Section A-Review Paper

RECENT ADVANCEMENTS IN THE DEVELOPMENT OF PROBIOTICS AS PHARMACEUTICALS: A REVIEW

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ABSTRACT:

Probiotics are live microorganism used to improve microbial balance in gastrointestinal tract. Probiotics have a long history of traditional use. Recently, probiotic products are gaining popularity in the last two decades because of scientific evidence showing their synergistic effect on humans. The mechanism of probiotics is diverse, strain-specific, and heterogeneous, which have received the attention on scientific development and research. This review aims to discuss the needs, health benefits with a main emphasis on pharmaceutical interventions done to improve the viability of probiotics as pharmaceutical dosage forms such as capsules, enteric coated tablets and encapsulation techniques. A brief review of available patents, market capture of the probiotic and its potential in the future, the use of probiotic as immunomodulators during the COVID-19 pandemic, and clinical trials happening in the field of probiotics are also discussed.Through the pharmaceutical approach, probiotics can be delivered efficiently and the viability can be maintained throughout the products shelf life.The production of effective probiotic products will rely both on evidence of a probiotic effect and on the development of products that, at the time of consumption, shelter high numbers of viable species. To increase the efficacy of the probiotics, there is a need for pharmaceutical intervention in terms of formulation manipulations. Persistent experimental studies focused on understanding the health-promoting effects of probiotic bacteria at a molecular level be a key focusing aspect.

Keywords: Probiotics, Health benefits, Tablet, Capsule, Encapsulation, Market, Viabilityy.

1. INTRODUCTION:

"Let food be thy medicine and medicine be thy food," which was said by Hippocrates to be wholly related to today's ideology of health-responsive population (1). A sharp rise in immune-related gut problems, pathogen-resisting antibiotics, and treatment costs have made humankind realised to go towards natural health care therapy. Probiotics are gaining attention in the field of healthcare treatment (2). Probiotics are derived from the Greek word meaning "for life," which defines the host's non-living pathogen's beneficial effects (3).

The first use of probiotics was dated to 2000 BC when people started to use the fermentation process of milk to dairy products. People used fermented products based on two principles: they contain microorganisms that can fight against certain infections and are nutritive. The journey of probiotic development has been started by undocumented way through several histories from ancient history, theological history, Roman history, Turkish history, Mongolian history to the modern era. The list of contributors to probiotics is described in **figure 1** (4).

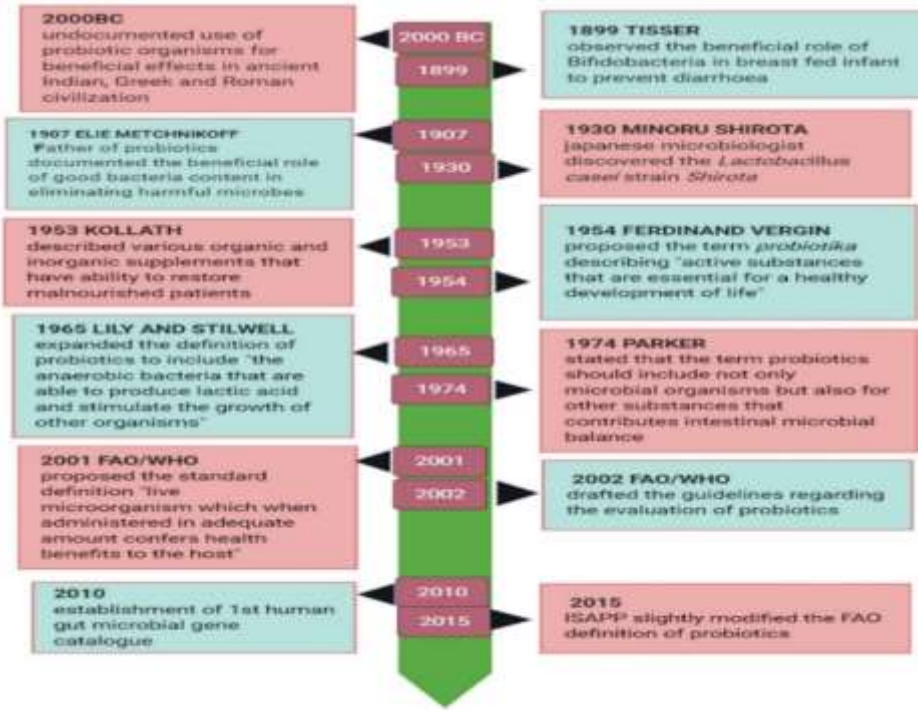

Figure History of probiotic discovery and milestones

2. PROBIOTICS:

Probiotics are products that contain a single strains or multiple strain of microorganisms(5). Generally used microorganisms as probiotics are *Lactobacillus and Bifidobacterium*. A recent study showed the benefits of *Enterococcus and Streptococcus* in probiotics. Some of the major strains used in the probiotic formulation are listed in **Table 1** (6)**.**

The other therapeautic benefits from probiotics includes irritable bowel syndrome, infections due to parasites, gut infection, hypercholesterolemia, traveller's diarrhoea, renal failure, colon and rectal cancer, dental disorders, food allergies, and ulcerative colitis. The mixture of

*lactobacilli, bifidobacteria, streptococci, E. col*i was used to maintain the antibodies induced pouchitis (an ileal pouch inflammation caused due to ulcerative colitis (7). All probiotic shows are strain-specific effect and does not endorse the concept of **cure-all**(8)**.** The crucial aspects of probiotics are that they should remain viable. The significant factors affecting the viability of probiotics in dairy products are storage temperature, pH, dissolved oxygen, hydrogen peroxide content, and even the concentration of lactic acid and acetic acid. Pharmaceutical preparations as probiotics with suitable modifications can overcome these problems by delivering them in adequate quantities (9). The pharmaceutical approaches include enteric coated tablet, polymerisation of pellets, capsules, microencapsulation and liquid orals. These dosage forms have shown good tolerability in acidic conditions, which improves delivery and increases probiotics in the intestinal gut(10). The group of microorganisms present in the human body can be said as the human microbiota. These microbiotas found in the nasal layer, mucosal tissue in larynx or throat, buccal region, and intestinal region(11). The presence of microflora in different parts of the gastro intestinal tract and their content is shown in **Figure 2.**

