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EVOLUTION OF THE USE AND MANUFACTURING OF LACTIC ACID BACTERIA PROBIOTIC AND ITS EFFECT ON HUMAN HEALTH

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ABSTRACT

Background: Probiotics are defined as live microbial food ingredients that have a beneficial effect on human health. There is an increasing interest in probiotics during the last years; the fermentation bacillus (Lactobacillus) positively influenced the micro flora of the colon, decreasing toxic microbial activities. Lactic acid bacteria (LAB) have a long history of application in fermented foods because of their beneficial influence on nutritional, organoleptic, and shelf-life characteristics. LAB including Lactobacillus specie. **Methodology:** is this research we review about lactic acid probiotic bacteria (LAB) using literature review method, 85 references has been use in this research from different source. **Result:** it was found that LAB occur in different biochemical (shape, gram staining, growth temperature, fermentation status) and molecular characteristic (extraction method, annealing temperature, DNA sequencing). It was found that probiotic have beneficial effect and has been used to protect the gastrointestinal tract, with minimum side effect but not sever that match and can be manage. **Conclusion:** Probiotics are a potential tool for decreasing intestinal contamination that cause by disease and foodborne bacteria. This microbial population of the intestinal tract is a complex natural resource that can be used in an effort to reduce the effect of pathogenic bacteria.

KEYWORDS: Lactic acid, Bacteria Probiotic and Microbial population.

1.1. INTRODUCTION

Probiotics are defined as live microbial food ingredients that have a beneficial effect on human health.[1] In Greek Probiotic means "for life".[2] During the last years, there has been an increasing interest in probiotics which have been defined by a joint expert consultation.[3] The concept of probiotics evolved at the turn of the 20th century from a hypothesis first proposed by Nobel Prize winning Russian scientist Elie Metchnikoff, who suggested that the long, healthy life of Bulgarian peasants resulted from their consumption of fermented milk products. He believed that when consumed, the fermenting bacillus (*Lactobacillus*) positively influenced the microflora of the colon, decreasing toxic microbial activities. The historical association of probiotics with fermented dairy products, still true today, stems from these early observations. Investigations in the probiotic field during the past several decades, however, have expanded beyond bacteria isolated from fermented dairy products to those of intestinal origin. The probiotic bacteria most commonly studied include members of the genera *Lactobacillus* and *Bifidobacterium*.[1] In the United States, probiotics are just now receiving attention by the food industry as healthful ingredients for an increasingly health-conscious consumer. The passage in 1994 of the Dietary Supplement Health and Education Act invigorated the sale of probiotic products as dietary supplements.[1]

Lactic acid bacteria (LAB) have a long history of application in fermented foods because of their beneficial influence on nutritional, organoleptic, and shelf-life characteristics.[4]

(LAB), including *Lactobacillus* species, which have been used for preservation of food by fermentation for thousands of years, can serve a dual function by acting as agents for food fermentation and, in addition, potentially imparting health benefits.[5]

For more than a century, germ theory focused on the idea that bacteria caused disease. In fact, many common bacteria do cause illness in humans and animals, but many others are harmless, and still others are actually beneficial. Years ago, scientists believed that human beings and the bacteria in their bodies had a commensal

relationship — meaning that they exist together without harming each other. Advances in medicine helped to clarify that this relationship is mutualistic; that is, both your body and the bacteria in it benefit from each other.[6]

Fermented milks are the best example of early probiotics. Milk turns sour in hot climates, so especially in the days before refrigeration many people deliberately fermented milk to make curd, or yogurt. Today, the same curd or yogurt is made in a controlled environment by adding live cultures such as *Lactobacillus acidophilus* or *Lactobacillus bulgaricus.* Bacteria are categorized using Latin nomenclature that identifies genus and species. So *Lactobacillus* means a bacterium that produces lactic (or milk) acid, and acidophilus means the bacterium is a species that survives well in acid environments, such as stomach.[6]

Probiotic microflora display numerous health benefits beyond providing basic nutritional value. They cooperatively maintain a delicate balance between the gastrointestinal tract and immune system. When this balance is disrupted, disease and inflammation result. Inflammation and over stimulation of the immune system by pathogenic bacteria are competitively inhibited by mucosal adherence of normal beneficial microflora. A healthy gastrointestinal tract with adequate mucus production and appropriate bacterial colonization prevents the overgrowth of pathogenic bacteria, modulates disease processes, and prevents widespread inflammatory disorders. The understanding of the function of probiotics in the maintenance of health and their importance in preventing disease serves to enhance the overall health of patients. With increasing understanding that beneficial microbes are required for health maintenance and disease prevention, probiotics may be commonly used as a therapeutic tool by health care practitioners in the not-too-distant future.[7]

1.2. Justification

We want to display what are the proven benefits of probiotics today specifically lactobacillus probiotics. Antibiotics, the best antimicrobial weapon, have lost their effectiveness gradually or rapidly in the face of many bacterial infections, exposing some patients to the risk of being uninfected and life-threatening, The Centers for Disease Control and Prevention has described the threat of germs and antibody resistance to antibiotics as a very fast-growing and very serious problem. Many scientists around the world have been working too, on a long-term solution to this problem, and it has been found that the appropriate solution is probiotic, and To battle the increase in health care costs, the development of probiotics products is being advanced. So we try in This research to discusses the potential beneficial effects of probiotics in preventing and treating certain diseases as well as we make our own efforts to keep up with these researches that focused in all age groups to confirm the results of the published study, to know the real benefits of probiotics and to learn more.

1.3. Objective

General objectives

To assess the evolution the use and manufacturing of LAB probiotic and its effect on human health.

Specific objective

1. To determine the biochemical and molecular characterization of lactobacillus species.
2. To know what probiotics are and their mechanisms of action.
3. To Identify potential side effect and contraindication to using LAB probiotics.
4. Production of functional LAB probiotics bacteria isolated from different sources.
5. To identify regulatory status of probiotics in different medication regulatory bodies.

1.4. Methodology

Study desig

Review article.

Number of study

86 study.

Study trace

Google scholar, PubMed, Science direct, Springer, NCBI.

Studies reviewed

From 1993-2019.

2.4.1. Biochemical and molecular characterization of lactobacillus species

LAB are common in nature and are often associated with plant materials.

Lactobacillus are, fermentative, organotrophs. They are usually straight, although they can form spiral or coccobacillary forms under certain conditions. They are often found in pairs or chains of varying length. Lactobacilli are classified as lactic acid bacteria, and derive almost all of their energy from the conversion of glucose to lactate during homolactic fermentation. In this process 85-90% of the sugar utilized is converted to lactic acid. They generate ATP by nonoxidative substrate-level phosphorylation.[32]

2.4.2 Biochemical characteristic

Homofermentative: LAB ferment glucose with lactic acid as the primary by-product. Homofermentative LAB include *Lactococcus spp*. include yogurt strains consisting of rod and cocci and thermophilic strains that might be used in cheese. Other homofermentative cocci that might be found in milk and dairy products, but are rarely used as starter cultures include other Streptococcus spp., Enterococcus, Pediococcus and Aerococcus.

Heterofermentative: LAB ferment glucose with lactic acid, ethanol/acetic acid and carbon dioxide (CO2) as by-products. Testing for heterofermentative fermentation generally involves the detection of gas (e.g., CO2). With the exception of certain fermented milk products, heterofermentative LAB are rarely used as dairy starter cultures.

Heterofermentative: LAB include Gram-positive cocci and Gram-positive rods, Other Lactobacillus species are considered "facultat ively" heterofermentative.[33]

(A) Lactobacillus fermentum

Several research studies have confirmed that some strains for Lactobacillus fermentum have natural resistances to certain antibiotics and chemotherapeutics, Also, it has beneficial effects on the health of the gastrointestinal tract. On the other hand known to be a hetero-fermentative lactic acid bacterium Gram-positive rods, 0.5-0.9 x 3.0 um, occuring singly or in pairs .Cultural characteristics Colonies are generally flat, circular or irregular to rough, often semitransparent nonpigmented, but rare strains produce rusty orange dye. Grow at 41-42°C, No growth at 15 °C. Can grow at 45 °C.

It is regularly isolated from mucosal surfaces of healthy humans and fermented foods.[34]

Positive results for arginine hydrolysis, (NHb from arginine), fermentation of: fructose galactose, glucose (with gas production), glconate, lactose, maltose, mannose (weak reaction), melibiose, raffinose, ribose & sucrose

Negative results for nitrate reduction, fermentation of: adonitol, amygdalin, arabitol, dulcitol, erythritol, esculin, glycerol, inositol, inulin, mannitol, melezitose, rhamnose, salicin, sorbitol, sorbose & starch. Variable results for fementation of:arabinose, cellobiose, trehalose & xylose.[35]

(B) Lactobacillus acidophilus

Methods: Probiotic Lactobacillus strain was isolated from a trade yogurt and the characterization of the bacteria was carry out using gram stain, motility, catalase, biochemical tests and morphological features were confirmed using (SEM). Finally the identification was proven by BioLog system.

Effect of inoculation methods and implant on circumstance on the growth and yield of the bacteria were studied.

Results: The isolated strain was Gram positive cocco-bacilli, nonmotile and catalase negative. It ferment maltose, lactose, sucrose, and glucose, but unable to ferment arabinose and sorbitol. The SEM examination exhibit the extent of the cells ranged from 2.02 - 5.49 × 0.50 - 0.59 µm. From all the results it is proven that the species is Lactobacillus acidophilus. In addition, Biolog rapid identification system detect the presence of L. acidophilus in the designed samples with 90% indexed likelihood, Pour plate show a comparatively higher viable count than the spread plate, while there is no considerable differences were noted between aerobic and anaerobic conditions.

The bacterial strain was successfully isolated in accordance with a set of purification. It was identified as Lactobacillus acidophilus. It can be concluded that, a carbon utilization microplate assay system advanced by Biolog, has the possibility to simplify the identification schedule of lactic acid bacteria to the genus level.[36]

(C) Lactobacillus casei

Lactobacillus casei are a remarkably adaptive species, and sometimes it could be isolated from raw and fermented dairy products, fresh and fermented plant products, and the reproductive and intestinal tracts of humans and other animal to utilize and works as human probiotics.

L. casei are Gram-positive, facultative anaerobic, non-motile and non-spore-forming, rod-shaped, its cell size range = 0.7-1.1 x 2.0-4.0 µm, L. casei form part of the facultative heterofermentative.

Species cluster, which produce lactic acid from hexose sugars via the Embden-Meyerhof pathway and also from pentoses by the 6-phosphogluconate/phosphoketolase pathway.

