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SOURCE, ISOLATION, PRODUCTION AND FUTURE DEVELOPMENT OF *LACTO BACILLIUS* AND *BIFIDOBACTERIAL* PROBIOTIC STRAINS

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ABSTRACT: Probiotics are active microbes found in fermented milk products that support the host's health when taken in sufficient amounts. Probiotics are typically made from strains such as *Lactobacilli* and *Bifidobacteria* isolated from the human intestine, breast milk, and conventional fermented milk products. The choice of the optimal probiotic microorganisms is a crucial initial step. Probiotics are utilized to treat allergies, intestinal, liver and numerous metabolic illnesses, and acute and antibiotic-associated diarrhea, which they prevent and treat clinically. Probiotics have the power to control intestinal permeability, maintain a healthy balance of the host's microbiota, enhance the gut immune system functioning and balances the activation of pro- and anti-inflammatory cytokines. Providing consumers with probiotic formulations that provide the required dose at the end of their shelf life is a major challenge for pharmaceutical companies. Probiotic *Lactic acid bacteria* and *Bifidobacteria* have historically been added to fermented dairy products, which are refrigerated and have a short shelf life. Probiotics can now be added to a variety of 'dry' food matrices, such as dietary supplements, and are expected to be stable for up to two years at a specified temperature and humidity. Molecular characterization and strain improvement, advanced *in-vivo*, *in-silico*, and *in-vitro* methods aimed at revealing the effects of probiotics on targets and identifying key molecules that benefit host mediation.

INTRODUCTION: Millions of microbes, collectively called human microbes, inhabit the human body. In the human body, these bacteria develop more intricate, tissue-specific and adaptive ecosystems that impact the host's physiology. The physiology of both humans and animals is significantly influenced by microbes, which aid in the development of digestion, the treatment of gastric disorders, and the creation of vitamins [1, 2, 3, 4, 5].

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For a long time, fermented foods have mostly used *Lactobacillus*-like species as natural bio preservatives, demonstrating the favorable effects of some live bacteria, which are classified as Probiotics, have a good impact on the host's health when given in sufficient doses [6]. Better clinical health outcomes result from research on producing probiotics and associated bacteria.

Numerous clinical studies show the benefits of strain-specific probiotics for various health issues, such as respiratory infections, diarrheal bowel movements, maintaining body weight, and bone mineralization. The industries create large amounts of probiotic cultures in a stable form in response to the rising demand for probiotics and the introduction of probiotics in new food markets.

The easiest form to store, handle and transport for adding probiotic cultures to functional meals is dried concentrated forms, especially for shelf-stable functional goods. Many challenges arise in producing probiotic cultures, and it's stable maintenance [7].

Sources of Probiotics: Probiotics can be found in large quantities in milk and dairy-related products [8]. *Lactobacillus* species (LAB), *Bifidobacterium*, and other bacteria found in fermented milks have been used as probiotics for a long time. These conventional fermented milks contain combinations of probiotic-rich *Lactobacillus* species. In a recent study, 148 *Lactobacillus* bacteria were discovered in Kurut, a traditional Chinese product made from naturally fermented yak milk. Additionally, Lactobacillus and Yeast probiotic strains have been found in kefir grains, Maasainamed milk and a particular type of fermented milk beverage called Koumiss; these microbes can affect immunological responses [9]. Cheese, for instance, is a dairy product that can introduce probiotic microorganisms into the human colon. Breast milk is not always sterile, despite being collected in an aseptic environment that contains a naturally occurring bacterial inoculum, according to study studies [10]. Skin fecal contamination has traditionally been a source of bacteria in breast milk. The Lactobacillus strains found in breast milk have also been found in newborns' faeces, indicating that they are genetically distinct from other germs isolated from the skin [11].