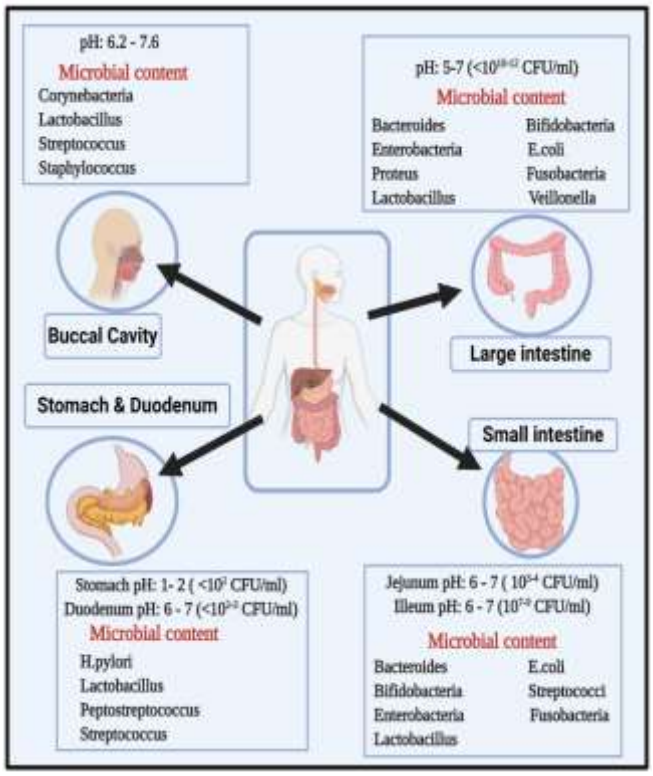

Figure 2: Microbial flora in different parts of GI

Table 1: List of Probiotic Microorganisms

PROBIOTIC MICROORGANISM		
Lactobacilli species	***Bifidobacterium* species**	**Other species**
Lactobacilli Acidophilus	*Bifidobacterium Animalis*	*Saccharomyces boulardii*
Lactobacilli Casei	*Bifidobacterium bifidum*	*Lactococcus lactis subsp. Lactis*
Lactobacilli Crispatus	*Bifidobacterium Breve*	*Enterococcus durans*

Lactobacilli Gasseri	*Bifidobacterium Lactis*	*Streptococcus Thermophilus*
Lactobacilli Johnsonii	*Bifidobacterium infants*	*Bacillus Coagulans*
Lactobacilli Reuteri	*Bifidobacterium Longum*	*E. Coli Nissle*
Lactobacilli Rhamnosus	*Bifidobacterium Adolescentis*	*Streptococcus salivarius*
Lactobacilli bulgaricus		*Saccharomyces boulardii*
Lactobacilli Sakei		

3.HEALTH BENEFITS:

Several health benefits are attained after probiotic administration, and they have been shown in **Figure 3.** The list of probiotic strains that are used for beneficial purposes is explained in **Table 2.** These probiotics provide health benefits in the small and large intestines of humans. The responses are grouped into three stages: nutrients and cofactor production, fighting against the pathogen for binding sites and stimulating the host immune system(12). Probiotics show the potential to improve the immune system and enhance the wound healing process by accumulating in the cells like macrophages, lymphocytes at the wound's site.(13)

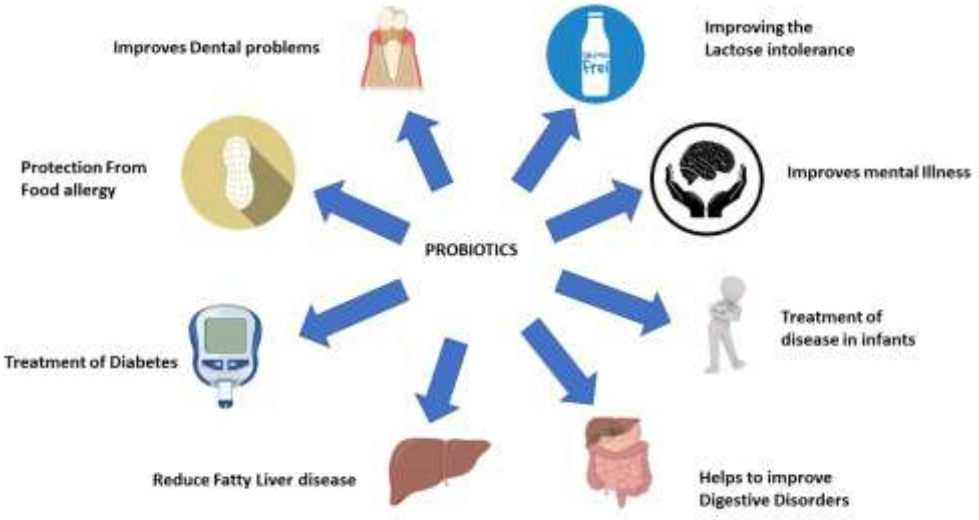

Figure 3: Health Benefits of Probiotics

Table 2: List of probiotic strains and their beneficial effects

Problems	Probiotic Strains	Beneficial Effect
Dental Caries	*Lactobacillus salivarius, Streptococcus Salivarius, Lactobacillus rhamnosus*	Preventing from tooth decay, reduces the dental plaque
Diabetes Mellitus	*Lactobacillus Rhamnosus GG*	Controlling the glycemia
Non-Alcoholic Fatty Liver	*Lactobacillus acidophilus, Bifidobacterium lactis, Bifidobacterium bifidum*	A decrease in alanine aminotransferase, Aspartate aminotransferase,

		Cholesterol, Low-density lipoprotein C
Mental Health	***Lactobacillus Helveticas, Lactobacillus Casei* DN 114001, *Bifidobacterium Longum***	**Improving cognitive functions, Stress management, Decision making**
Gastrointestinal Disorders	*Lactobacillus acidophilus*, *Lactobacillus rhamnosus GG*, *Lactobacillus delbruckii*, *Lactobacillus fermentum*, *Lactobacillus Plantarum*, Streptococcus *Boulradii*	The decrease in Antibiotic-Associated Diarrhoea, Irritable bowel syndrome, traveller's diarrhoea
Food Allergy	*Lactobacilli Murinus*	Modulating IL 12 and OX40L expression in the intestine and promoting Th1 immunity to stop the allergic response
Infant's Disease - Necrotising enterocolitis, Infantile colics	*Bifidobacterium breve* M16-V, *Bifidobacterium longum* DSMZ24709	Reducing the production of butyric acid and the incidence of death, Producing antimicrobial activity against gas-forming coliforms
Lactose Intolerance	*Bifidobacterium longum*, *Lactobacillus acidophilus*,	For metabolising the complex oligosaccharides for carbon and energy, and it also produces glycosyl hydrolases

4. MECHANISM OF ACTION:

Probiotics produce the desired therapeutics action in numerous ways in the Gastrointestinal Tract and Gut-associated lymphoid tissue by modulating the intestine's immune responses and function, enhancing the immune system's activation, resisting and exclusion of pathogen, production of IgA and production of antimicrobial substance (14). The primary mechanism of action includes an increase in adhesion towards the intestinal mucosa, stopping the pathogen's adhesion, increasing the integrity and enhancement of the epithelial barrier, bacteriocin

productions, and modulation of immune system and elimination of pathogens from the intestinal barrage(15). These mechanisms have been illustrated in **Figure 4**.