8 Growth of L. casei occurs at 15 but not 45oC, and requires riboflavin, folic acid, calcium pantothenate, and niacin growth factors.[37]

(D) Lactobacillus plantarum

Lactobacillus plantarum are Gram-positive, non-motile rod cells, non-spore-forming, catalase, Oxidase and urease negative.[38] L. plantarum is facultative anaerobe hetero-fermentative.[39] L. plantarum was stable at 121o C for 10 min. L. plantarum was found to be stable at pH 2.0 to 6.0.[40]

Colonies are creamy-white, circular, smooth, low arched and nitrate reduction is negative.[38] It has been found in a wide variety of fermented foods and it is part of the natural microflora of human and other animals.[39]

No ammonia from Arginine and also there is no gas produced from glucose by Lactobacillus plantarum.[38] Lactobacillus plantarum often it ferments hexoses via the EMP metabolic pathway give rise to the formation of D and L- lactic acids however, pentose are fermented to form lactic and acetic acid in the existence of inducible phosphoketolase.[39]

(E) Lactobacillus brevis

Lactobacillus brevis is a species of lactic acid bacteria, each of them are Gram-positive.[41] it's stable at pH range

of 2.0 to 8.0,[40] non-sporeforminig organisms, which it's major metabolic pathway involve fermenting hexose sugars to produce lactic acid.[41]

Bacteriocin formed by L. brevis has the highest heat stable; at 121 Co for up to 60 min, were characterized by the broad-spectrum inhibition of microorganisms.[40]

Lactobacillus brevis strains are usually isolated from the spoilage microflora in wine and beer fermentations, otherwise it also exist in fermented foods and feed such as sourdoughs, sour starch, cheeses, olives and silage[42] showed antimicrobial activity against the food-prone pathogens Escherichia coli, listeria monocytogenes, salmonella typhimurium, and staphylococcus aureus.[43]

(F) Lactobacillus salivarius

L. salivarius strain is Gram-positive, oxidase-negative, has rod shaped, catalase negative and induce production of acids from D-glucose, D-fructose, lactose, mannitol, sorbitol, inulin and starch. It's not able to hydrolyze red blood cells, It has the ability to survive in gastric and small intestinal fluids at pH 2.0 and bile salt 1.0% for many hours and it can also grow at 45°C. although, this strain exhibit an inhibitory activity against different pathogens that transported by food.[44]

L. salivarius produce many immunomodulatory agents capable of inhibiting H. pylori-induced production from gastric epithelial cells.[45]

2.4.3 Molecular characteristic

(A) Lactobacillus fermentum

This study done by:Ann Catherine Archer, and Prakash M. Halami-:

It's method-:

Twelve L. fermentum isolates (nine from (homemade curd) and three from infant feces, has been chosen from group of acid and bile-tolerant lactobacillus (n=30) particularly taken from fecal samples of infant (n=10) and also from dairy products (n=30). After isolation the L. fermentum were grown in Rogosa, de Man, Sharpe (MRS) broth media.

The isolates were selected based on non-pathogenic traits like absence of hemolysis, lecithinase, gelatinase activity and antibiotic sensitivity tested in vitro. alot of molecular methods are utilize to differentiate and characterize lactobacilli from different food and human fecal sources for variation studies up to the strain level of lactobacillus species. These studies Done through, eribotyping, PCR repetitive sequence-based (rep) PCR, randomly amplified polymorphic DNA(RAPD), 16S rRNA gene sequencing, in addition to pulse field gel electrophoresis (PFGE).

The temperature condition for PCR reaction in a thermal cycler were found as following : 95°C for 5 min followed by 34 cycles of 95°C for 1 min, annealing temperature for about1min, and 72°C for 1 min. Final extension temperature was completed at 72°C for 10 min.[46]

(B) Lactobacillus acidophilus

This study done by: Vahid Jabbari, Reza Rezaei Mokarram, Mahmoud Sowti Khiabani, Fereshteh Askari, Elham Ahmadi, Azad mohammad Hassanzadeh, Sanaz buick Aghazadeh, Mohammad Asgharzadeh and Hossein Samadi Kafil-:

It's method-:

Molecular method by PCR. Fully diffusion method was used and the inhibitory action on the growth of pathogenic bacteria was determined.

DNA extraction method: The DNA of the bacteria was extracted from one colonies that grown on MRS agar (DE MAN, ROGOSA, SHARPE agar) medium. Tissue buffer was used to extract DNA, tissue buffer compounds consist of Sodium Dodecyl Sulfate (SDS) and NaOH.

20ml of tissue buffer shed in eppendorf and for tissue buffer solution some bacterial colonies were added, then after that eppendorf was positioned at 95°C on hotplate for 10 minutes. At the end, solution centrifuged for 1 min at 13000 g and 180 ml of deionized water was thrown into it, extracted DNAs were taken directly to -70°C freezer.

PCR reaction and amplification of 16S rRNA gene: 16S rRNA gene fragment specific primers for Lactobacillus acidophilus were obtained from Sinaclon.[47]

(C) Lactobacillus casei

This study done by:MojtabaAlimolaei, and Mehdi Golchin-:

It's method-:

The isolated whole DNA samples were applied as template for particular amplification of DNA from the 16S rRNA gene of L.casei. PCR reaction was achieve by using 5 µL of the extracted DNA with 25 µL of ready-to-use PCR master mix 2x(PR901638, SinaClon, Iran), 2.5 µL (20 pmol/µL) of each primer and dH2O till 50 µL volume was reached. DNA Amplification from the 16S rRNA gene of L. casei was done. Amplicons of cpb were obtained with 35 cycles following an initial step of denaturation at 95°C for 10 minutes. Every cycle included denaturation at temperature 94°C for one minute, annealing at 52°C for at least 1minute, synthesis at 72°C again for one minute, and therefore the final extension step at 72°C for 10 minutes. The PCR products were then analyzed for clarity and intensity. The amplified products were electrophoreses in 1.7% agarose geland detected with gel documentation system.[48]

(D) Lactobacillus plantarum

This study done by: Svetoslav Dimitrov Todorov a b, and Bernadette Dora Gombossy De Melo Franco-:

It's method-:

Molecular characterization of L. plantarum strains is of interest and is often achived by species-specific PCR and sequencing of partial 16S rDNA gene.

Many different primers for species-specific PCR have been suggested and effectively applied.

Characterization methods are being performed, namely, peptidoglycan structure, DNA base composition, DNA homology, electrophoretic mobility of L-lactate dehydrogenase, species-specific PCR (derived from rRNA sequences), RAPD-PCR, PFGE, and restriction enzyme analysis. To differentiate and characterize species, phenotypic methods together with genetic methods was applied because many species are very similar phenotypically but different genotypically.

Antimicrobial compounds is able to be produced by L. plantarum, contributing to the safety of the final product. The capability of L. plantarum or its metabolites to inhibit the growth of human pathogens, Mycobacterium tuberculosis and some sort of viruses was well documented. However, L. plantarum as probiotic likely will be the new center of application of this species in the near future.[49]

(E) Lactobacillus brevis

Lactobacillus brevis one of most vital biological organism that are able to produce bioactive γ-aminobutyric acid biologically.[50]

This study done by:HeeSeon Lim, In-Tae Cha, SeongWoonRoh, Hae-Hun Shin, and Myung-Ji Seo-:
It's method-:

- GABA production is mainly run by fermentation of microorganisms such as fungi, bacteria and yeast. Lactic acid bacteria (LAB) specially have been announce to be GABA producers.
- Microbial GABA production is rely on different fermentation factors, e.g pH, temperature, medium composition, glutamate concentration and time taken for fermentation.

In a way to genetically test and resolve the 16S rRNA gene and GADgene included in GABA biosynthesis, genomic DNA of GABA producing microorganisms extracted in this study was isolate with the HiYield Genomic DNA Mini Kit (RBC, Taiwan) it was conducted to identify the isolated GABA producers by using of 16S rRNA gene sequence analysis. The full-length GAD gene from the genomic DNA also was amplified by using specific primer groups depend on early reported GAD sequences of particular L.brevis strains.

On the other hand condition that should be applied on The polymerase chain reaction (PCR) for GAD amplification were begin with denaturation at 94°C for 2 min, after that followed by 30 cycles at 94°C for 30 sec, 52°C for 1 min, and 72°C for 1 min, and final extension temperature at 72°C for 5 min.[51]

(F) Lactobacillus salivarius

This study done by: Zhong Wang, Ximin Zeng, Yiming Mo, Katie Smith, Yuming Guo, and Jun Lina-:
It's method-:

Lactobacillus salivarius was isolated from chicken intestine it usually grown in de Man-Rogosa-Sharpe (MRS) broth or on agar (Difco Laboratories, Detroit, MI) at 37°C under anaerobic situations, they were generated by an AnaeroGen gas pack in an enclosed jar. As a host for gene expression, Escherichia coli strain have been used. The Ultra Clean microbial DNA isolation kit (MoBio) was used to extract the genomic DNA from L. salivarius NRRL B-30514 and size ruptured into 400- to 500-bp fragments, which subsequently were blunt-end repaired, ligated to special adaptors, immobilized and amplified on the DNA capture beads, and after that was sequenced using the PicoTiter Plate in the 454-FLX instrument, according to FLX Titanium protocol. The genomic DNA of this strain was sequenced using a 454 GS FLX sequencer with Titanium series reagents.[52]

Table 1: PCR primers.