An intriguing source of probiotic *Lactobacilli* and *Bifidobacterium* for use in infant formulae and meals recommended for newborns is breast milk. Since breastfed newborns are less likely than formula-fed infants to develop allergies and gastrointestinal illnesses, the intestinal microbiota of breastfed infants is thought to be in perfect health. Several types of bacteria, including *Micrococci*, *Staphylococci*, *Streptococci*, *Lactococci*, *Lactobacilli*, *Bifidobacterium*, and *Enterococci*are commonly found in human breast milk. Two *Lactobacillus* strains derived from human breast milk improved innate and acquired immune responses by stimulating the production of regulatory T cells and natural killer and T-cell lymphocytes [12].

Selection of Probiotic Strains for Production: Specific requirements should exist for a strain to be categorised as a probiotic. The strain should ideally be GRAS-certified (generally recognized as safe). It should be non-pathogenic, resistant to gastric acids, bile juices, and gastric enzymes, and have a long history of safe use in food products and dietary supplements [13]. Probiotics are defined as living organisms administered to a host in sufficient quantities to impact health positively. For a product to be popularized, the proper probiotic strain must be chosen carefully, and generally speaking; probiotic microorganisms must still be viable in the product even at the end of its expiry. Processing and subsequent ingestion provide certain difficulties. For instance, the bacteria are subjected to various challenges, like osmotic, cold, heat and drying stress. The genera *Lactobacillus* and *Bifidobacterium*, as well as *Pediococcus*, *Propionibacterium*, *Enterococcus*, *Bacillus*, *Streptococcus* and *Saccharomyces*, to a lesser extent, are connected to probiotic strains. Different criteria exist for choosing the right strain for meals [14].

1. Probiotics activity in the GI tract.
2. Industrial production of Probiotics.
3. Microorganisms safety.
4. Health benefits of probiotics.

Probiotic Activities Ingastrointestinal Tract: After being consumed in the stomach, the probiotics move into the small intestine. After eating, the pH of the stomach ranges from 1.5 to 6.0. Proteolytic activity of pepsin, bile acids, pancreatin in the small intestine, and gastric acid exposure during the movement through the gastrointestinal tract (git), cause viability losses to probiotic strains due to reduced bacterial cytoplasmic pH [15]. *In-vitro* techniques have been established to help choose the strains that can tolerate the harsh conditions of the stomach. Although there is disagreement on the pH range that should be considered when selecting a potential probiotic, values between 1 and 5 have been extensively studied. HCL is an alternative to phosphate buffer.

When challenged with broth, *Acidophilus* evaluated at pH 3-7 showed greater acid tolerance by having optimal growth at 6 [16]. Similarly, when given milk, *Lactic acid bacteria* (LAB) lived longer in the stomach at low pH levels than when given buffered saline *in-vivo*. By choosing *Bifidobacterium* that can withstand acid by subjecting human faeces to low pH for an extended period [17]. The isolated pH-tolerant bacteria were exposed to high sodium chloride and bile salt concentrations. Promising probiotics could be isolated relatively successfully using this method of selection. Others looked over L. thirteen spore-forming bacilli from the *Bacillus lavolacticus, Sporolactobacillus, Bacillus coagulans* and *Bacillus racemilacticus;* species were tested for resistance to acidic environments and bile in another study. Strains were examined for probiotic qualities such as acid (HCL) and bile tolerance, adherence to epithelial cells, antibacterial activities, and cholesterol-lowering, and a wide range of diversity was found.

At pH 5, only five bacteria were able to grow in MRS and all five were *Bacillus coagulans* and racemilacticus, which all maintained bile levels above 0.3(%w/v). *Propionibacterium* is more frequently used in functional food. Transit tolerance tests *in-vitro* show that these bacteria had strain-specific acid resistance (pH 2–4). Analyzed *Propionibacterium* all made it *via* little gut simulations. Bile salt concentrations between 0.15 and 0.3(%w/v) have been suggested for replicating the small intestine. This is related to the GIT concentration in the body.

