as a new paradigm in neuroscience.

Recently from the germ-free studies, scientists have found that gut microorganisms play a pivotal role in brain function, the establishment of the blood-brain barrier, myelination, and functioning of neurotransmitter systems such as serotonin. There are various parallel routes through which gut microorganisms and the brain can communicate with each other. One big example is the Vagus nerve channel. Lactobacillus strain produces GABA that binds to GABA receptors and shows significant impact on behavior, but those who were vagotomised had no behavioral change. Gut microorganisms like Bifidobacterium produce tryptophan, which we also acquire from foods, get metabolized by IDO1 and results in an increase in Kynurenine level. Some microbial strains like Lactobacillus reuteri can modulate Kynurenine by downregulating IDO1.

**Gut and Hypothalamic Pituitary Adrenal (HPA) Axis:** The HPA axis is the central stress response system. Under stress corticotrophin-releasing hormone (CRH) gets released from the hypothalamus and induces ACTH (Adrenocorticotrophic hormone) release from the adrenal cortex and pituitary gland. It can lead to activation of sympathetic and parasympathetic nerves for extended periods which in turn slows down the digestive process and also alters gut barrier.

**Memory and Gut Micro Flora Link:** One major property that controls cognitive function is neuroplasticity. It is a lifelong change which involves the alteration of neural synapses, neurons, pathways, vesicular cell, and glial cells. It involves deletion of the neural connections, those are no longer necessary, to strengthen the useful memories and neural connections. Brain-derived neurotrophic factor (BDNF) contributes largely to Neuroplasticity. A type of ionotropic glutamate receptor called N-methyl-D-aspartate (NMDA) receptors found in nerve cells when binds to glutamate and glycine allow cations to flow through cell membrane potential of 0 mV.

Dysfunction of the NMDA receptor has found to be very common in various nervous system disorders, including ischemic brain injury and neurodegenerative diseases, depression, schizophrenia. Hyperactivity or hypoactivity can contribute to physiopathology of the diseases. Bifidobacterium infantis, Lactobacillus farciminis, and Lactobacillus helveticus were found helpful in reducing stress and improving cognitive health. The significantly lower amount of Bifidobacterium sp. was found in individuals with major neurogenic disorders than healthy people. Administration of probiotics like Lactobacillus acidophilus, Lactobacillus casei and Bifidobacterium bifidum help in improving insulin metabolism and decrease oxidative stress. Significantly high improvement was seen in the condition of a patient diagnosed with Alzheimer’s disease after administration of probiotics like L. acidophilus, L. casei, B. bifidum, L. fermentum. Probiotics containing L. rhamnosus and Bifidobacterium animals were found to be especially helpful for patients diagnosed with schizophrenia. Some studies suggest that ingestion of Bifidobacterium longum 1714 helps in improving memory.

**Gut Microbiota & its Link to Autism-Like Disorders:** Various studies have been done to establish the effect of gut microorganisms in diseases like Autism. Autism-like behavior was
induced in Pups when pregnant BALB/c females were to valproic acid. Afterbirth of offsprings they were exposed to the social environment for social behavior test in which they showed less social behavior. After examination of inflammatory markers in the brain and intestinal tissue, an increase in expression of markers was observed. Serotonin level in the cortex, amygdala and small intestine of male pups decreased 48.

In another experiment behavior, of germ-free (GF) mice who were colonized by bacteria after weaning was compared to mice those were conventionally colonized. As a result GF mice were showing less social involvement and novelty compared to conventional mice. But these behaviors normalized after bacterial colonization 49.

**Effect of Gut Microbiota on Amygdala and Hippocampus:** P. Luczynski et al., demonstrated how the gut microbiota could influence brain morphology. Amygdala and hippocampus are two vital regions of the brain those regulate both behavioral and physiological characteristics.

They performed a germ-free experiment with GF (germ free) and CC (collaborative cross) mouse model. GF & CC mice were divided into two groups, one for stereological study and another for the study of dendritic morphology. Results found after the study was quite unavoidable. GF those lacked gut microbiota had enlarged amygdala & hippocampus where total brain volume was the same. So, definitely enlargement of amygdala & hippocampus did not happen due to whole brain expansion. With this dendritic hypertrophy in BLA inhibitory aspiny interneurons and excitatory pyramidal neurons were seen in GF mice. 81% more axospinous synapses were detected in BLA pyramidal neurons of GF mice. The germ-free status also induced atrophy in both hippocampal pyramidal neurons and dented granule cells. These findings showed that gut microbiota is essential for proper amygdalar and hippocampal morphology 50.