PCR primers and conditions used to isolate specific lactobacillus species:

Species	Primer sequence (5' to 3')	PCR annealing temp (°C)	References
L. fermentum	GCCGCCTAAGGTGGGACAGAT CTGATCGTAGATCAGTCAAG	55	[24]
L. acidophilus	AGCTGAACCAACAGATTCAC ACTACCAGGGTATCTAATCC	62	[24]
L. casei	CAGACTGAAAGTCTGACGG GCGATGCGAATTTCTTTTTC	52	[24]
L. plantarum	GCCGCCTAAGGTGGGACAGAT TTACCTAACGGTAAATGCGA	55	[24]
L. brevis	TGTACACACCGCCCGTC TAATGATGACCTTGCGGTC	480	[25]
L. salivarius	GCC TAA CAC ATGCAA GTCGA CGT ATT ACC GCGGCT GCT GG	55	[26]

2.5 Probiotics and Their mechanism of action

2.5.1. General mechanism of action of probiotics

Although the exact mechanisms of action of probiotics are not known, several have been proposed. Probiotic microorganisms are considered to support the host health. However, the support mechanisms have not been explained. There are studies on how probiotics work. So, many mechanisms from these studies are trying to explain how probiotics could protect the host from the intestinal disorders. The most frequently used probiotics include lactic acid bacteria, particularly Lactobacillus and Bifidobacterium species. These bacteria produce lactic acid, acetic acid, and propionic acid, which lower the intestinal pH and suppress the growth of various pathogenic bacteria, thereby reestablishing the balance of the gut flora.[8,9]

Some of These mechanisms include:

- Production of inhibitory substances: Production of some organic acids, hydrogen peroxide, bacteriocins and biosurfactants that are toxic to pathogenic microorganisms, which have an inhibitory effect on both gram-positive and gram-negative bacteria.[8]
- Blocking of adhesion sites: They are a competition between a Probiotics and pathogenic bacteria. Probiotics inhibit the pathogens by adhering to the intestinal epithelial surfaces by blocking the adhesion sites and decrease colonization of pathogenic organisms in the urinary and intestinal tracts.[8]
- Probiotics involves immunomodulation : some of probiotic strains increase the immune response by stimulating the phagocytic activity of lymphocytes and macrophages. Probiotics also increase immunoglobulin A (IgA) and stimulate cytokine production by mononuclear cells. It is found that children with acute rotaviral diarrhea who were given Lactobacillus rhamnosus strain GG (LGG) had an increased IgA, immunoglobulin G, resulting in a shortened duration of gastroenteritis symptoms.[9]
- Competition for nutrients: probiotics inhibit the pathogens by consuming the nutrients which pathogens need.[8]
- Stimulating of immunity: Stimulating of specific and nonspecific immunity is mechanism of probiotics to protect the host from intestinal disease. This mechanism is not well documented, but it is thought that specific cell wall components or cell layers acting as adjuvants to increase the humoral immune response.[8]
- Degradation of toxin receptor: S. boulardii protects the host against C. difficile intestinal disease. Due to degredation of toxin receptor on the intestinal mucosa.[8]

2.5.2 Effect of probiotics for selected diseases

In the face of widespread diseases and ageing societies, the use of knowledge on microbiocenosis of the gastrointestinal tract and on the beneficial effect of probiotic bacteria is becoming increasingly important.

Probiotic formulas and products may protect people against enteral problems, and influence the overall improvement of health.

The below classification clarify diseases that can treated using probiotics and their mechanism :

(A) Diseas

Inflammation/Arthritis.

Mcroflora

Lactobacillus GG and Lactobacillus. casei.

Mechanism of action

- Probiotics has both direct and indirect effects.
- Direct effect: including modulation of resident bacterial colonies and vitamin production locally in GI tract.
- Indirect effect: exerted at sites outside the GI tract, including the joints.

Indirect effects most likely result from an impact on immunity, via changes in inflammatory mediators such as cytokines. Modulation of inflammatory responses may be related to regulating or modulating the immune system both loacally in the GI tract and systemically.

It has been found that orally administered Lactobacillus GG has potential to reinforce mucosal barrier mechanisms in this disorder. When inflamed, the gastrointestinal tract becomes permeable and serves as a link between inflammatory diseases of the GI tract and extra inflammatory disorders such as arthritis.

- Oral administration of L. casei significantly decreased the proinflammatory cytokines.

L. casei has Inhibition of COX-2 via inhibiting the proinflammatory cytokines is an understanding of the complex interactions involved in these pathways.[7,10]

(B) Disease

Allergies/Eczema.

Microflora

Lactobacillus rhamnosus.

Mechanism of action

- Probiotic bacteria are important in down regulating inflammation associated with hypersensitivity reactions in patients with atopic eczema and food allergy.
- Perinatal administration of *Lactobacillus rhamnosus GG* decreased subsequent occurrence of eczema in at risk infants by one-half.
- In mildly hypersensitive patients, probiotics down regulated a milk-induced inflammatory response. This was found to be secondary to prevention of increased receptor expression in monocytes and neutrophils. individuals with- out milk sensitivity

were not found to have receptor down-regulation when taking probiotics.
- Supplementation of lactobacillus rhamnosus cause improvement in quality of life, skin symptoms and day- and night time irritation scores in children.[7,11]

(C) Disease
Hepatic disease.

Microflora
Bifidobacteria, Lactobacillus acidophilus and L. Plantarum, L. Casei,

Mechanism of action
- Oral bacteriotherapy could improve microbial balance and lower portal pressure with a reduction in the risk of bleeding.
- Instead of antibiotics, probiotics should be administered and demonstrate this could be a safe way to regulate portal pressure. They conclude that not all bacteria should be regarded as pathogenic in liver cirrhosis and portal hypertension.
- Probiotic therapy was associated with a significant end of treatment reduction in ALT, AST, GGT, lactate dehydrogenase, and total bilirubin. Patients with alcohol-induced liver injury have altered bowel flora compared to healthy controls. Short-term oral supplementation with B. bifidum and L. plantarum was associated with restoration of the bowel flora and greater improvement in alcohol-induced liver injury than standard therapy alone.[7,12]

(D) Disease
Diarrhea.

Microflora
LactobacillusGG, L.reuteri and L.acidophilus.

Mechanism of action
- Diarrhea is a severe reason of children death in the worldwide and rotavirus is its common cause. In the treatment of rotavirus dierrhea, Lactobacillus GG is reported really effective.
- Probiotics appear to benefit viral diarrhea, possibly by increasing secretory IgA and decreasing viral shedding, suggesting an immunological mechanism.
- Probiotics increase intestinal mucin production, which prevents the attachment of enteropathogens.
- The best documented probiotic effect is shortened duration of rotavirus diarrhea using Lactobacillus GG.
- Addition of L. acidophilus to oral rehydration therapy was effective in the treatment of children with acute diarrhea by decreasing the duration of diarrhea.
- Effectiveness of probiotics for preventing travelers' diarrhea have been inconsistent, possibly due to the probiotic strain used and the various trip destinations.[7,8,9,13]

(E) Disease
Helicobacter pylori Infections.

Microflora
Lactobacillus salivarius.

Mechanism of action
- H. pylori may not always be eradicated with antibiotics and acquisition of resistance is often a serious problem.
- Some strains of Lactobacillus are able to inhibit H. pylori growth through the release of bacteriocins or organic acids, and may also decrease its adhesion to epithelial cells. In addition, probiotics have a possible role in the stabilization of the gastric barrier function and the decrease of mucosal inflammation. Other aspects that are considered are the contribution of probiotics to the healing of the gastric mucosa linked to their antioxidant and anti-inflammatory properties.
- Lactobacillus salivarius capable of producing high amounts of lactic acid, which can inhibit the growth of H. pylori in vitro. This was subsequently confirmed in vivo in a murine model.
- It was found that the higher the level of lactic acid production by Lactobacillus, the more potent was the effect on reducing H. pylori's urease activity.
- Lactobacillus salivarius was found to be most effective due to high level of lactic acid production.[7,14]

(F) Disease
Cholesterol reduction.

Microflora
Lactobacillus reuteri and Lactobacillus acidophilus.

Mechanism of action
- Another unexpected benefit of probiotics is serum lipid reduction.
- Lactobacillus have ability to assimilate cholesterol, demonstrated by identification of cholesterol in cells during growth and decreases in the concentration of cholesterol in the growth medium.
- The mechanism of this effect could not been explained definitely. There are two hypotheses trying to explain the mechanism. One of them is that bacteria may bind or incorporate cholesterol directly into the cell membrane. The other one is, bile salt hydrolysis enzymes deconjugate the bile salts which are more likely to be exerted resulting in increased cholesterol breakdown.
- This may have important implications in preventing reabsorption of cholesterol back into systemic circulation.[7,8]

(G) Disease
Lactose intolerance.

Microflora
Lactobacillus bulgaricus.

Mechanism of action

- Milk is the richest source of calcium and Ca requirement of the body is met only through milk. These lactose intolerant people cannot metabolize lactose due to the lack of essential enzyme β-galactosidase. When they consume milk or lactose-containing products, symptoms including abdominal pain, bloating, flatulence, cramping and diarrhea ensue. If lactose passes through from the small intestine, it is converted to gas and acid in the large intestine by the colonic microflora. Also the presence of breath hydrogen is a signal for lactose maldigestion.
- Calcium absorption is better and more in acidic conditions; hence, if lactose is converted to lactic acid, pH of the gut decreases, i.e. it becomes acidic favouring enhanced absorption of calcium. So, if probiotics are fed to lactose intolerance patients, then milk lactose is hydrolysed by probiotic strains and lactose is assimilated and calcium absorption is also favoured.
- The beneficial effects of probiotics on lactose intolerance are explained by two ways. One of them is lower lactose concentration in the fermented foods due to the high lactase activity of bacterial preparations used in the production. The other one is; increased lactase active lactase enzyme enters the small intestine with the fermented product or with the viable probiotic bacteria.
- Also that yogurt can be a best alternative to milk.[8,15]

(H) Disease
Inflammatory bowel disease.

Microflora
Saccharomyces boulardii and Lactobacills GG.

Mechanism of action

- Inflammatory diseases of the digestive tract include UC, Crohn's disease, and pouchitis.
- Probiotics may improve the microbial balance of the indigenous flora. Although studies have been conflicting, probiotics seem to be an attractive option in the treatment and prevention of inflammatory bowel disease, providing an appealing alternative to the use of antibiotics.
- It has been reported that probiotic combination therapies may benefit patients with inflammatory bowel disease.
- Saccharomyces boulardii in patients with Crohn's disease was found to extend remission time and reduce relapse rates.
- Both Saccharomyces boulardii and Lactobacillus GG have been reported to increase secretory IgA levels in the gut. Ongoing investigation into the use of probiotics in inflammatory bowel disease has generated considerable interest.[8,9]

(I) Disesase
Irritable bowel syndrome.

Microflora
Lactobacillus plantarum and Lactobacillus bulgaricus.

Mechanism of action

- IBS is characterized by abdominal pain, bloating, flatulence, and altered bowel habits. These symptoms may be due to bacterial overgrowth in the small intestine, causing increased fermentation activities and gas production.
- Irritable bowel syndrome, Lactobacillus plantarum and were shown in clinical trials to reduce abdominal pain, bloating, flatulence, and constipation. It was also observed that Saccharomyces boulardii decreased diarrhea in irritable bowel syndrome.[8,9]

2.5.3. Safety and Side effect of lactobacillus probiotics
Several probiotics have a long history of safe use. However, new probiotic strains will not have such a history and therefore need to be assessed for safety on a strain-by-strain basis.

The scientific evidence on the health benefits of probiotics continues to increase.