One out of 35 *Bifidobacterium* breve strains examined, including B., failed first acid tolerance testing in modified MRS (pH 3). *B. infantis, B. Longum, B. bifidum, B. adolescentis, B. animalis, B. Breve*, and species of *Pseudocatenulatum* showed improved adhesion properties. They demonstrated enhanced survival capacity when exposed to 0.5(%w/v) pepsin and 1(%w/v) pancreatic [18]. In addition, adhesion to epithelial cells is considered a desirable probiotic property to resist stresses encountered in the stomach and small intestine. Several studies have shown that probiotics adhere to epithelial cells in *in-vitro*, most using HT-29, HT-29MTX, Caco-2, and Int-07 cell lines. The morphological and physiological characteristics of typical human intestinal cells are demonstrated by the HT-29, Caco-2 and Int-407 cell lines [19, 20]. An HT-29 variation that produces mucus is called HT-29MTX [21]. These cell lines were cultivated to research the adherence to probiotics and enteropathogens. Although colonization of the gut *in-vivo* has not been demonstrated for all probiotics, adherence is considered an important probiotic property. It has been manifested that *Lactobacillus rhamnosus GG* can stay in the human GIT for up to a week after treatment is discontinued [22, 23]. It has been proven that some probiotics can inhibit pathogen adherence to epithelial cells without being active. Using competitive exclusion, heat-killed *Lactobacillus* acidophilus, for instance, lessens the adhesion of diarrheagenic *Escherichia coli* to Caco-2 cells *in-vitro*. Probiotics, including *Lactobacilli* and *Bifidobacterium*, have been shown in numerous studies to be able to prevent or reduce pathogen adherence [19, 24].

Two other ways for choosing probiotics are screening for antibacterial activity and the capacity to elicit the host immune response. In studies on animals, probiotics were found to boost the immune system. Although these assays are highly useful, using them as a screening tool requires much effort and money. On the other hand, a more practical selection technique is antimicrobial activity selection utilizing traditional microbiological procedures against a particular disease. The relatively affordable cost of genome sequencing has made studying the genomic functioning of a variety of probiotics possible, providing insight into the genetic basis for probiotic properties like adherence, environmental adaptation, and the manufacture of antimicrobial compounds. These developments might lead to creative methods for genome-level probiotic screening [15, 25, 26].

Isolation: Before incubating in suitable media, the probiotic sample must be maintained in suitable conditions as the initial stage in separating probiotic microorganisms. Since many probiotic bacteria are facultative or anaerobic, the samples were cultured anaerobically and processed as quickly as possible, *i.e.*, within three hours. The samples must be promptly homogenized, diluted, and sub-cultured in particular media [27]. A selective medium for the isolation of oral and fecal

Lactobacilli and *Bifidobacterium* that has a Columbia agar base enriched with propionic acid was developed from several media for the elective or selective isolation of *Bifidobacteria* and *Lactobacilli* [28, 29]. The *Lactobacilli* and *Bifidobacteria*-tolerated lower pH of this medium limits the growth of other dominant organisms found in human excrement, such as the *Bacteroides* genus and eubacteria species. The probiotic strains were cultured on plates for 48–72 hours at 37°C in an anaerobic environment to grow *Bifidobacteria* and other anaerobic species or in a CO_2-rich atmosphere for the growth of *Lactobacilli*. After that, the colonies are separated and moved to fresh new agar plates or broth.

***Bifidobacteria*:** *Bifidobacterium* species are not as fastidious as *Lactobacilli* species, lack oxygen, and have significant nutritional requirements in the media. Several vitamins, nucleotides, and some minerals are needed for *Bifidobacterium* species to grow in semi-synthetic media, which solely contains lactose as a carbon source and free amino acids like glycine, cysteine, and tryptophan. The media used by *Bifidobacterium* contain nutritive growth additives, have minimal oxidoreduction, at pH maintained between 5 and 8, with a pH between 6 and 7 being ideal, and grow at a temperature between 37 and 41 degrees Celsius with growth not occurring below 25 or 45 above [30, 31].