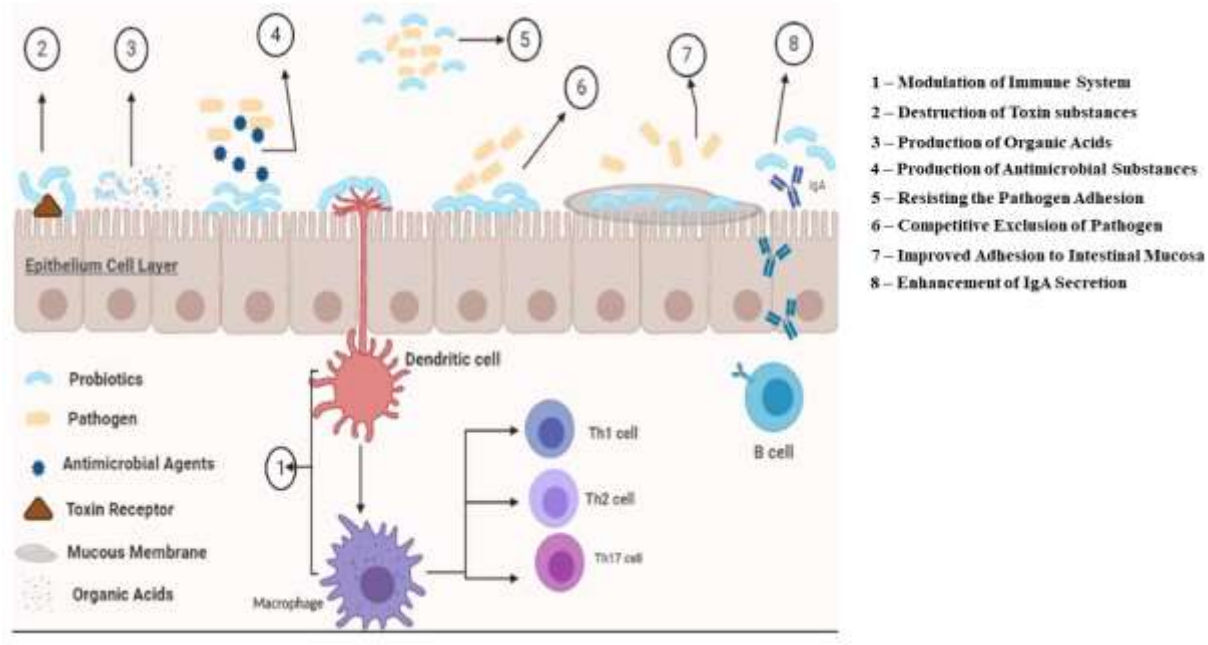

Figure 4: Mechanism of Action of Probiotics

Probiotics stimulating the immunomodulatory effect leads to cytokine production, increases mucin secretion, enhances the activity of Natural killer cells, stimulates the IgA antibody, and reduces T cell proliferation (Table 3) (16). Each action is specific to specific bacterial strainsThe primary response towards the pathogen is made by Patterns Recognition Receptors (PRRs), which attaches to Pathogen Associated Molecular Pattern (PAMPs)(17). The PRRs comprise Toll-like receptors, a transmembrane protein expressed on various cells such as dendritic cells, macrophages, natural killers, epithelial cells, and NOD-like receptors (NLRs) which guard the cytoplasmic space and transmit signals for interaction with bacteria. The list of Probiotic strains having immunomodulatory actions is explained in **Table 3**

Table 3: List of probiotic and their process in modulating the immune responses

Probiotic Strain	**Action**
Salmonella typhimurium VSL #3	Reducing the IL-8 secretion or degrading the counterregulatory Factor IkB by the process of blocking the pro-inflammatory molecules
Lactobacillus rhamnosus	Teichoic acid, a component of this probiotic strain, has anti-inflammatory activity
Lactobacillus rhamnosus GG	Promotes the growth of epithelial by inhibiting TNF-α-mediated apoptosis by activating Protein kinase B and Anti-apoptotic Factor Akt
The probiotic strain of *E. coli* and *Lactobacilli Jhonsonii*	Restoring the depleted Cd4+ and CD8+ cells, spleen and increasing the frequencies of regulatory T cells

Lactobacillus casei Shirota	Increasing the expression of CD69 marker and mucosal salivary IgA1, IFN, IgA2 concentrations

The removal of toxic substances is another mechanism of Probiotics. Botulinum Neurotoxin stereotype A (BONT/A) is a toxin, and probiotics have shown effective means to eliminate them from the body. The probiotics such as *Saccharomyces boulardii*, *Lactobacillus rhamnosus* LGG, *Lactobacillus acidophilus* and *Lactobacillus Reuters* block BONT/A by inhibiting the binding with $Caco_2$ cells in a dose-dependent manner(18).

Another mechanism of action of Probiotics is the competitive exclusion of pathogens. The competitive exclusion refers to one bacterium which competes vigorously with other bacteria for receptor sites of the intestinal tract(19). The mechanism of this exclusion is mostly still unknown. However, the proposed mechanisms create a hostile environment by adjusting the pH, eliminating other receptor sites for pathogens for binding, producing bacteriocins and antimicrobial like substances, depleting the supplement of nutrients for pathogens(20).

Probiotics produce inhibitory substances such as organic acids and bacteriocins. Lactic acid bacteria can produce antibacterial peptides and bacteriocin from gram-positive bacteria such as lactacin, plantaricin, nisin. They can be active against the foodborne pathogen. The bacteriocin mediated elimination of pathogen is by forming a pore by destroying the target cells and inhibiting the cell wall synthesis (21). *Bifidobacterium longum* BL1928 is the only characterised strain against gram-negative bacteria. The bacteriocin was categorised in several databases by structure and antimicrobial spectrum of BAGEL and BACTIBASE(22). A list of some Probiotic strains having the bacteriocin actions are listed in **Table 4.**

Table 4: Probiotic Strains and their bacteriocin actions

Probiotic Strain	Bacteriocin Action
Nisin	binds to cell wall precursors and form complex which leads to inhibition of the cell wall in spore-forming bacilli
Lactobacilli Rhamnosus	inhibits the internalisation of Enterohemorrhagic E. coli in human intestinal cell
Lactobacillus salivarius strain UCC118	Protects from foodborne pathogen Listeria monocytogenes
L. Plantarum and L. acidophilus	Inhibiting the growth of rotaviruses, multidrug-resistant Shigella spp. Helicobacter, and E. coli in some gastrointestinal conditions
Lactobacillus reuteri	It Produces antibiotic reuterin (3- Hydroxy propionaldehyde). Active against Gram-positive bacteria, fungi, protozoa, Virus, Gram-negative bacteria, yeast.