**Probiotics as a Treatment of Alzheimer’s Disease:** Recent studies suggest that deposition of amyloid beta (Aβ) lies at the heart of Alzheimer’s disease. Lactobacillus is one of the widely used probiotics. Lactobacillus reuteri ELF has been reported to be able to decrease amyloid beta (Aβ) and postsynaptic density protein (PSD). In fact, it can reduce Tau protein. It is also reported that Lactobacillus reuteri can suppress the expression of the glial fibrillary acidic protein (GFAP). It can also increase COX activity in brain mitochondria significantly. By this way, it promises an effective treatment to prevent degenerative brain disease including Alzheimer’s disease 51, 52, 53, 54.

**Gut Microbiota in Prevention of Parkinson’s Disease (PD):** Another widespread neurodegenerative disease is Parkinson’s disease. It is the second most common neurodegenerative disease in the United States with an average of 1 in every 500 people. 6 million people are carrying this disease worldwide. Aggregation of protein α-synuclein (αSyn) results in motor dysfunction, which is the hallmark pathology of this disease. During the experiment under GF (germ-free) condition or when microbiota depleted with antibiotic treatment, transgenic mice expressing excess human αSyn led to a reduction in microglial activation, αSyn inclusion, and motor deficit. But these things do not happen in healthy controls. Healthy mice were inoculated with microorganisms derived from the body of a Parkinson’s patient; it resulted in motor dysfunction 55. It suggests that gut microorganisms regulate signaling pathways.

**Rise of Psychobiotics:** Psychobiotics are good bacteria when ingested in adequate amount impart good mental health and helps in brain and neuronal development. The Nobel laureate Metchnikoff, for the first time, introduced the concept of psychobiotics when he noted a fact that individuals of Bulgaria who used to consume fermented milk in large quantity had a longer life span than the rest.

Some recent studies included probiotic fibers in Psychobiotic because it works as a growth-promoting factor for gut microbiota 56. Bifidobacterium longum 1714 strain was reported to improve cognitive function & behavior. Improvement in hippocampal visuospatial memory was noted. Frontal midline electroencephalographic mobility was also enhanced 57.

In a study, pregnant women were treated with Lactobacillus rhamnosus HN001 which resulted in lower anxiety and depression in the postpartum
period than those who did not receive the above Psychobiotic 58.

Thereby, above findings open up the door for a new field of study and promises new genera of personalized medicines, where based on individual’s need each person will get their personalized combination of probiotics.

**Influence of Probiotics on Gastrointestinal Health:** Use of probiotics for the betterment of intestinal health is not new. The contribution of gut microorganisms in maintaining intestinal health has been supported by a large number of published literature. Widely studied probiotic microorganisms include *Lactobacillus* sp., *Bifidobacterium* sp., *Escherichia coli*, *Bacillus* sp., and *Streptococcus* sp. Probiotics shield us from various intestinal disorders in many ways. All the mechanisms lead to ‘Colonization Resistance’. Probiotics prevent pathogens from colonizing in our intestine.

**TABLE 4: MECHANISMS OF DISEASE PREVENTION BY GASTROINTESTINAL MICROFLORA** 59,61

<table>
<thead>
<tr>
<th>Actions</th>
<th>Mechanisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Competition for nutrients</td>
<td>Probiotics compete with pathogenic microorganisms for food and exclude them by preventing from nutrient uptake</td>
</tr>
<tr>
<td>Production of inhibitory substances</td>
<td>Probiotics produce various substances like hydrogen peroxide, organic acids, bacteriocins that eliminates live pathogens and also eliminate toxins</td>
</tr>
<tr>
<td>Degradation of receptors</td>
<td>Probiotics like <em>Saccharomyces boulardii</em> help in degrading toxin receptors on intestinal mucosa</td>
</tr>
<tr>
<td>Blocking of adhesion</td>
<td>Probiotics not only compete for the nutrients but also compete for adhesion site. So, probiotics adhere to epithelial mucosa. In this process, they exclude pathogens from the gut</td>
</tr>
<tr>
<td>Immunity boosting</td>
<td>Increase in good bacteria also boosts immunity by inducing both specific and nonspecific immunity. Example: <em>Lactobacillus</em> sp. is capable of inducing an immune response during rotavirus infection</td>
</tr>
</tbody>
</table>