Lactobacillus nutritive media, which are often employed for the growth of *Bifidobacterium*, make up the majority of the nutrition medium used for the growth of *Bifidobacteria*. The yield and growth performance of *Bifidobacteria* can be improved by adding specific growth factors to the growth medium in addition to specific antibiotics that are selective for *Bifidobacteria*. There is more than one ideal medium for *Bifidobacteria*, and the ideal medium should be developed for each strain. There are many documented methods for medium optimization for *Bifidobacteria*. It lists a few typical bifidogenic nutrient supplements used to encourage *Bifidobacteria* growth. The media should ideally have a lower redox potential so that reducing agents like ascorbic acid or cysteine-hydrochloride can be utilised. When coupled with cysteine, b-glycerophosphate promotes the growth of *B. infantis* and *B. bifidum* in milk but does not affect *B. longum*. Milk products with growth-promoting properties include casein and whey. Casein hydrolysates aided *B. infantis, B. breve* and *B. longum* in growing. While Threonine, Peptone, Trypticase, Dextrin, Maltose and Short-Chain Fatty Acids had a higher growth effect on Bifidobacteria, extracts from other sources of beet like (potatoes, carrots) and corn had the opposite effect. Yeast extracts, which are typically added between 0.1 and 0.5 (% v/v), have been demonstrated to be efficient growth promoters. When 0.25 (%v/v) yeast extract was added to the medium, *B. infantis* did not grow more, but more acid was generated [15].

***Lactobacillus*:** *Lactobacillus* needs complex nutrition media. Low oxygen pressure, proteins, nucleic acid derivatives, polyunsaturated fatty acids, vitamins, and minerals (magnesium, manganese and iron) are needed in a medium for *L. acidophilus*. The *Lactobacilli* only like amino acids that are peptide-bound [32]. Using whey boosted the development of *Lactobacilli,* and added peptone and trypsin promoted acid generation by increasing the Sulphur groups in the production environment [33]. Low pH during fermentation can gradually impede growth, and to prevent this growth-impairing acid generation, a buffer like a phosphate buffer with an adjusted pH can be employed. Large phosphate buffer concentrations can act as inhibitors due to the binding of metal ions (magnesium, calcium, and manganese), which play a crucial role in bacterial growth, so a minimal amount can be added to the media [34].

Therefore, the phosphate content must be carefully adjusted for the strain. The ideal conditions were discovered to be pH 6, 30°C, the media composition of 40(g/l) glucose, 20 (g/l) peptone, 20 (g/l) yeast extract, 5 (g/l) sodium acetate, and 3 (g/l) sodium citrate. This was done using an experimental design to determine the best-growing media for *L. acidophilus*. The combined effects of glucose (carbon source), yeast extract (nitrogen source), vitamins, and pH on the growth of *L. rhamnosus ATCC7469* were studied using the response surface methodology. pH of 6.9–7, 1.3(%v/v) vitamins, 5(%w/v) glucose and 6(%w/v) yeast extract were the ideal circumstances.

It has been demonstrated that increasing the inoculum size and adding prebiotics like fructooligosaccharide and Maltodextrin boosts

Lacto bacillus species ability to produce acid [35]. Most of the nutrients needed for *Lactobacilli* to develop are supplanted by milk. *According to research, Lactobacilli can develop up to 108–109 (CFU/ml) when cultivated in milk*. There are numerous strategies to enhance the growth of *Lactobacilli* in milk, such as adding growth agents to the milk or increasing the inoculum size if more lactobacilli are needed after a particular amount of time. Supplements that have been reported to encourage lactobacilli growth include manganese, acetate, fatty acids (like oleic acid), tomato juice, casein powder, whey protein, and simple fermentable carbohydrates (like sucrose, fructose). By adding 0.5(%w/v) yeast extract and 1.1(%w/v) glucose to skim milk media, *L. acidophilus* growth was improved. Maltose, salicilin, raffinose or melibiose supplements can be utilized to enhance the basic MRS media used to produce *L. acidophilus* from yoghurt instead of dextrose [36, 37]. Although *Lactobacilli* can tolerate oxygen, low oxygen concentrations are best for their growth. Whey protein concentrates, acid casein hydrolysates and ascorbic acid supplements all helped *L. acidophilus* and *Bifidobacterium* grow better in yoghurt. Ascorbic acid was also utilised as an oxygen scavenger, promoting the growth and stability of *L. acidophilus* [15].