The benefits provided by probiotics are likely to outweigh any potential risks, but this risk benefit ratio should be determined for each strain and target population. Lactic acid bacteria in general have a good safety record. They are rarely involved in disease. But There are some theoretical adverse risks that have been raised with respect to the use of probiotics in humans. These theoretical risks include the potential for transmigration and the fact that colonization with probiotics may have a negative impact on gastrointestinal physiology and function, including metabolic and physiologic effect. There could also be adverse immunologic effects, both localized and generalized.[16,17]

2.6. Side effect of probiotics
There are 3 theoretical concerns regarding the safety of probiotics :
(1) The occurrence of disease, such as bacteremia or endocarditis.
(2) Toxic or metabolic effects on the gastrointestinal tract.
(3) The transfer of antibiotic resistance in the gastrointestinal flora.[16]

(1) The occurrence of disease, Such as bacteremia or endocarditis
Bacteremia and endocarditis potential. We do know that lactic acid bacteria, including bifidobacteria, have been

isolated as causes of bacteremia and also as causes of endocarditis. The list of organisms that have been associated with endocarditis or bacteremia includes L. rhamnosis, L.plantarum, L. casei, Lactobacillus paracasei, Lactobacillus salivarius, L. acidophilus, and many other lactobacilli. Bifidobacterium species have also been isolated from the blood and in patients with endocarditis. Enterococcus species, of course, are well known as causes of bacteremia and endocarditis.

With respect to sepsis related to probiotics, there have been 3 reports of Lactobacillus GG–associated bacteremia in children with short gut syndrome.

L. acidophilus bacteremia in a patient who had HIV infection and Hodgkin disease and a case of Lactobacillus infection after a bone marrow transplant. Many of the cases had some degree of molecular identification and confirmation of the probiotic strain.[16]

Platelet aggregating activity has been considered to be a required test in the assessment of safety. Aggregation of platelets by bacteria is thought to contribute to the progression of infective endocarditis.[17]

(2) Metabolic effects on the gastrointestinal tract

- Probiotics should not produce harmful compounds. In addition, some enzymic activities such as some specific glycosidic and proteolytic enzymes that could help in the invasion or translocation through the epithelium or in the colonization of some host tissues must not be present. In general, these harmful activities have not been found in the traditional probiotic strains, but must be assessed in the new strains. For example, mucus degradation capacity, which has been considered a marker for invasive potential, was not found in specific commercial probiotics.
- Additional potential toxicities, there is also a theoretical possibility that d-lactate production might occur, with the development of lactic acidosis. Studies have been performed in healthy humans with an ileostomy.
- Other risks related to the metabolic activities of the strains are the deconjugation of bile salts. Secondary bile acids may exhibit carcinogenicity. L. acidophilus and Bifidobacterium species have been shown to transform conjugated bile acid into nontoxic secondary salts. In patients with short small bowel syndrome, it is possible that the conjugated bile acid metabolites might accumulate and lead to malabsorption. This might lead to the risk of the lactate accumulation and a theoretical risk of colon cancer. There is also the theoretical possibility that there may be degradation of intestinal mucus. Thus, the use of probiotics with a high capacity for deconjugation /dehydroxylation of bile salts must be carefully evaluated.[16,17]

(3) The transfer of antibiotic resistance in the gastrointestinal flora

It has recently been reported that more than 68% of the probiotics isolated from different products were resistant to two or more antibiotics.[17] However, transferable antibiotic resistance is another matter because of the possibility of resistance spreading to other, more harmful bacteria.

A major area of concern has been the potential for antibiotic-resistance transfer in the gastrointestinal tract that might take place between probiotics and pathogenic bacteria. When one examines the potential for transferable antibiotic resistance in lactic acid bacteria, one can find the presence of plasmids with antibiotic-resistance genes, including genes encoding resistance to tetracycline, erythromycin, chloramphenicol, and macrolide-lincosamide- streptogramin. These resistance plasmids have been found in L. reuteri, L. fermentum, L. acidophilus, and L. plantarum in raw meat, silage. Streptomycin resistance, tetracycline resistance, and chloramphenicol resistance, have been found in L. lactis in raw milk and soft cheese. Tetracycline resistance has been found in L. plantarum.[16]

In most circumstances the available data suggest that probiotics colonize the human intestine transiently. Nevertheless, concern exists regarding the possible transfer of antimicrobial resistance from probiotic strains to more pathogenic bacteria in the intestinal microbiota. Many Lactobacillus strains are naturally resistant to vancomycin, which raises concerns regarding the possible transfer of such resistance to more pathogenic organisms, particularly enterococciandm Staphylococcusaureus. However, the vancomycin – resistantgenes of Lactobacillus spp.are chromosomal and, therefore, not readily transferable to other species. Conjugation studies have not found the vancomycin-resistant genes of lacto- bacilli to be transferable to other genera.[18]

2.6.1 Precautions and Contraindications of probiotics

Since probiotics contain live microorganisms, there is a slight chance that these preparations might cause pathological infection, particularly in critically ill or severely immunocompromised patients. Probiotic strains of Lactobacillus have also been reported to cause bacteremia in patients with short-bowel syndrome, possibly due to altered gut integrity. Caution is also warranted in patients with central venous catheters, since contamination leading to fungemia has been reported when Saccharomyces capsules were opened and administered at the bedside.

Lactobacillus preparations are contraindicated in persons with a hypersensitivity to lactose or milk.

S. boulardii is contraindicated in patients with a yeast allergy. No contraindications are listed for bifidobacteria,

since most species are considered nonpathogenic and nontoxigenic.

- Extremely Low birth weight, clinical instability, abnormal abdominal examination, the presence of congenital abnormalities, post necrotizing enerocolitis (NEC). stage iii asphyxia, and umbilical catheters.[9,19]

2.6.2 Drug interaction

Since probiotics contain live microorganisms, concurrent administration of antibiotics could kill a large number of the organisms, reducing the efficacy of the Lactobacillus and Bifidobacterium species. Patients should be instructed to separate administration of antibiotics from these bacteria-derived probiotics by at least two hours. Similarly, S. boulardii might interact with antifungals, reducing the efficacy of this probiotic. According to the manufacturer, Florastor, which contains S. boulardii, should not be taken with any oral systemic antifungal products. Probiotics should also be used cautiously in patients taking immunosuppressants, such as cyclosporine, tacrolimus, azathioprine, and chemotherapeutic agents, since probiotics could cause an infection or pathogenic colonization in immunocompromised patients.[9]

2.7 Manufacturing of probiotics

The increasing demand for probiotics and the new food markets where probiotics are introduced, challenges the industry to produce high quantities of probiotic cultures in a viable and stable form. Lactobacillius require in general complex media containing a range of nutrients. It is reported that LAB prefer peptide bound amino acids rather than the free form by increasing the thiol groups in the media.[53] Lactic acid bacteria are well known to be beneficial for food production and, as probiotics, they are relevant for many aspects of health.

Many physiological traits of these microorganisms, evolved for optimal growth in their niche, are also valuable in an industrial context.[54]

Isolation of various LAB producing microbes is an important way to obtain excellent LAB producers. LAB have been predominantly used in food industries to preserve food and inhibit microbial contamination. Due to their traditional use in the food industry and their non-pathogenic character, lactic acid bacteria have been given a unique Generally Recognized As Safe (GRAS) status.[55] Selection of LAB for its uses as a probiotics is based on its ability to survive in diverse and extreme conditions and its ability to produce bioactive compounds for host that also work against other bacteria. Tolerance to a wide range of pH is one of the desired properties in the probiotic bacteria, facilitating the survival of such probiotics in host gastrointestinal system.[56]

The development of formulations containing probiotics needs

- ❖ The selection and characterization of strains with proven therapeutic properties.
- ❖ The selection of excipients.
- ❖ The processes suitable for large-scale production of the final dosage form.
- ❖ Factors that affect viability and stability of probiotics.
- ❖ Quality control test for different dosage form.

After this whole process is completed, probiotics can then be commercialized as medicinal products.[20]

2.7.1. The selection and characterization of strains with proven therapeutic properties

Selected strain should help in the healthy functioning of human body systems. Strain used should be a fully characterized one using scientifically valid techniques to confirm the strain identity and its critical characteristics Each strain should be pure and when used in combination of pure strains, the proportion should be known. The strain should have ability to pass through digestive system in live condition and possibility of its intestinal implantation. The culture should maintain its original properties while preparation of formulation and subsequent treatment to prepare in suitable dosage form.[21]

Their functionality during storage. The reduction of viable cells during storage, processing, and passage through the GIT is a significant challenge in formulating dosage forms containing probiotics1manu.

Although the viability of probiotics is essential for functioning of probiotics, it is a difficult task to maintain the viability from fabrication/storage to the target site in the GI tract. For this reason, a majority of probiotic delivery studies focus on how to improve the probiotic viability. Because several pharmaceutical industrial processes may induce an increase in the temperature of the handled material and may expose it to moisture variations, the viability of probiotics must be studied at different conditions of temperature and relative humidity. The first probiotic products available were mostly liquid formulations that showed low cell viability after oral administration, mainly because bacteria did not survive in the stomach conditions. Nowadays, the development of suitable solid dosage forms allows obtaining higher levels of bacterial survival.[20,22]

2.7.2. The selection of excipients

Most of the commercially available probiotic preparations contain different excipients along with the active ingredient (probiotics). These are used in virtually all drug products and are essential for product performance. Product performance depends extensively upon the physical and chemical properties of these excipients. The functional purposes of excipients are many, to act as a diluent, lubricant, colorant, binder,

coating agent, sweetening agent, anti-caking agent, suppository base, etc. Most commonly used excipients for probiotic preparations like bulk powder, tablet and capsules include microcrystalline cellulose (as binder/diluent), rice maltodextrin (as binder/ diluent), silicon dioxide (gliding/anti-caking agent), magnesium stearate (as lubricant), hydroxy propyl methyl cellulose (as suspending/viscosity agent).[21]

The particle size of the excipients is also important. So the smaller the microorganism and the larger the particle size of the excipients, the better the survival of microorganisms during compression will be. The explanation is that the microorganisms are able to "hide" in spaces which are formed when using excipients with large particle size. With increasing particle size the number and the size of void spaces will increase, and the micro-organisms will thus escape mechanical damage during the compression and consequently have better survival.[23]

2.7.3. The processes suitable for large-scale production of the final dosage form.

These processes include

i. Selection criteria for potential probiotics.
ii. Method to produce suitable probiotics
iii. Different dosage form of probiotic