Organic acid, particularly lactic acid, acetic acid has a potent inhibitory effect on gram-negative bacteria. The intestinal *lactobacilli and bifidobacteria* strains have been shown to produce Conjugated Linoleic acids, a potent anticarcinogen(23). Probiotics can also improve butyric acid production, which will neutralise the carcinogenic effects from dietary sources such as nitrosamines. When a specific strain acting as probiotic, it should have adhesion to intestinal mucosa as a prerequisite for colonisation and interaction between the host and administered. This interaction is required to produce an antagonist action against pathogens and modulates the immune system. For preventing the pathogen from adhesion, the intestinal epithelial cells secrete mucin, which is the main component of the mucosa, and it is made up of a complex glycoprotein mixture(24). A list of probiotic strains and their mechanisms has been shown in **Table 5**.

Table 5: Probiotic strains and their process for enhancing their adhesion.

Probiotic Strain	Process
Lactobacillus reuteri	The adhesion is by mucus binding protein. This protein's role is to enhance mucus adhesion through lipid moieties; it is anchored to the cell membrane or embedded in the cell wall.
Lactobacilli Plantarum	It induces Mucin2, Mucin3, enhances the mucous layer and glycocalyx in intestinal epithelial cells, and inhibits the adhesion of enteropathogenic E. coli.
A mixture of several Probiotics strains and VSL 3	Modulating the mucin gene expression and synthesis of cell surface mucin in the intestinal epithelium.

5.SELECTION CRITERIA OF PROBIOTICS:

Various criteria should be considered for selecting the probiotics before progressing towards formulation. The selected strains should be nontoxic and non-pathogenic. The strain should be generally recognised as safe (GRAS) list according to USFDA and according to European Food and Safety Authority (EFSA) selected strain should be listed in Qualified Presumptions of Safety (QPS). Apart from that, toxicological studies and several tests should be performed, such as:

- Side effect assessments in previous human studies
- Metabolic activity assessment such as D-Lactase production, bile salt deconjunction
- Post-market surveillance of adverse incidents on consumers.

Finding a species having all these criteria is highly unlikely. Therefore, it has led to an approach on using multispecies/strain in products for betterment of the user of probiotics is divided into 4 criteria which have been illustrated in **Figure 5.**

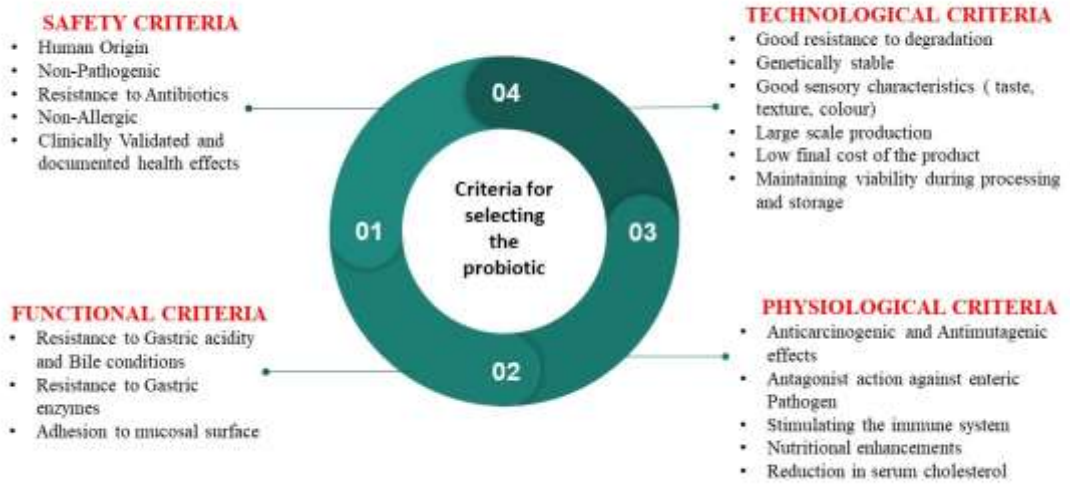

Figure 5: Formulation considerations for Probiotics

6. PROBIOTICS IN PHARMACEUTICAL DOSAGE FORM:

For oral delivery, many strategies have been developed to target the intestine because of its basic pH, reduced enzymatic activity. long transit time, The intestine-specific drug delivery system increases the bioavailability of probiotic formulations. The major oral drug delivery includes tablets, capsule, and powder dosage forms.

6.1 *TABLET*:

Tablet has several advantages over other dosage forms such as ease in large scale production and administration, good acceptance by patients, accurate dosage, minimising the penetration of acid fluid in acidic pH, has good protection of probiotic strain during delivery and can be developed to deliver to the colon. The tablet dosage form can be designed efficiently and make probiotics enhance the adhesion and colonisation to epithelial mucosal of the host with proper excipients.

Howerer, there are some factors which needed to be considered before formulating tablet dosage form for probiotics are

- Water activity level of those ingredients which comes in contact with probiotic species should be under 0.20aW. The higher water activity can able to provide good microbial growth but the improper growth in final dosage form can be considered as unstable and will face difficult to achieve desired shelf life and viability.
- These critical step that needed to be maintained during the use of non-spore probiotic species are tableting speed, flow agents, and diluents used.
- The speed of tablet press must be slow in order to reduce the friction and minimize the heat produced. An average of 15 KN force is used.

Guowei shu et al. developed a probiotic goat milk tablets with *Lactobacillus* acidophilus LA-5, *Bifidobacterium bifidum* BB01 and *Lactobacillus Plantarum* LP69 as a probiotic strain in freeze-dried form. The tablet formulation developed was stable, and the viable count was found to be 10^6 CFU/ml (Colony Forming Unit/millilitre) with good sensory characteristics.(25). Animal studies have also suggested that probiotics could have a

modulating role in neurodocrine and neurochemical responses outside the gut.In animal depression models, probiotic treatment has proved beneficial(28).Using the probiotic strain *Lactobacillus fermentum* CECT 5716, *Villena MJ et al.* have developed a gastro-resistant tablet with eudragit L-100- sodium alginate as an excipients which showed that the tablet survives gastric conditions and exerts its action in its appropriate site(26).