Basic Steps Involved in Manufacturing of Probiotics: *Bifidobacterium*, a species similar to *Lactobacillus*, colonises in the human colon and generates lactic acid. These probiotics are good microbes that aid in forming a friendly microbial barrier against dangerous bacteria in the intestine. Several *Bifidobacterial* species including *Bifidobacterium infantis, Bifidobacterium breve* and *Bifidobacterium longum* cling to the intestine's mucosa and prevent the attachment of undesirable bacteria.

Step 1: Strain Selection: Probiotics are used to create a dietary supplement that aids in digestive function, immune system function, and a healthy reaction to periodic stress. The primary step in the production of probiotics is species selection. Each species has unique qualities and benefits. Certain species support a healthy immune system, while others ease lactose digestion. Therefore, the creation of premium supplements requires premium probiotics raw ingredients [38].

Step 2: Media Designing: Selecting the correct digestive fluid and acid-tolerant species and a good formula to assess their enteric capabilities is crucial. The selected probiotic strains are then solidified and stabilized. Examine the probiotic strain in a bioprocessing environment to observe which growth factors and nutrients should be modified. This analysis is performed automatically by the probiotic manufacturer. Once a particular combination of different nutrients and technical parameters has been determined, production on a large scale commences [39].

Step 3: Fermentation Process: Continuous fermentation necessitates a costly concentration stage; hence batch or fed-batch fermentations are the main production methods in the dairy business[40]. The culture of probiotic microorganisms will take up to six weeks. As populations increase, you must not accelerate the agricultural process. Typically, specific strain identification codes are exclusive to the material source. However, some raw materials may not be immediately available in sufficient quantities to the contract producer, hence extending the period [41]. All nutrients and equipment are disinfected throughout the fermentation process to eliminate unnecessary and inadvertent contamination. In a huge tank, the pressure is elsewhere. The pressure is increased in a tub containing abundant nutrients and heat until the required calculation (CFU-colony-forming units) is attained. Metabolites, which are by-products of microbial metabolic activity, are also produced during this process. Probing probiotics is arduous, necessitating extensive overuse to ensure that every species supplemental needed [39].

Step 4: Centrifugation: After cultures are prepared, probiotic species and metabolites are separated by centrifugation. The stability of probiotics is another crucial aspect of the probiotics production approach that requires control. Once instant probiotics are packaged, their consistency and freshness degrade by the end of the day. Multiple methods are utilized to ensure the supplement's stability and efficacy for extended shelf life. These stages are essential and impact the survival and connectivity of probiotic species [39].

- Refrigeration: The Probiotic microbial unit maintains a frozen state.

- Avoiding sudden processes protects microorganisms from exposure to moisture. Several drying procedures are applied to damp objects.
- Freeze-drying is an extended technique that facilitates the preservation of probiotic species by lyophilizing the probiotic bacteria.
- Spray drying is a speedier technique with higher temperatures that do not appear to be too high for microbe survival. Later the probiotic is transformed into a powder form.

Blending and Bottling the Formulation: The powder produced by spray drying contains the same species. Combining a second probiotic powder with the multiple compositions produced a balanced, evenly distributed mixture. Other components, such as prebiotics, flavourings and binders, are added to the probiotics to provide varied probiotic types that complement the health objective. The mixture is ready in infinite quantities in tablet, pill, and powder form. Temperature, humidity, and sunlight are all crucial for the growth of probiotics. These conditions affect the assembly's expiration date and are distinct for stress. Therefore, they must be packaged and carried with care. Avoid direct sunlight, extreme temperatures, and damp conditions [42].