Crohn’s disease and ulcerative colitis are two inflammatory bowel diseases. Crohn’s disease is caused by inflammation of the digestive tract. Inflammation can take place in different areas of the digestive tract, spanning from mouth to anus. Crohn’s disease can be genetic, if it acquired hereditarily or it can be due to an autoimmune disorder, where mistakenly immune cells detect digestive tract microflora as foreign. Non-steroid anti-inflammatory drugs like Ibuprofen and birth control pills can increase the chances of getting Crohn’s disease 60.

Ulcerative colitis is long lasting inflammation of the colon. It can be caused by bacteria like *C. difficile* or autoimmune disorder, malnutrition. *E. coli* Nissel strain (Serotype O6;K5: H1) was found to be helpful in the treatment of colitis. *Bifidobacterium* sp. was found to be especially helpful in Crohn’s disease 62.

Irritable bowel syndrome (IBS) is caused by hypersensitivity of the colon, which causes spasm, leading to chronic pain, diarrhea or constipation. In physiological studies, it was found that alteration in intestinal microbiota has a profound effect on IBS 63. A study in Poland suggests that *L. planterum* can significantly reduce abdominal pain in IBS patients 64.

*Helicobacter pylori* are the etiological agent of chronic gastroenteritis and gastric ulcer which can lead to stomach cancer. Studies showed that *Lactobacillus* plays an antagonistic role against *H. pylori* 65.

Not only was that *L. acidophilus* found helpful in hepatic encephalopathy because of their ability to reduce urease which is first and foremost reason behind hepatic encephalopathy. Ammonia gets produced in the intestine by bacterial urease, and increased level of ammonia in the blood leads to this disease 66.

**Effect of Gut Microbiota in HIV:** HIV is one of the major challenges for medical science. Worldwide 36.9 million people are HIV positive. Among them, 1.8 million are children (age <15) 67. Till today there is no complete cure for HIV. One hallmark pathology of HIV is decreasing in CD4+ count. It is because they bind to CD4+ T-lymphocytes, invade them and grow inside them. At the end of the life, cycle T-lymphocytes get ruptured, and new progeny of HIV get released.
This process is repeated until all the CD4+ T cells are destroyed. Almost all HIV infected patients encounter impaired gastrointestinal (G.I.) tract, which in turn causes microbial translocation, immune activation, and progression of the disease. Other symptoms like diarrhea and low immunity are also seen.

One good news is recent studies on the effect of probiotics in HIV infected individuals were very promising. A study with 44 HIV patients showed that when they were supplemented with *Saccharomyces boulardii* for over 4 weeks, there was a significant decrease in LPS binding protein and IL-6 those were responsible for translocation and inflammation respectively, compared to those supplemented with placebo. In another study, *Lactobacillus rhamnosus* effectively elevated G.I. tract, improved nutrient uptake and increased tolerance to ARV (Antiretroviral) treatment. Another study performed with 25 HIV positive women; among them, 18 women were experiencing serious diarrhea, flatulence, and nausea. They were supplemented with probiotics and their condition improved within 30 days of probiotic uptake. But those symptoms reappeared after 3 months of discontinuation. So, it suggests continuous use of probiotics. A meta-analysis has shown that probiotics can decrease diarrhea and fever in HIV positive children.

During a study 8 out of 12 HIV patients consumed probiotic yogurt for 15 days and there was a four-fold increase in CD4+ count. Another group of HIV positive patients receiving ARV (Antiretroviral) treatment up took probiotic in the daily diet and count of CD4+ cells increased by 62 CD4+ per year. Another study showed HIV positive women with less than 200 CD4+ cells had a profound increase in CD4+ cell with a mean of 93 cells/µl where those who received placebo had a decrease in CD4+ cells by mean of 69 cells/µl.