For protecting the probiotics from gastric acid, *Whee-soo Kim et al.* developed pH-sensitive Phthayl inulin tablets consisting of probiotic strain *Lactobacillus Reuters*, which showed adequate protection in simulated gastric fluid (SGF). Its swelling index is more in SIF than the SGF of its pH sensitivity, and the fast release was shown in intestinal conditions.(27).

For colon-specific drug delivery, *Ghosh PK et al.* evaluated the effect of probiotics in drug release in patients having disturbed human microbiota. Guar gum matrix tablet with 5–fluorouracil as a drug and *Lactobacillus acidophilus, Bifidobacterium bifidum, Lactobacillus sporogeneses* as a probiotic strain were used. The *in-vitro* data showed that 90% drug was released in the colon with probiotics when compared to the tablet with the absence of probiotics(28).

For oxygen-sensitive strain, direct compression method is suitable for tablet preparation. For probiotic strain, *Faecalibacterium prausnitzii*, *Allouche et al.* has developed a probiotic tablet through direct compression with microcrystalline cellulose, Hydroxypropyl methylcellulose, Hydroxypropyl methylcellulose phthalate as excipients. The tablet exhibited a survival of more than 10^8 CFU of the probiotic strain. *J.p. Sousa e silva et al.* has incorporated *Lactobacillus paracasei* L26 in whey protein microparticles using spray drying technique and enabling good viability for probiotic cells, which is more than 10^7CFU. These microparticles were used to make a tablet with cellulose acetate phthalate and sodium croscarmellose as an excipient, and the tablets were made through a direct compression. The tablet was able to protect the probiotic strain in acidic conditions and has maintained its stability for 60 days(29). Probiotic tablets were made through direct compression process. Thus, it should be noted that the compression force reduces the bacterial viability to one log due to the shear forces linked and an increase in temperature inside the compression die. The preconsolidated stage has improved the bacterial survival because of low shearing force and lesser temperature rise.

6.2 *ENCAPSULATION*:

Microencapsulation techniques are gaining significant interest because they incorporate viable probiotic bacteria in food and nutraceutical products. The microencapsulating materials should offer adequate protection during storage, formulation and passage through the gastrointestinal tract. The microencapsulating material should be nontoxic so that probiotic strains and the host should not be harmed by it. The encapsulation technique can be done through spray drying, emulsion techniques, and extrusion techniques

A wide range of polymers are used as an encapsulating material such as pectin, starch, cellulose, gelatin, chitosan, alginate and gum-like locust bean gum, guar gum, and their composites. Among these, chitosan and alginate are often used because chitosan dissolves in low pH, thus releasing in the intestinal region. The alginate with calcium ions as cross-linker forms a matrix, so the strain encapsulated with alginate and chitosan has shown better viability and remained stable during storage conditions(30).

6.2.1 *EXTRUSION PROCESS*:

It is one of the oldest methods and involves a two-step principle (a) the probiotic strain present in the internal phase is dispersed in small drops, then (b) solidification of drops occurs by gelation or through the formation of a membrane on the surface of drops. It involves pumping the core material and polymeric solution through a syringe (lab scale) or extruder (pilot scale) into the hardening agent. The resultant formed is hydrated hydrogel particles(31). The process of encapsulation through the extrusion process is shown in **figure 6.**

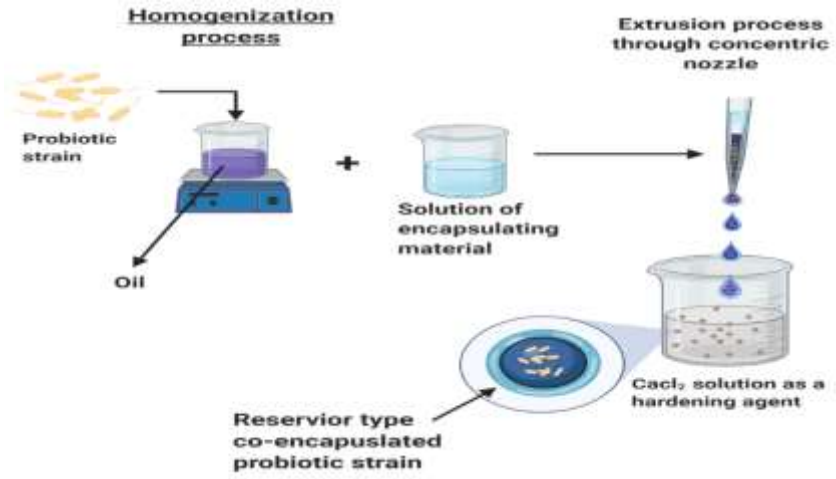

Figure 6: Process of encapsulation using an Extrusion process

Cook et al. have developed microcapsules through external gelation (extrusion technique) as an enteric delivery vehicle of *Bifidobacterium breve*. The microcapsules are made up of alginate, which is coated with chitosan polymer. Because of high charge density, these polymers allow increasing the capsules residence, which can be used for controlled release.

Seema B Chauhan et al. have developed an enteric-coated microbead for colonic delivery consisting of *Lactobacillus rhamnosus* GG as a probiotic strain. The encapsulation was done with pectin, sodium carboxymethyl cellulose, eudragit S100, cellulose acetate, and phthalate as a coating agent. The resulting formulation has shown better adhesion to the mucous layer and better release in the simulated colonic fluid.(32).

Bifidobacterium bifidum is susceptible to gastrointestinal conditions and has good storage stability. So, Tahreem Riaz et al. coated *Bifidobacterium bifidum* with sodium alginate and zein (a maise protein). Using 7%, zein has shown better-encapsulating efficiency of nearly 95%. It has a viable count greater than 10^7 Log CFU/ml and stability showed the probiotic free cells were more extraordinary significant than 10^6 Log CFU/ml after 32 days storage at 4˚C(33).

Hanady A. Albadran et al. proposed a novel approach of using surfactant as a coating agent for alginate microcapsules containing *Lactobacillus Plantarum*. Cationic and Zwitterionic surfactants have shown better cell coating in cell survival in acidic conditions and better cell release in alkaline conditions. Whereas, anionic surfactant coating has shown the worst survival of cells (34).