Future Developments of Probiotics: The approaches in probiotic formulations and their mass production greatly influence the microbiota and interact with the host. Although the recent observational studies and advanced technologies have controversial results from the perspective of early career researchers innovating in these probiotics areas. This major opening started at the 2019 meeting of the International Scientific Association for Probiotics and Prebiotics- Student and Fellows Association (ISAPP-SFA). Probiotic research studies are being driven by molecular characterization and improvement of strains by genetic modification through experimental in-vitro method and *in-vivo* experimental method by using living organisms and Insilco experimental techniques are designed to present the effects of probiotics on their targets, and metabolomic tools to identify the key molecules that mediate benefits on the host. Scientists need to acquire this diverse equipment or form inter-connected teams to perform experiments and systematic analysis of data.

Identifying microbial structure at body sites and determining how administered probiotic strains and prebiotic substances influence the host is very difficult. These alternative ways projected during this review can pave the approach for translating the health edges ascertained throughout analysis into real-life outcomes. Probiotic strains and prebiotic merchandise will greatly improve world problems threatening society. This text intends to produce an Associate in Nursing early career researcher's perspective on wherever the most important opportunities delude advance science and impact human health.

Considerations for Research in Probiotics:

Genetic Modification and Characterization of Probiotics: Bioinformatics computational studies and *in-silico* research techniques contribute to the detailed understanding of beneficial microorganisms, thereby allowing their targeted usage and safety assessment. As whole genomic sequencing (WGS) is inexpensive, we advise that qualitative WGS and rigorous annotation should become the quality observed before selling new probiotic strains.

Fresh sequenced genomes ought to be deposited and created in public via normal central databases (e.g., GenBank1, DDBJ2, ENA3). A rigorous sequence quality control and annotation should be carried out, identifying the mobile and other genetic elements (e.g., CRISPR arrays) and predicting their functional properties, thereby estimating the safety of probiotic candidates concerning virulence factors and attainable antibiotic resistance gene transfer [43]. WGS is also helpful to assess genetic instability and ensure the retention of regions joined to the strain's health edges, as incontestable for the *Lacticaseibacillus rhamnosus* GG variants with and without spaCBA pili genes [44].

Genetic manipulation is an important tool to study probiotic mechanisms of action e.g., by using isogenic mutant strains, and to potentially create improved strains. However, a scarcity of enough genetic tools out there for a few probiotic species,

particularly food-grade systems for *Bifidobacteria*, and a legal framework for the utilization of genetically manipulated/enhanced organisms limits the analysis progress above [45]. However, It is not common to send genetic constructs to biological repositories. The hassle it takes to form these constructs and laws among the analysis establishments are the most probable reasons to stay this genetic material in-house. To advocate for a far better sharing of genetic constructs by providing the genetic material to the scientific community through existing repositories.

This could save a considerable investment of your time and analysis funds and can serve to enlarge the genetic tools box for probiotics. Specifically, the event of safe vectors (e.g., food-grade vectors) could be a necessary step in genetically tweaking probiotic strains for industrial and pharmaceutical applications, as well as specialized probiotics designed to deliver bioactive compounds to a lot of effectively target specific diseases. Each elementary and applied probiotics analysis would take pleasure in a massive investment in genetic components and a much better understanding of their mechanism of action and specificity. Genetic and bioinformatics coaching and skill within these knowledge base areas can therefore be key for researchers creating progress in the probiotics field. *In-vitro* Models in Probiotics Analysis Animal models don't seem to be strictly necessary for presymptomatic assessment of probiotics. Whereas humanized animal models will be enforced, developing ones that simulate microbe-host interaction in humans for niches demonstrating distinctive physiological options like the Lactobacilli-dominated epithelial duct niche characterized by an occasional pH scale is extremely difficult. Human-based in vitro and ex vivo models followed by little studies with healthy volunteers and bigger clinical intervention studies are invariably needed to draw precise and relevant conclusions on probiotic safety, action, and health edges. Recent advance *in-vitro* and *ex-vivo* approaches supported human cells and tissues pave the means on the far side *in-vitro* cell lines and animal models. **Table 1** below illustrates *in-vitro*, *in-vivo*, and *in-silico* techniques for probiotic research.