2.7.4. Selection criteria for potential probiotics

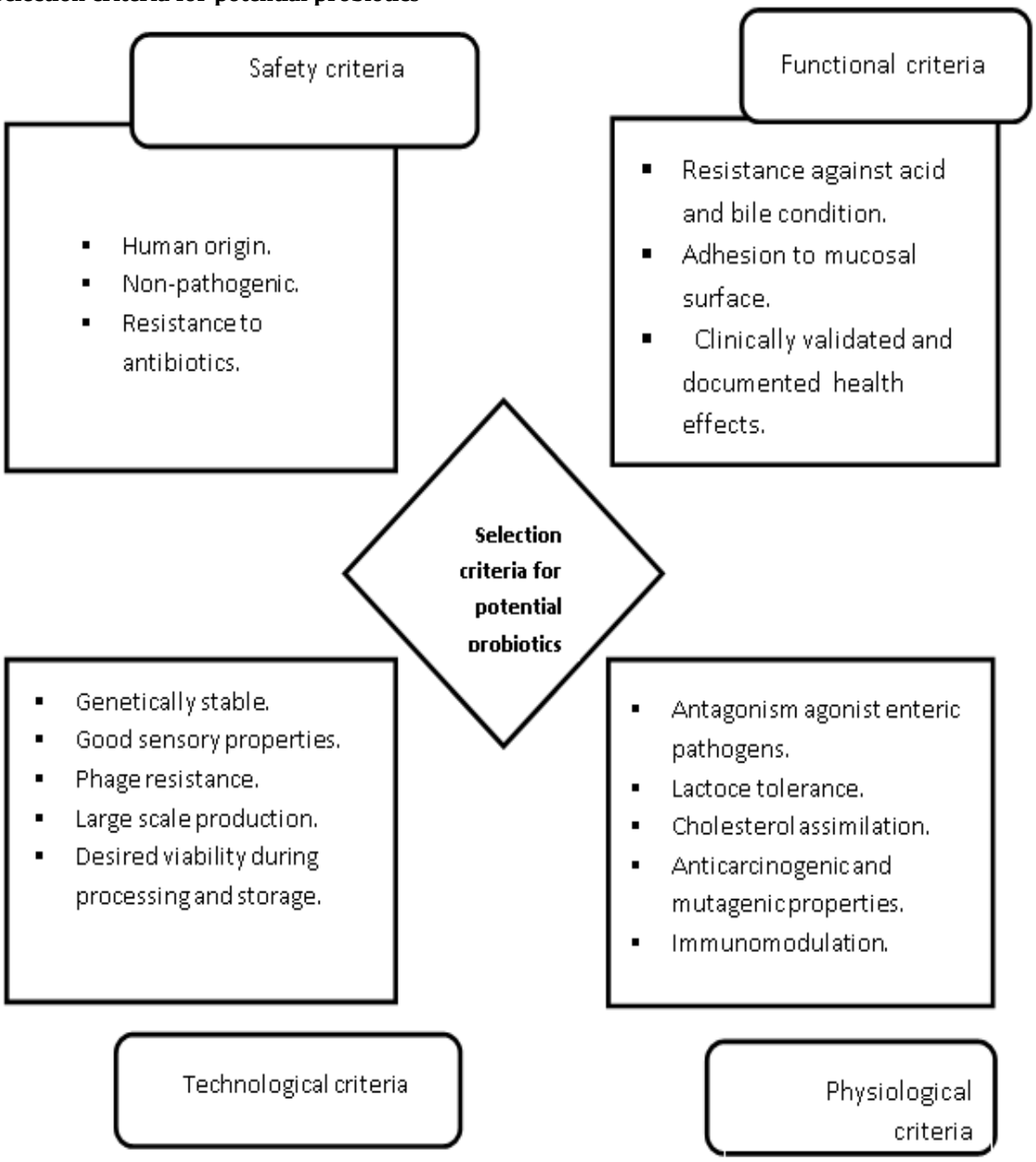

Figure 1: Criteria for potential probiotics.

2.7.5 Method to produce suitable probiotics

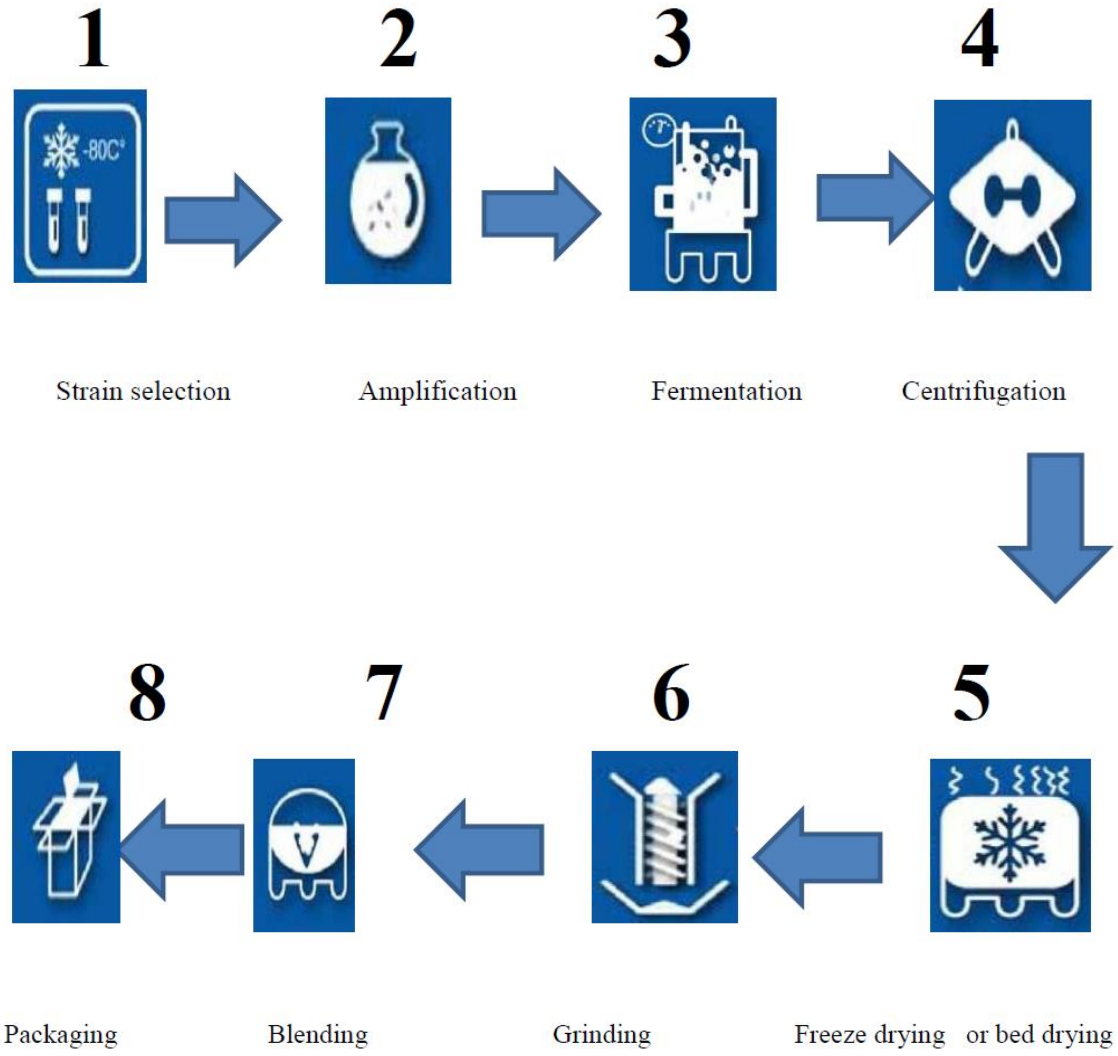

Figure 2: Method to produce suitable probiotics.

1- Selection of strain

The strain should have ability to pass through digestive system in live condition and possibility of its intestinal implantation. The culture should maintain its original properties while preparation of formulation and subsequent treatment to prepare in suitable dosage form.[21]

2- Amplification

Amplification usually starts from a few amounts of bacteria from the cell bank vial, which will be grown step-wise from a lab-flask to the industrial fermenter. The inoculum is inserted in a flask containing the suitable culture media, under sterile conditions. Then the bacteria become activated and start to multiply in large amount. And finally the culture transferred to a larger container.[57]

3- Fermentation

The conversion of carbohydrates into alcohols or acids through the action of bacteria is called fermentation.[58] Probiotic cells are usually produced by large scale fermentation. The cultivation conditions are directly affect the growth, culture stability, and activity as well as drying and finally storage. The most widely used medium for cultivation of LAB is MRS agar.[59]

Types of Fermentation Involved in Probiotic Production :

A- Membrane bioreactors (MBR)

In a membrane system with continuous supplying of fresh media, cells are then kept in the bioreactor by an ultrafiltration or microfiltration membrane where the small molecules spread out through the pores of the membrane depends on their size. The concentrated cell fraction may be collected batch-wise or continuously with no, or minimal, extra downstream treatment for cell concentration before freezing or freeze-drying, however, these systems could result in high cell yields and volumetric productivities. Cell functionality and physiology were not studied yet. Although, cells are exposed to a lot of tension in membrane bioreactors, represented in low nutrient concentration, oxygen, osmotic and mechanical stresses that may affect sensitive

bacteria, but also it could lead to cross-protection effects for other different stresses.[60]

B- Immobilized cell technology (ICT)

The target of ICT is either to defend cells from a hostile environment or to maintain high cell concentrations within the bioreactor, the transporter substance should be non-toxic, easily accessible and with relatively low cost. Also it have to lead to high-cell loading and the cells should have an extended viability in the support.[61]

IMC divided into four major categories based on their physical mechanism

- Self- accumulation by flocculation (natural) or with unnaturally induced cross-linking factors.
- Mechanistic contaminant beyond a barrier that may be either a microporous membrane or a microcapsule.
- Entrapment inside a spongy matrix because of cells permeation, while their ability to move is blocked by the existence of other cells or by the formation of porous substance in situ into a cell culture.
- Appendage or adsorption on rigid transporter surfaces by somatic adsorption because of electrostatic strength or by covalent binding between the cell membrane and the transporter.[62]

Advantages of IMC

- The concentration of cell in immobilization is greater than that in free suspension.
- Collection of high cell concentration and high flow rate permit, high volumetric productivity.
- Suitable micro environmental situation .
- Get better genetic stability.
- Protect versus cut damage.[63]

Figure 3: Probiotics fermentation.

4- Centrifugation

Centrifugation is the process that includes the application of centripetal stimulation to detach the microalgae from the culture media and is may be the quickest cell-recovery procedure depends on density tendency. The centrifuge rollers are simply cleaned and sterilized, and centrifugation can be applied to any type of microalga.