**Effects Probiotics in Cancer:** Cancer is considered as a second most fatal disease in the entire world with 14.1 million cases which are expected to increase up to 20 to 21 million with up to 13.2 million casualties worldwide by 2030. Cancer is the uncontrolled proliferation of cells with less sensitivity to growth factors, thus resulting in low quality of the cells. A hallmark of cancer is metastasis. Metastasis is the ability of cancer cells to detach from the primary site and develop secondary tumors on a distant tissue organ. After thorough research on different cancer cell lines probiotics were found to have antiproliferative effects on various cancer cells like myeloid leukemia cells, stomach, breast, cervix, colon cancer cells. It was reported that *Lactobacillus kefir* showed an apoptotic effect on myeloid leukemia cells. Probiotics like *L. rhamnosus* strain GG & *B. adolescentis* SPM0212 had large antiproliferative and inhibitory effects on gastric cancer cells, three colon cancer cell lines SW 480, CaCO2 and HT-29.

*Lactobacillus casei* Shiroti (LcS) was found effective for breast cancer, cervical cancer, and HPV positive intraepithelial lesion. *Enterococcus lactis* IW5 obtained from human gut found to be able to decrease viable cancer cells of different cell lines like HeLa, MCF-7, HT-29, CaCO2 and AGS. Mechanisms responsible for the anticancerous effects of probiotics are poorly known, but some reports suggest that mechanisms like enhancements of gut barrier function and immune system function, degradation of carcinogens and modulation of gut microbiota are behind these actions. From the above information, it is quite evident that probiotics are crucial in maintaining our health. It not only opens a new frontier, but also promises a better future. Thus, demands more attention and research.

**DISCUSSION:** From the first part of the review it is evident that microencapsulation by the formation of microsphere and microparticle can significantly control the release of drug or probiotic. By microencapsulation, drug release can be reduced by approximately 20-25%. It is also able to protect drug or probiotic from the harsh effect of gastric fluid and bile salt. With the increase in molecular weight of encapsulating polymer, the rate of drug release decreases. One major noticeable matter is microsphere used for both drug and probiotics, but microparticles are mainly used for drugs. One possible reason is the presence of less amount of water in microparticles than microsphere, and this low water activity can be fatal for the microbial cell.
That’s why lyophilization is detrimental to the survival of bacteria, so protection from the harsh effects of freeze-drying is most often needed. From the second part of the review, we also get a brief idea about how pivotal role gut microbiota plays in maintaining our health.

Probiotics not only contribute to gastrointestinal health, but also helps to fight various neurodegenerative diseases like Alzheimer’s disease, Parkinson’s disease and also modulates our behavior. Probiotics are also proven to be beneficial in the treatment of diseases like HIV and boost immunity.

CONCLUSION: From the above study, we can easily conclude that probiotics are and will be very effective in the treatment of many complicated diseases. That is why it has now become more important to improve administration processes of probiotics so that we can effectively introduce a less amount of probiotics and get better results. For that encapsulation is considered an excellent idea which is novel and demands more study.

Encapsulation of probiotic microorganisms or drugs increases their shelf life. It protects them from gastric juice; thus the uptake of less amount of medicine becomes more effective for the most amount of administered drug reaches to destination and gets absorbed. It is also effective in targeted drug delivery.

Microspheres appeared to encapsulate more drugs than micro-particles due to greater surface area. From the above study it is evident that increase in the concentration of the polymer increases entrapment efficiency due to increase in surface area but tends to decrease the release due to complex polymeric mesh-work and that is why micro-particles are recommended for drug encapsulation, small particles of drugs can diffuse more quickly through those mesh works than microorganisms. Micro-particles are used more than microspheres for drugs, not microorganisms also because of their less water content and less surface area. To entrap microorganisms microspheres are preferred than micro-particles. Microorganisms survive well in microspheres because they get the required moisture content to survive.

FUTURE ASPECT:
- Enhancement of the efficacy of probiotic, targeted drug delivery.
- Immune modulation.
- Treatment of diseases like intestinal colitis, IBS, neurodegenerative diseases, HIV and cancer.
- Exploration of new beneficial organisms.
- Use of microencapsulation as a gene carrier.
- Improvement of food supplement.

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