Chitosan coated alginate microcapsules consisting of *Lactobacillus rhamnosus* can exhibit thermal tolerance and higher freeze-drying. However, cell release remains low because of gastric susceptibility. So, *wean sin Cheow et al.* added Xanthan gum and locust bean gum to alginate capsules containing *Lactobacillus rhamnosus*, and these capsules were made through an extrusion technique. The results showed that locust bean gum alginate capsules showed better thermal tolerance, freeze-drying than chitosan-coated alginate capsules. In comparison, xanthan gum has shown better acid tolerance. The locust bean gum coated alginate capsules had shown better cell release in SIF. Because of its high swelling capacity, the speed of dissolution can also be delayed(35).
Jimenez Pranteda et al. proposed using polymer mixtures as encapsulating material for *Lactobacillus Plantarum* CRL 1815, and *Lactobacillus rhamnosus* ACT 53103 electrostatic drop generator extrusion technique is used for formulating capsules. The polymer mixtures used were xanthan gum with gellan gum(X:G) and jamilan gum with gellan gum(J:G). X:G polymer mixture has shown better protection of strains from simulated bile fluid and improved the viability of 1-2 Log CFU compared to G polymer mixture .
S.B. Doherty and the team have used gelled whey protein as an encapsulating material for encapsulating *Lactobacillus rhamnosus* GG, by extrusion technique. These encapsulated beads showed better survival rate than free cells in pH of 2.0 – 3.4, and these beads have had adequate adsorption capacity and controlled release of viable probiotic cells within 30 minutes when exposed to intestinal pH.
Silva Marluci P et al. compared the extrusion and co-extrusion technique to protect the probiotic strain *Lactobacillus acidophilus* LA3. The co-extruded beads gave 6.2 and 7.2 Log CFU/ml cell viability after 60 days of storage, whereas the extruded beads gave 5.3 and 6.2 CFU/ml cell viability.This technique is inexpensive, easy to handle, requires gentle formulation conditions for cell viability retention, and the particles formed are more than 1mm. It does not cause any cell damage. It is used less on an industrial scale because of the slow formation of microspheres.

6.2.2 *EMULSION PROCESS*:

This method involves emulsifying the core material with a polymeric solution in a base, followed by breaking the emulsion and adding the hardening solution. The beads formed are subsequently washed and filtered out to remove the residual oil content. This method is costly because of the use of surfactants and oil. The particle size obtained is smaller, which can be scaled up. It has a better size distribution. The process of encapsulation is shown in **Figure 7.**

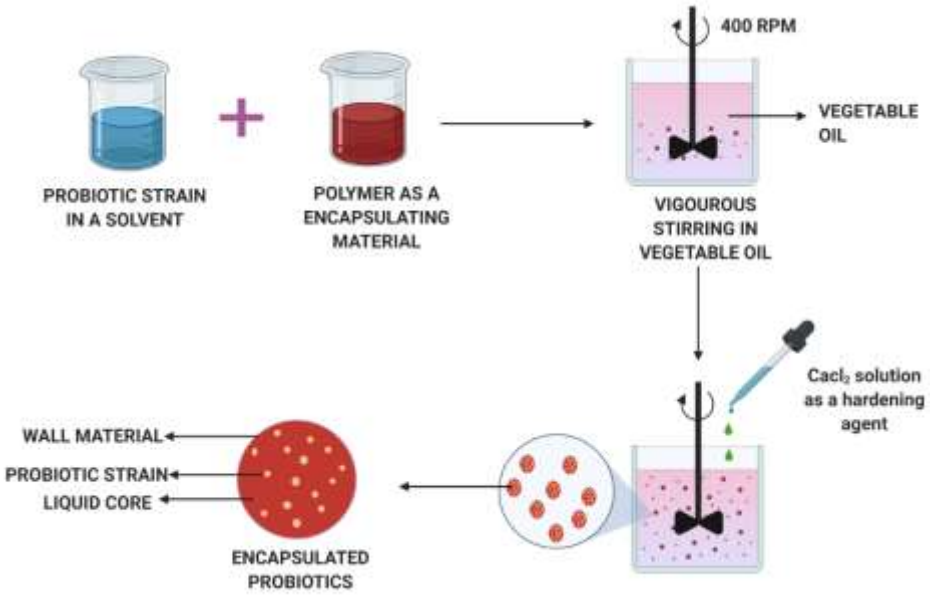

Figure 7: Encapsulation process through emulsion technique

Philippe E Ramos et al. developed a new system to immobilise the folate producing probiotic *Lactococcus lactis ssp. Cremoris*. The strain was encapsulated using Alginate Poly-L-lysine Alginate Chitosan Microcapsules (APACM). The encapsulated strain produced 95.25±26 µg/L of folate. The APACM has shown its ability to protect the strain against harsh conditions, and the probiotic strain showed the viability of 6 Log CFU/ml(36).

Bifidobacteria species were having a significant beneficial impact in health but faces difficulty of survivability in gastric fluid, and it is sensitive to high temperature. *Rui Ji et al.* came up with encapsulation of B*ifidobacteria longum* with sodium alginate as wall material and chitosan as a coating material. At 25ºC, sodium alginate and chitosan as encapsulating material showed a decrease of 1.44 Log CFU, whereas free cells showed a decrease of 3.143 Log CFU(37).

Lactoprotein, a natural material possessing a high nutritional value, can be used as a wall material to encapsulate the probiotic strain and later used in the food industry. *Lili Ma et al.* used lactoprotein as an encapsulating material using the emulsion technique. The microcapsules had an entrapment efficiency of 86.59%. The microcapsules on exposure to SGF for 90 mins showed a survival rate of 53.3%, and it was better than free cells (38).

Georgia Frakolaki et al., have developed a novel double emulsion technique in which double emulsion encapsulation is done before the extrusion technique for better stability and protection of *Bifidobacterium animalis subsp. Lactis*. The double emulsion technique had shown the viability of 6 Log CFU/gram during the storage of 4 ºC and -18 ºC for a time of 4 weeks. The formulation has shown 86% of release characteristics, and cell survivability was 98%(39).

6.2.3 *SPRAY DRYING PROCESS:*

Spray drying is an encapsulating technique was used when a probiotic is encapsulated by forming an emulsion or suspension. It involves atomisation of probiotic suspensions or emulsion with the carrier material into a drying gas, resulting in evaporation of water. The

final product obtained is in dry powder form. The solvents generally used are gelatin, vegetable gum, dextrin, and non-gelling protein. The main advantage of spray drying is, it is a highly reproducible, low cost, and suitable for industrial applications. There is still some disadvantage with significant advantages because of the use of high temperature, which is not compatible with the survival of probiotic microorganisms. For probiotic survival, protectants can be added to emulsion or suspensions before proceeding to the drying stage(40).

As probiotic faces several challenges in liquid preparation such as pH, oxygen, water activity and environmental stress. Therefore, a powdered probiotic can be a suitable alternative to overcome these challenges. In industry, probiotic powders can be done either by spray drying or freeze-drying. *Anyi wang et al*. developed a novel method of formulating probiotic powder of *Lactobacillus salivarius* NRRL B-30514 suspension with spray-dried lactose ration of 1:15, 1:25 and it had shown greater viability with a small reduction of 0.49 and 0.56 Log CFU/ml for 180 days storage at 4ºC. It can be useful in the development of probiotic functional foods(41).