Disadvantages of centrifugation

- Cell structure could be altered because they are exposed to a high gravitational force.
- The recovery of weak microalgae biomass requires low-speed centrifugation; rapid corrosion of equipment could be caused by the salt contained in the microalgal culture media equipment.
- Large-scale procedures require equipment with high costs, like continuous centrifuges.[64]

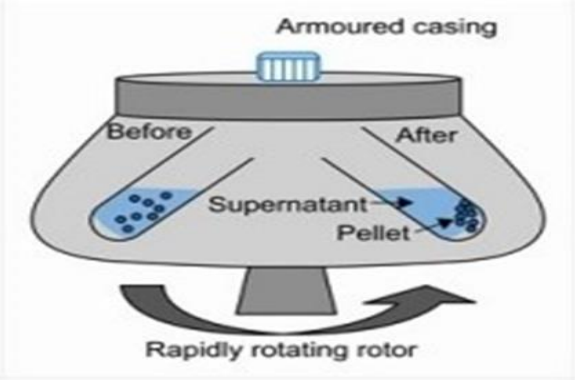

Figure 4: Centrifugation.

5- Freeze-drying or bed dryer

Freeze-drying

Freeze-drying or lyophilization is the process of choice if valuable and sensitive products have to be stabilized in a dry solid state in the field of food- and pharmaceutical manufacturing and research. Freeze-drying process is defined as a process, where the solution is frozen in first step that called freezing and thereafter should be dried under a reduced pressure.[65] unluckily, the drying process takes a prolonged period of time.[66]

Freeze drying is a process where the ice is sublimed from the substance straightway to a gas, under pressure and with a little degree of heat. Freeze drying is usually used to produce a very small amount of dried spongy powder with the support of a physical foundation procedure.[67]

In general freeze-drying process composed of three steps, there order is freezing followed by primary drying and finally secondary drying.[65]

Advantages of freeze-drying include

I. Abstraction of water without high degree of heating.

II. Chemical dissolution is reduced.
III. Suitable with sterile process more than dry powder filling.
IV. Ability of processing a liquid, facilitate aseptic handling.
V. Promoted product stability in a dry state.

Disadvantages of freeze-drying include

I. Vacuum could remove volatile compounds.
II. Consume more handling and processing time.
III. Need for sterile diluents in reconstitution.[68]

Figure 5: Freeze drying step.

Freeze-drying steps

1. Freezing to −50 Co
2. Primary drying (−20 Co, 20 Pa)
3. Secondary drying (25 Co, low pressure)

1. Freezing

The freezing step is the first and the shortest step of the freeze-drying process. It controls ice crystal size, distribution, and morphology.[69]

During freezing, the starting liquid solution is supposed to be homogeneous, part of the water breaks away from the dissolved materials to take shape in the clear state. The rest of the water then become hard little by little in a blend with the solvents to compose an interstitial substance -between the ice crystals- which adopts an indeterminate structure.

Actually, the bigger the ice crystals formed, the bigger the pore diameter and therefor, the higher the drying rate. So It is very important from a technological point of view to produce small crystals of ice by quick freezing.[70]

Freezing of the aqueous formulation in flasks under the thermodynamic freezing degree of the solvent, give rise to most of the water to form ice crystals and the solvents to become crystallized or converts into a solid amorphous system.[71]

2. Primary drying (Sublimation)

Primary drying, where the frozen water (ice crystals) get sublime. usually, most of the water in the substance is extracted at this stage.[72]

The frozen substance is exposed to a main space with fractional steam compression a little under the fractional equilibrium steam compression of the ice at the temperature of the substance, this is carried out by sublimation of the ice crystals.[73]

The sublimated water is then moved out with a particular rapidity in the way to a condensation system and enclosed on it, If the rapidity of the water steam from the substance to the cold trap and the condensation rate of water at the trap face are very large.

To make a greater sublimation rate (and therefor lowering the drying time), it is very important to transfer heat to the sublimation interface. The most effective way to obtain this in a freeze-drying process is to rise the shelf temperature.

However, the temperature of the sublimation interface should be maintained under the eutectic or glass-transition temperature to keep away from the risk of breaking down, which can cause significant material shrinkage and/or incomplete extraction of water.[74]

3. Secondary drying

After primary drying process is complete, and all ice has sublimed, restricted moisture is still exist in the substance. The product may appears to be dry, but the residual moisture content may reach to 7-8% so continued drying process is required at lower temperature to decrease the residual moisture content to ideal values.

Secondary drying is usually takes approximately 1/3 or 1/2 of the time that required accomplishing primary drying. Secondary drying, in which most of the residual water is desorbed from the glass where the temperature of the product is progressively increased while trying to achieve low pressures. Perfectly, the final product is a dry, easily reconstituted cake with a high surface area.[68]

Secondary drying is usually carried out with elevated shelf temperature than that in primary drying, and awareness should be paid to progressively increasing the shelf temperature so as to keep away from any damage to the freezes substance such as shrinkage and/or collapse.[75]

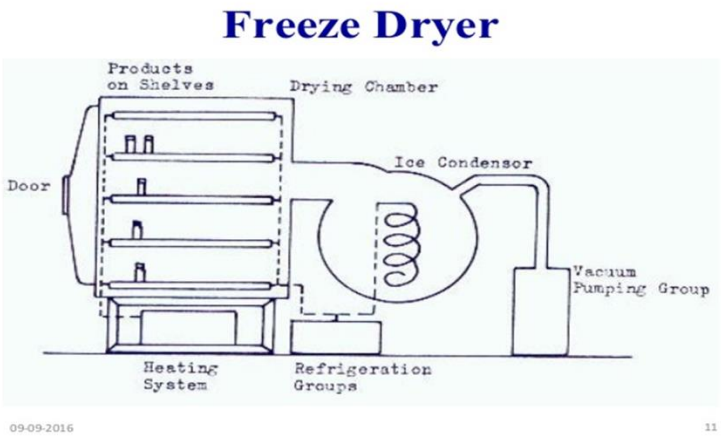

Figure 6: Freeze dryer machine.

Fluidized bed drying

Fluidized bed dryer (FBD) has been used widely for drying various products due to its many advantages, including high drying rate due to an excellent gas-solid contact, high thermal efficiency, relatively low cost of operation.

In fluidized bed drying, hot air is forced through a distributor into bed at a sufficiently high velocity to overcome the gravitational forces on the products. When the air velocity is greater than the gravitational force and the bed resistance, the products will suspend. The particles are fluidized in bed when the drag force created by the gas flow through the bed is equal to the weight of the particles.[76]

It should be noted that fluidized bed drying cannot be used as the sole drying technique for probiotics.[77]

Fluidized-bed drying was processed by layering the microorganisms on spherical pellets. The impact on cell viability of atomizing air pressure, processing temperature and time was investigated. Using 1.5 bar atomizing air pressure, 37 Co processing temperature, and 15 min processing time provided optimal dehydration condition. Fluidized-bed drying caused more substantial losses of cell viability. However, viability of cells pre-treated with membrane protective agents was less affected by fluidized-bed drying than by freeze-drying, resulted in a minor degree of membrane damage after 2 months storage.[78]

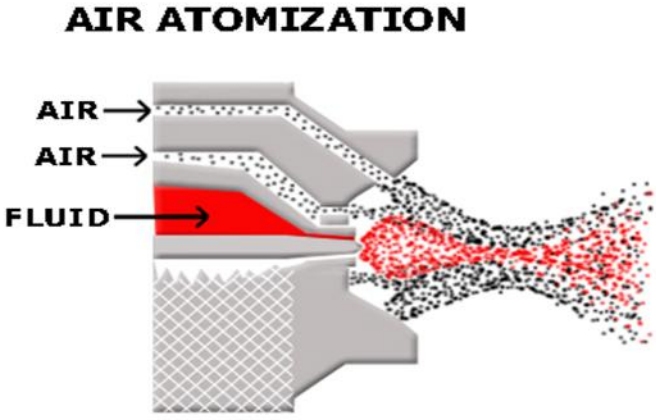

Figure 7: Air atomization.

6- Grinding

Grinding is used in recent automated production to get accurate shaped, high-quality surfaces. The results of the grinding processes mostly depends on the grinding wheel cutting strength, defined as the tool's potential to take off the machined material through the grinding process.[79]

Particle size reduction is a common unit operation in pharmaceutical formulation. Reasons vary from improving homogeneity and dose uniformity content to enhance bioavailability to ensure and make drug products suitable for parenteral delivery. Particle Sciences has the complete group of milling technologies in-house including jet, ball and high energy milling.[80]

A number of agent influence the conditions in the grinding wheel zone of contact (with the work piece), such as: the grinding wheel properties, the grinding

wheel type, process parameters, kinematics, the work piece kind and the consumption and mode of application of the coolant, amongst others.[79]

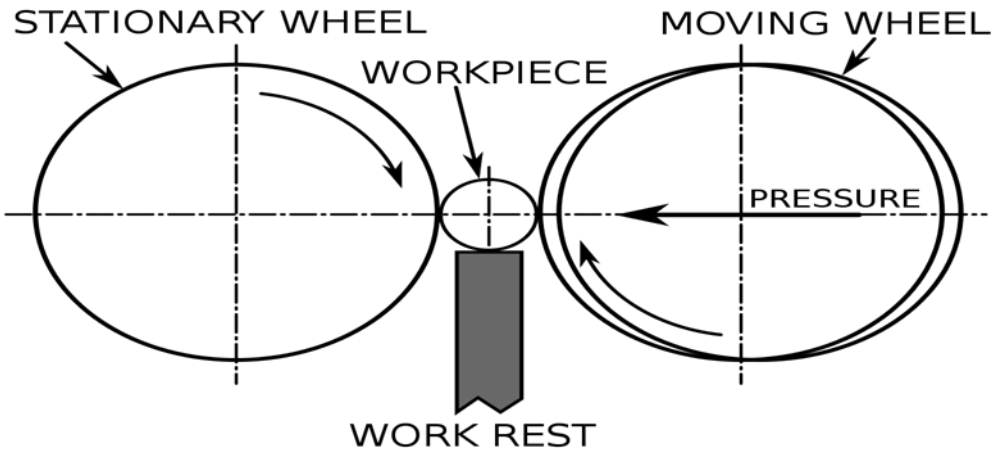

Figure 8: Grinding process.