Duplicable human organoids are wont to recapitulate irritable viscus syndrome manifestations and the therapeutic effects of *L. rhamnosus*. Sophisticated organs-on-chips combine advances in human cell culturing with microelectronics and microfluidics to discover the anti-cancer potential of probiotic and symbiotic formulations; however, they are limited due to lack of exposure to host defenses [46]. Cervico-vaginal tissue explants and organotypic tissue models circumvent this by combining epithelial tissue of human and immune cells and have antecedently allowed spotting antiHIV-1 effects of an untamed kind and genetically changed *Lactobacilli* [47, 48]. The U.S. Environmental Protection Agency free a memorandum stating that studies on mammals will be eliminated by 2035. This has implications for early career scientists developing their future line of analysis, and gap opportunities for implementing different *in-vitro* models without using any *in-vivo* models.

TABLE 1: *IN-VITRO, IN-VIVO* AND *IN-SILICO* TECHNIQUES FOR PROBIOTIC RESEARCH

Techniques	Uses
Full shotgun metagenomics sequencing (Microbiome and *in-silico* method)	To sequence the genome of untargeted cell in a community in order to determine the composition and function of the community [49]
1H-NMR-spectroscopy (Metabolite/protein detection methods)	Metabolite detection in biological samples [50]
CRISPR-Cas9 (Genetic manipulation)	Targeted genetic engineering [51, 52]
RNAseq (*In-vitro* techniques)	The RNAseq approach analyses gene transcription in bacterial communities and hosts and offers information regarding gene expression under various ecological situations [53]
Humanized animal models (*In-vivo* technique)	Performing an analysis of microbe-host and prebiotic-host interactions [54]

CONCLUSION: The ever-increasing demand for probiotics and the introduction of probiotics into new food sectors present the industry with the difficulty of producing large amounts of viable and stable probiotic bacterial cultures. The growth conditions significantly impact the survivability and activity of probiotics during and following processing. The storage conditions that maintain

characteristics such as temperature, vapour pressure, oxygen levels, and moisture content are crucial for preserving probiotics' viability until their shelf life expiration. The most suitable and preferable form of probiotics for storage and transportation is the dried form. Spray drying at maximum pressure and freeze drying with the aid of lyophilization are the most often used drying procedures in the industry. Parameters such as temperature, rate of rehydration and osmolarity of the solution are essential for the survival of bacteria.

The genomics of probiotics can provide information about the survival mechanisms and processes involved in producing probiotic strains, drying and storing live bacterial cultures, hence facilitating the development of more effective probiotic products. *Bacillus* is a spore-producing probiotic bacteria. The spores offer a protective enclosing that enables nearly infinite storage until the food is ready to be digested.

According to studies, Bacillus species contain a sperm-like protein coating that can resist and retain stomach acid, reach the small intestine, germinate and thrive. *Bacillus* species have been demonstrated to tolerate bile, which remains in the small intestine in *Streptococcus* and *Bacillus* as well. *Streptococcus thermophilus*, one species of this bacterium, is employed in the dairy industry to ferment yoghurt and various cheeses. Modern technology techniques enable research studies in humans and the tracking of a probiotic bacteria as it integrates with an existing microbiota and system. Future physical examinations will incorporate the interaction with the host and the influence of environmental elements such as medications, nutrients, *etc*. Modern sampling technologies will highlight how an applied probiotic interacts with the host's immune system, metabolism, and all microbiome components on several levels. We aim to be a part of a society that employs probiotic formulations to solve problems, such as reducing the risk of sickness caused by viruses and removing medicines and poisons from food and the environment.

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