Probiotic as a dry powder form can be easily handled and stored, which later can be used in functional foods. *Divyasree Arepally et al*. studied *Lactobacillus acidophilus* encapsulated using spray drying technique to form probiotic powder containing 20% of maltodextrin with varied concentrations of guar gum. The processing condition was having an inlet temperature of 130-155ºC. The encapsulated probiotic powder upon exposure to SGF, the viability report shows that H1 free cells were reduced to 1.43 Log CFU/ml whereas encapsulated showing 5.6 Log CFU/ml. At pH 2, the encapsulated probiotic powder showed a 6.46 Log CFU/ml value, which protects from an acidic environment (42).

6.3 *CAPSULES*:

An enteric-coated tablet can effectively deliver probiotics, but the effects can be reduced if tampering happens, like often patients tend to break the tablet or crush the tablet to make it easier to swallow(43). This problem can be overcome if probiotic can be delivered through the banded capsule. Banded capsules are the one which is tamper-free and provides an airtight seal which prevents leaking until the desired pH is reached.

Capsules allows the product showing higher potency and longer stability whereas the powder can have higher potencies. There are certain factors needed to be considered before formulating capsules which can lead to manage shelf life effectively

- Most stable raw should be used.
- The facility where products are being manufactured should have humidity less than 30%.
- Use of low water activity excipients.
- Use of glass packaging material or high density poly ethylene material.

Capsules with a high level of pea protein and a small amount of alginate calcium have proposed to create a less porous matrix for protection of probiotics and provide a novel release mechanisms via proteolysis in the lower GI tract. thus, *Karla J. Klemmer et al*. have used this capsule to entrap *Bifidobacterium adolescentis* for targeted release in intestinal pH. The results showed that, in SGF, protein concentration underwent shrinks, but viability was not reduced. The *Bifidobacterium adolescentis* showed burst release first and then prolonged

release in SIF. This study shows that plant-based material could play a major role in the pharmaceutical industries and food industries(44).

Recently Hypromellose, which is vegetarian-based capsules has been available commercially. It has become a choice of capsule shell because of low relative humidity and maintaining their mechanical stability with changing temperature. The water content is 2.5 times lesser than gelatin capsules. This assures the best choice for using a capsule shell for better probiotics stability and reduces the risk of brittling, which happens in a hard gelatin capsule. Several commercially available Hypromellose capsule brands are Vcaps®, Vcaps®plus and DRcaps™. Among which, DRcaps™ has better resistant to gastric conditions at pH 1.2 . (45).

7.THE MARKET OF PROBIOTICS:

The global probiotic market value is around 46.20 Billion US Dollars, and it is estimated to reach around 76 Billion US Dollars at Compound Annual Growth Rate (CAGR) of about 7.4% during 2021-2026. Global companies have come up with collaborations and novel technologies to deliver probiotics in dietary supplements.The USA's legislation allows probiotic supplements under the Dietary supplement Health and Education Act of 1994.

Asian- Pacific (APAC) probiotic market size is estimated to grow 9.5% CGAR during the period of 2020-2025. China is still holding Asia's biggest market, and the probiotic market is predicted to grow 25 billion US Dollar by 2025. With India and south-east Asia being emerging hotspots.Indian probiotic market size will reach 961.865 million US Dollar in 2025. Probi, a biotechnology research company collaborates with USV India leading pharmaceutical company and will launch Vibact IBS based on the probiotic formulation of Probi Digestis.List of marketed probiotic products and their details are listed in **Table 6.** European probiotic market had a value of 1654.3 Million US Dollars in 2019. By 2024, the CGAR will be increased to 4% with Italy, Germany, and France having a share of 45.5%(46)

Table 6: List of Marketed Probiotic products in India

DOSAGE FORM	BRAND	STRAIN
Lyophilised Powder	Lactivest™	*Saccharomyces boulardii*
Powder	Econorm	*Saccharomyces Boulardii*
Capsule	Eubioz	*Lactobacillus Acidophilus, Bifidus, Lactobacillus Casei, Lactobacillus rhamnosus, Saccharomyces Boulardi, Strptococcus thermophilus*
Capsule	Evebact	*Lactobacillus Crispatus, Lactobacillus Rhamnosus, Lactobacillus Gasseri, Lactobacillus Jensenii*
Capsule	Actigut	*Bifidobacterium bifidum, Lactobacillus rhamnosus, Bifidobacterium longum, Saccharomyces boulardii, Saccharomyces thermophilus, Lactobacillus acidophilus.*

Capsules	Vizylac Rich	*Streptococcus faecalis, clostridium butyricum, Bacillus mesentericus, Lactobacillus sporogenes, Saccharomyces boulardii*
Capsule	Flora sante	*Bacillus subtilis, Bacillus coagulans, Streptococcus thermophilus*
Tablet	Carbamide Forte	*Lactobacillus Acidophilus, Saccharomyces Boulardii* *Lactobacillus Rhamnosus, Bifidobacterium Longum,*

8.PATENT INFORMATION:

Companies spend a remarkable amount of time, energy, money, resources to convert a new idea into a marketable product. The field of technology and innovation, showing rapid advancements. Thus, Intellectual Property Rights (IPR) has become fundamental for corporate success and the future growth of a company. When a Company's core idea is protected by IPR, it increases the company's run in the market for the long term. In the field of probiotics, the patents had not shown any noticeable rise before the year 2000. However, the market saw the transformation in favour of functional foods. So, the patents filed on probiotics had been improved in the last two decades. Earlier, the probiotics were focussed only on health benefits, use, safety and its composition, but the increased interest in the generation of IPR by academia or industry has resulted in some novel technologies in the field of probiotics(47). Significant patents related to probiotics are mentioned in **Table 7**.

Table 7: List of some patent on probiotics

PATENT ID	DESCRIPTION	PROPRIETOR NAME
CN101579038A	The invention reveals a method for the production of aquatic microcapsule feed containing probiotics intended to resolve the probability of survival of probiotics in high temperature and high humidity environments and to introduce probiotics to aquatic microcapsule feed for the production of aquatic microcapsule feed suitable for shrimp, crab, young fish	ZHEJIANG HENGXING FEEDSTUFF CO., LTD.
CN103893100A	The invention provides a multi-probiotic moringa oleifera mask composed of the following components as a percentage by weight: 60% of yeast powder, 20% of multiple probiotics, 10% of moringa oleifera plant extract, 5% of natural honey and 5% of fresh milk.	LIU FULI
AU2007245002A1	Dietary supplements containing at least one probiotic, Vitamin E&C, carotene, zinc, magnesium and copper proteinate, at least one animal digest. The supplement contains probiotics and other ingredients in adequate amounts to improve the probiotics' palatability and the formulation comprising the probiotics, improve the immune	NESTEC SA

system to enhance the beneficial effects of the probiotics, or extend the life of the probiotics.