7- Blending

Blending treatment one of the most importance in the pharmaceuticals industrial production industrial or food commodities. Blending is ticklish in ensuring uniformity of content in the final dosage form, as part of numerous unit operations included in the manufacturing process of pharmaceutical solid dosage forms. When a blend process is examine, two main objectives can be of advantage: first objective, optimizing the process parameters to progress the blending operation effectively and, second, discovering when the blending has reached an homogeneity standard that develop by external regulations or by the manufacturer.[81]

In the pharmaceutical industry, product homogeneity is quite important element in powder mixing because of small scale of powder used per dose. To accomplish product consistency and provide safety, precise mixing is require, which is sometime very difficult to relatively achieve in practice due to the size, shape and physical property variation between powders used to produce pharmaceutical formulations. In the blending of pharmaceutical formulations, shear forces are required to ensure that the often small percentage (1–2 wt.%) of large cohesive drug particle clumps are de-agglomerated and dispersed throughout the bulk excipient.[82]

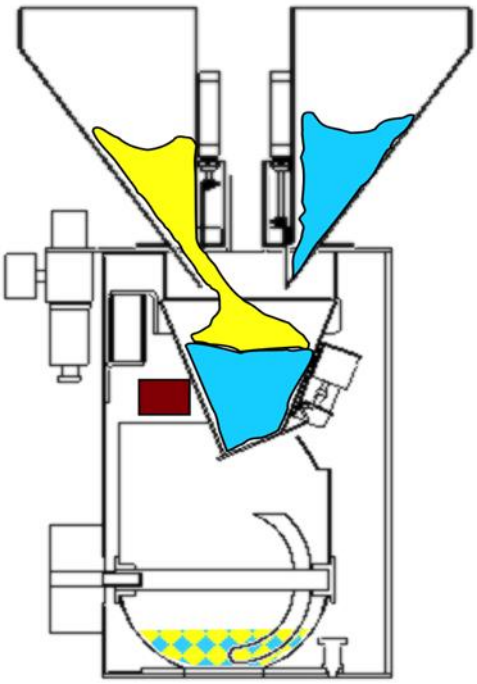

Figure 9: Blending process.

8- Packaging

The packaging plays a fundamental role in maintaining the quality and shelf-life of foods. The package is an integrated part of the preservation system and functions as abarrier between the food and the external atmosphere. The package should be designed and developed not only to hold the food product, but also to protect it and add value to it, since its design may directly affect the purchase decision of the consumer. Consider that the protection provided by the packaging is the factor of greatest importance, since it is directly related with the safety of the consumer.

The packaging materials and the storage conditions are important factors for the quality of products containing probiotic microorganisms.

The level of oxygen within the package during storage of the product should be as low as possible to in order to avoid toxicity and death of the microorganism and the consequent loss of functionality of the product.[83]

Powder

The best material for packaging this product is a desiccated wall bottle, sachet or stick pack. This packaging will pull moisture from the product, supporting longer-term stability compared to using a desiccant component tossed into the bottle.[84]

Tablet and Capsule

There are several different ways to package pills and capsule supplements. The most effective method is a trap blister with a foil barrier. Using this technique, supplement pills and capsules are first pre-packaged in "foil" blisters, before the packages are completed with rigid trap card blisters. The method improves upon traditional face blister alternatives, providing better protection and merchandising advantages.[8]

2.7.6 Different dosage form of probiotics

Expectations for product development of an LBP do not differ from other regulated products that consist of live microorganisms, such as live vaccines or microbial vectors for gene therapy. The development and manufacturing of dietary/food supplement dosage forms is similar to medicines; nevertheless, they do not need to follow the stringent guidelines used in a medicinal product.[20]

Powder dosage form

Powders are basic types of pharmaceutical and food formulations for direct use or as starting point for technical processes.[24]

Oral powders are dosage forms consisting of solid, loose, dry, finely divided particles that are intended for internal use. They may contain bioactive compounds and/or other excipients such as coloring, flavoring, and sweetening agents.[20]

The administration of oral powders could overcome the difficulty in swallowing tablets experienced by children or even some adults.

From a production point of view a production of oral powders measures are taken to ensure an adequate particle size, which is generally done by milling and/or sieving. Powders containing probiotics are frequently used to disperse in water. Only the choice of excipients and mixing are needed for a product to be ready for packing.[23]

Some examples of marketed powders for oral suspensions containing probiotics are presented in Table:[20]

Table 2: Powder product example.

Composition	Probiotic product
UL-250®	Sacharomyces boulardii
Bacilor®	Lactobacillus acidophilus
Antibiophilus®	Lactobacillus casei
Lyobifidus®	Bifidobacterium bifidum

Granulation

Granulation is the process in which primary powder particles from (approx. 0.5 mm up to 1 mm -2 mm) These granulate grains consist of active ingredients (lactic acid bacteria) and excipients are made to adhere to form large, multi-particle entities and collecting particles together by creating bonds between them Bond are formed by compression or by using a binding agent . Binders used in connection with granulation are usually dissolved in water or an organic solvent and thereafter added to or sprayed on the powder which is thus made into granulate and then dried. As the use of water as well as solvents is harmful to freeze-dried lactic acid bacteria other and less traditional methods must be used for granulation. Bond are formed by compression or by using a binding agent. Granulation is extensively used in the manufacturing of tablets and pellets.[23]

Tablet

Tablets are solid dosage forms usually obtained by compressing uniform volumes of particles. Tablets are the most widely used pharmaceutical dosage form.

Tablets are prepared by the application of a high pressure to uniform volumes of powders (direct compression) or granules (obtained by wet or dry granulation) using tablet presses. Tablets may be coated in order to facilitate its administration, to protect its bioactive content, or to modify its release.

To obtain a tablet, it is usually necessary to use some type of excipients such as diluents, binders, disintegrants, lubricants, and glidants. The design of tablets using functional polymers improves the stability of probiotics, contributes to accurate dosing and ease of administration, and provides the ability to produce dosage forms containing probiotics at a large scale. Generally, tablets

containing probiotics are produced by direct compression of mixtures of excipients and freeze-dried probiotics.

Tablets can be designed to modify the release and enhance the adhesion and colonization of the probiotic microorganisms to the epithelial mucosa of human host by using suitable kinds of tablet excipients.

Another technique to enhance the production of tablet is enteric coated technique.

That techniques have been explored in order to protect living bacteria from the unfavorable interaction within the dosage form, adverse gastrointestinal environments (pH, enzymes, bile salts, etc), and to deliver bacteria to the human intestine.[20,25]

Capsules

Capsules are solid dosage forms with hard or soft container or shell made from gelatin or, less often, from other suitable material. There are two types of capsules, hard and soft (one-piece). Hard capsules are generally preferred in order to administer probiotics. The hard capsules, which consist of two cylindrical pieces (body and cap) closed at one end, are typically used to contain powders.

The filling of hard capsules may employ machines that form powder plugs and eject them into the capsule bodies after having separated the cap of the shell.[20]

Encapsulation is the process of forming a continuous coating around an inner matrix that is wholly contained within the capsule wall as a core of encapsulated material, immobilisation refers to the trapping of material within or throughout a matrix. Encapsulation tends to stabilize cells, potentially enhancing their viability and stability during production, storage and handling. An immobilized environment also confers additional protection to probiotic cells during rehydration. The cell immobilization technology has evolved into cell encapsulation technology, which we refer to here as PET. The best application of PET in biopharmacy is the controlled and continuous delivery of cells in the gut. The potential benefit of this therapeutic strategy is to maintain greater cell viability despite the acidity into the stomach.[26]

2.7.7 Factors that affect viability and stability of probiotics

2.7.7.1. Factors that affect viability of probiotics

The majority of manufacturing procedures involves contact of strains with aqueous or organic solvents during the mixing or coating process, exposure to high temperatures during the drying process, and under pressure during the compression, which can cause considerable loss of viability during the preparation process.[22,25]

- **Thermal stress**

During a long-term storage the integrity of probiotics can be damaged by thermal stress as well as commonly applied manufacturing processes such as drying and pasteurization. At higher temperature probiotics are inactivated by denaturation of protein and subsequent cell damages.[22]

- **Oxidative stress**

Since many of probiotic strains are anaerobes or microaerophiles, the viability of probiotics can be deteriorated by the existence of oxygen. Reactive oxygen species are generated under oxidative condition and they interact with probiotic components such as proteins, lipid or nucleic acid.

The growth rate of some of probiotic species were inhibited in the presence of oxygen for example Bifidobacterium spp. In other species such as Lactobacillus acidophilus and Bifidobacterium the oxygen concentration dependent toxicity was observed.[22]

- **Osmotic stress(shock)**

During a drying process osmotic shock impairs the viability of probiotics. Dehydration that happens due to the drying process leads to efflux of water from a probiotic cell, which causes the osmotic shock by increased intracellular molarity in probiotic cells, resulting in damaged cell functions .For example, decreased viability of Lactobacillus plantarum was enumerated due to air drying in a desiccator and a spray dryer.[22]

- **Gastric juice**

After intake of probiotics, the first and biggest barrier for maintaining the viability of probiotics is the harsh environment in the stomach, more specifically the gastric juice, which is extremely acidic.

Probiotics cannot survive under the acidic conditions, owing to disruption in metabolic and cytoplasmic activities. Since the passage through the stomach is inevitable for probiotic to reach the target site, acid resistance is considered an indispensable property of an effective probiotic delivery system. Acid resistance can be tested in vitro using a simulated gastric juice which possesses characteristics of human stomach fluid, such as buffer capacity, osmolality and surface tension.[22]

- **Compression force**

Depending on the pressure applied, the compression of cells may cause damages to the cell walls and membranes or even loss of viability. It is clear that under mechanical stress some cells cannot tolerate such compression. Initially, the increase in the force applied will primarily damage the cell wall, and when such pressure is further increased, it will also reach the cell membrane. Therefore, it has been observed that cellular

viability decreases almost linearly with the applied compression force.[27]

2.7.7.2 Factors effecting stability of probiotics

These factors starting from the strain production process to the storage conditions of the final product, may have a profound effect on the stability and properties of probiotics.

Stability, not only in terms of viability but also in terms of metabolic and functional activity, is needed to maintain the desired sensorial attributes and to provide the claimed health benefit during the whole shelf-life of the product.[28]

Many different conditions present during the manufacture and storage of the product may affect the stability of probiotics; these include, among others, temperature, pH, (WA), oxygen content or the presence of chemicals, and other microorganisms.[28]

Industrial processes may be modified to enhance the stability of the strains. To this regard, the selection of the best suited culture medium composition and cell protectants may positively influence strain survival. The chemical composition of the product may also play a role in stability, the presence of antimicrobial compounds and certain food additives being detrimental. Most probiotic strains are obligate anaerobes or facultative anaerobic microorganisms and, therefore, oxygen content is also relevant. In this case, the solutions adopted have been either to reduce oxygen permeation into the food or introducing oxygen scavengers for reducing its redox potential. Cells can also be physically protected by means of microencapsulation, which has been reported to improve stability of strains and to confer tolerance to GIT conditions.[28]

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2.7.8 Quality control test for different dosage form

The quality in the pharmaceutical industry has become a very important and sensitive issue, there has been a growing awareness for the significance of the quality of the pharmaceutical products. In the pharmaceutical industry, it is essential for controlling the errors during the every stage in production process since total quality of the product must be ensured according to compendia of drugs (2).