BIO-tract® is a patented controlled drug delivery technology for probiotics, that can deliver the Lactobacillus in the upper part of small intestine and Bifidobacteria in the smaller amount of the lower intestine.

Cryotabletting™ technique patented by Nutraceutix, with this technique tablet cooled to preserve microorganisms after production, and this does not require refrigeration after storage.

STAR™ (Stomach Acid Resistance) technology developed by Institut Rosell-Lallemand. STAR™ consists of a natural protective water-based layer around a capsule and ensure ten-fold improvement in probiotic survival(48).

9.CLINICAL TRIALS OF PROBIOTICS:

Preclinical studies are performed to assess the drug safety profile and not a substitute for studies, the way the drug interact in the body. The clinical research majorly focuses on safety, efficacy, dosage, side effects, and monitoring adverse reactions.The components which should be considered during the phase design of a study are (a) targeting the interested populations (b) control groups (c) safety and efficacy outcomes (d) size and type of survey for addressing hypothesis and providing evidence to the target population. Validated animal models do not exist for the safety assessment of probiotics. As a result, for determining safety assessment, human studies are primarily done. Guidelines are available from the Organisation for Economic Co-operation (OECD). The Agency of Healthcare Research and Quality (AHRQ) with Office of Dietary Supplements (ODS), Food and Drug Administration (FDA), and National Center for Complementary and Alternative Medicine (NAACM) has bespoken for evidence reports on the safety of probiotics on human health. The report includes an overall examination of the human body which should act as evidence for a key research question on probiotic safety.Here, some clinical trial on using probiotics has been enlisted in **Table 8**.

TABLE 8: Clinical trial Data on Probiotics

REGISTRATION NUMBER	TYPE OF TRIAL	STUDY PROTOCOL	PHASE TRIAL	COUNTRY
CTRI/2008/091/000049	Interventional	Effect of probiotics to prevent sepsis in low body weight infants	Phase 2/Phase 3	India
CTRI/2019/04/018846	Interventional	Effect of probiotic in Parkinson's disease	Phase 4	India
CTRI/2018/08/015282	Interventional	Effect of Bacillus clausii suspension for treatment of upper respiratory infection	Phase 2	India
CTRI/2017/11/010539	Interventional	Effect of bacillus coagulans unique IS2 in adults with	Phase 3/Phase 4	India

		constipation		
CTRI/2017/06/008725	Interventional	Role of probiotic in glycemic control in children with type 1 diabetes	Phase 3	India
NCT01224132	Interventional	Effect of probiotics in atopic dermatitis	Phase 3	Turkey
NCT02520583	Interventional	Effect of probiotic supplements on muscle damage	Phase 2	Texas
NCT03967301	Interventional	Role of probiotics on preventing and decolonisation of multidrug-resistant bacteria	NA	Argentina
NCT03511365	Interventional	Effect of probiotics on inflammation and microbiota in NASH patient	Phase 1/Phase 2	New York

10.IMPACT OF PROBIOTICS AGAINST COVID-19:

On January 30, 2020, WHO (World Health Organization) has alarmed the PHEIC (Public Health Emergency of International Concern) because of novel coronavirus which is termed SARS- CoV -2, causes the acute respiratory syndrome. The virus disrupts epithelium gas exchange area. The epithelial cells serve Angiotensin-converting enzyme 2 receptor for SARS and digestion related enzyme in human enterocytes. Several possible treatments for coronavirus are antiviral drugs, vaccination or taking medication with probiotics such as bifidobacteria and lactobacillus which provide a significant chance of recovery. Consumption of probiotics can improve type 1 interferon, Natural Killer cells and T & B cells in lungs immune system and also improves the anti-inflammatory cytokines which help in reducing the cell damage in the lungs caused by the virus(49,50).

Baud et al. has listed some probiotic species which can reduce the burden caused due to COVID-19 such as *Lactobacillus gasseri, Lactobacillus rhamnosus, Bifidobacterium bifidum, Lactobacillus plantarum* and these probiotic strains were added in some products such as Shirota, Tribion harmonis, and Mediform. In one study it has been shown that people with COVID-19 has suffered severe hypoxemia and changes in the balance of gut microbiome which causes an increase in the pathogen. So, this finding indicates that the administration of probiotics will be able to bring the balance of gut microbiome which can improve the immune functions to fight against the COVID-19 (51,52).

11.FUTURE PERSPECTIVE:

Probiotics had a tremendously beneficial impact on improving the human immune system and their digestive health(53). Current technologies and methodology offer great possibilities for research and application of probiotics and prebiotics. Earlier scientists used the beneficial

effect of probiotics to reduce the risk and the impact of diseases and remove drug toxic substances. Hence, the future trend leads to the use of probiotics as a substitute for antibiotics, as antibiotic resistance problem is increasing day by day(54). The field of probiotics is growing broadly and many new terms and definition have been proposed (**Table 9**) which can be used in future research for better development of probiotic products(55).

Table 9: List of New terms an definition in the field of probiotics

SL. NO	TERM	DEFINITION
1	Next Generation probiotics	A beneficial microorganism is identified based on microbial assay and given to specific disease to confer its beneficial effect
2	Pharmabiotics	Microorganism with proven pharmacological action
3	Paraprobiotic/Ghost probiotics	A non-viable microorganism which on administration produces beneficial effects in animals or humans
4	Biogenic	Products from life forms which includes metabolites

12.CONCLUSION:

The entry of probiotics in pharmaceutical industries is evident that probiotic formulations can be available as over the counter products. The proper pharmacokinetic model approach should be developed if probiotic needs to be considered as a modern therapeutic drug. For using probiotics as an alternative for antibiotics, active involvement of medical practitioner is required because physicians are still sceptical about the use of probiotics as drugs. The market of probiotics in a pharmaceutical approach is still unclear. More regulations and clinical evaluation studies are needed to be performed on the final product to check its viability. Even though probiotics are performing well in nutraceutical formulation, still maintaining the viability remains a big challenge. Therefore, the pharmaceutical formulation can able to overcome these issues. There is a large scope in the probiotic market because of the prevailing health conditions and consciousness among the people for better living.

ABBREVIATION:

SGF - Simulated Gastric Fluid
SIF - Simulated Intestinal Fluid
CFU - Colony Forming Unit
CAGR - Compound Annual Growth Rate

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ETHICAL CLEARANCE:

No animals or humans were used in the present study. Therefore, ethical clearance does not apply to this manuscript.

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