The purposes of QC are to produce a perfect finished product by preventing or eliminating errors at every stage in production. QC is a team work and we have to remember that quality must be built into a drug product during product and process design and it is influenced by the physical plant design, space, ventilation, cleanliness and sanitation during routine production.

The quality control of probiotic cultures in foods traditionally has relied solely on tests to ensure that an adequate number of viable bacteria are present in the products throughout their shelf lives. Viability is an important factor, but not the only criterion for quality assurance. To be effective, probiotic strains must retain the functional health characteristics for which they were originally selected. Such characteristics include the ability to survive transit through the stomach and small intestine and to colonize the human gastrointestinal tract. In vitro test protocols can be readily adopted to examine the maintenance of a strain's ability to tolerate acidic conditions, survive and grow in the presence of bile, and metabolize selective substrates. Molecular techniques are also available to examine strain stability. So many types of in vitro testing that can be used to ensure quality control of functional probiotic strains.

It is necessary to test the stability of these characteristics during manufacture and storage.[29,30]

Important criteria for screening and testing

- Phenotype and genotype stability, including plasmid stability; carbohydrate and protein utilization patterns
- Acid and bile tolerance survival and growth, bile metabolism, intestinal epithelial adhesion properties.
- Antibiotic resistance patterns; ability to inhibit known gut pathogens, spoilage organisms, or both

This criteria is necessity for clarifying the ongoing quality control of probiotic bacteria during manufacture and use for continual monitoring of the effectiveness of probiotics in humans. It also indicates the need for selection of more stable probiotic strains for commercial use.[29]

Adhesion stability

Adhesion characterization may be an important quality-control method for assessing gut barrier effects and also considered important for stimulation of the immune system of probiotic microorganisms that adhere to M cells or Peyer's patches.

The properties of adhesion should be carefully monitored, including adhesion to intestinal cells and human intestinal mucus adhesion has been related to

immunogenic effects, shortening the duration of diarrhea, and other health effects.

The use of biopsies from the intestinal mucosa is more accurate means of determining colonization more than fecal samples that have been used in most colonization studies with probiotic bacteria. These, however, reflect only the bacteriologic situation in the fecal material and do not give an accurate picture of the different parts of the gastrointestinal tract or the mucosal layer of the gut. Lactobacillus strains were found to adhere to rectal mucosa obtained from volunteers who had consumed a fermented oatmeal soup.[29]

Acid and Bile stability

To survive passage through the stomach and small intestine, probiotic strains must tolerate the acidic and protease-rich conditions of the stomach, and survive and grow in the presence of bile acids. Acid tolerance is also important for the probiotics' survival Hence, acid tolerance is one of the first properties screened for when selecting probiotic strains.

In vitro assays examining to assess acid tolerance, the inhibitory effect of bile acids on the growth of probiotic strains are also relatively simple to perform and can be applied to ensuring the quality of probiotic cultures during manufacture and storage and throughout the shelf life of the product. Interspecies variation in the ability to grow in the presence of bile is often observed between potential probiotic strains. Such tests have been applied to lactic acid bacteria and Bifidobacterium strains used in the dairy industry and proposed as probiotics. In vivo validation of survival through the human stomach is more difficult to obtain and quantitative extrapolation to probiotic performance in vivo is difficult too.[29]

Viability and Properties during processing and storage

The viability of several strains in fermented milks is dependent on both the production method and the strain. In one study, 5 strains of L. acidophilus and Lactobacillus GG were tested to determine the effect of refrigeration on the viability of the strains in cultured buttermilk and yogurt. In cultured buttermilk, 3 of the strains showed no significant loss of viability during storage, but 2 strains had significantly decreased viability. Results were similar in yogurt. It is possible that cultures producing organic acids, diacetyl, or other inhibitory compounds in the fermented milk may influence the survival of some probiotic cultures. L. casei GG showed no loss of viability during storage of any of the cultured products. Thus, the results indicate that the production method for fermented milk needs to be carefully evaluated to offer consumers the right amount of viable cultures to obtain the reported health effects.[29]

Quality control test for tablet and capsules

The tablet dosage form accounts for approximately 50% of all dosage forms on the market.

Size and Shape

The size and shape of the tablet can be dimensionally described monitored and controlled. It is determined by the tooling during the compression process.[30]

Color and Odor

Many pharmaceutical tablets or capsules use color as a vital means of rapid identification and consumer acceptance. But it must be uniform within a single tablet, from tablet to tablet and from lot to lot. The presence of an odor in a batch could indicate a stability problem.[30]

Thickness

The thickness of a tablet is the only dimensional variable related to the process. Thickness of individual tablets may be measured by a micrometer. Other techniques involve placing 5 or 10 tablets in a holding tray, where their total thickness may be measured by a sliding caliper scale.[30]

Moisture content of granules

Granules should possess sufficient strength to withstand normal handling and mixing processes without breaking down and producing large amounts of fine powder. On the other hand, some size reduction during compaction into tablets is desirable to expose the areas of clean surface necessary for optimum bonding to take place so moisture content is the very important factor for producing good pharmaceutical product.[30]

Mass variation

It is an assay for the active substance(s) on a representative sample of the batch using an appropriate analytical method. The result of this value, expressed as percentage of label claim. Assume that the concentration (mass of active substance per mass of dosage unit) is uniform. Select not less than 30 dosage units, and proceed as follows for the dosage form designated.[31]

Uncoated or film-coated tablets

Accurately weigh 10 tablets individually. Calculate the active substance content, expressed as percentage of label claim, of each tablet from the mass of the individual tablets and the result of the assay.[31]

Hard capsules

Accurately weigh 10 capsules individually, taking care to preserve the identity of each capsule. Remove the contents of each capsule by suitable means. Accurately weigh the emptied shells individually, and calculate for each capsule the net mass of it contents by subtracting the mass of the shell from the respective gross mass. Calculate the active substance content in each capsule from the mass of product removed from the individual capsules and the result of the assay. Calculate the acceptance value.[31]

Soft capsules

Accurately weigh 10 intact capsules individually to obtain their gross masses, taking care to preserve the

identity of each capsule. Then cut open the capsules by means of a suitable clean, dry cutting instrument such as scissors or a sharp open blade, and remove the contents by washing with a suitable solvent.[31]

Hardness test

Is a laboratory test used by the pharmaceutical industry to test the breaking point and structural integrity of a tablet or capsules under conditions of storage, transportation, and handling before usage The breaking point of a tablet is based on its shape. For this test one of the earliest testers was Ketan tablet hardness tester, which is a type of the Monsanto hardness tester to evaluate tablet hardness tester.[30]

Friability test

Is a method, which is employed to determine physical strength of tablets upon exposure to mechanical shock and attrition. Friability test tells how much mechanical stress tablets are able to withstand during their manufacturing, distribution and handling by the customer. Friability of a tablet can determine in laboratory by Roche friabilator. For this test twenty tablets are weighed and placed in the friabilator and then operated at 25 rpm for 4 minutes. The tablets are then dedusted and weighed. As stated by USP if conventional compressed tablets that loss less than 0.5 % to 1 % (after 100 revolutions) of their weight are generally considered acceptable.[30]

Disintegration

Is a process of breaking down of the tablet into smaller particle or granules

The USP disintegration apparatus consist of 6 glass tubes that are 3 inches long, open at the top. To test for disintegration time, one tablet is placed in each tube and the basket rack is positioned in specified medium.

Move the basket containing the tablet up and down.

Uncoated USP tablet have disintegration time as low as than 5 minutes, but the majority have maximum disintegration time of 30 minutes.[30]

Dissolution

Tablet Dissolution is a standardized method for measuring the rate of drug release from a dosage form. Dissolution apparatus (Basket apparatus) consist of a cylindrical vessel with a hemispherical bottom, which may be covered, made of glass or other inert.[30]

2.8. Regulatory status of probiotics

United states (FDA) regulation

The field of probiotics is growing quickly in the United States, as is evidenced by the increasing interest of industry, consumers, and researchers. As professionals embrace this burgeoning area, they need to approach it in a manner that will ensure that product formulations and communications about these products are done responsibly and with the primary objective to benefit consumers. The US Food and Drug Administration (FDA) uses other terms for live microbes for regulatory purposes; live microbes used in animal feeds are called "direct-fed microbials, and, when intended for use as human drugs, they are classified as "live biotherapeutics"

However, no legal definition of probiotics exists in the United States or in other countries, which allows the marketing of products labeled as "probiotics" that do not meet the fundamental criteria stipulated in the scientific definition.[86]

Europe (EMA) regulation

The existing regulatory aspects or standards on probiotics differ from country to country. Among the European countries, probiotics are considered under foods/functional foods/novel foods/natural remedy. Probiotic foods and food supplements are covered by the Food Products Directive and Regulation (regulation 178/2002/EC; directive 2000/13/EU). Some probiotic products which were earlier marketed as herbal products and could not obtain a drug registration as per the directive of Herbal Medicinal Products Directive (2004/24/EC) are put under food supplements category. It is necessary that all probiotic food and supplement health claims are scientifically evaluated by the European Food Safety Authority (EFSA) prior.[21]

3.1. RESULT

- ✓ It was found that there is variation between different LAB probiotic characteristic, it differ in their growth temperature, shape, size, and their type of fermentation. But they are all staining gram positive, and anaerobic bacteria.
- ✓ It was found in this article demonstrate that probiotics were safe and effective for treating some gastrointestinal tract disorder by lowering PH, of the intestine, inhibition the growth of pathogenic bacteria, and also beneficial for dermal issues and arthritis.
- ✓ It was found that LAB probiotic supplement can be formulated in different dosage form including powder, tablet and capsule by specific procedure started from selection of functional strain for amplification and to ferment it till final packing in suitable container.
- ✓ It was found that there is potential to minimum side effect and contraindication for using this LAB probiotic.
- ✓ It was found that there is a huge development of probiotic utilization from the first discovery of it, since it has been used by population based therapies, focusing on problem encountered in developing countries
- ✓ It was found that the differentiate the biovariants based on their response to glucose fermentation analysis. Biochemical test that has been carried out were used to confirm lactobacillus sp.
- ✓ Biochemical characteristics:

Table 3:

Physical/ Biochemical test	L. Fermentum	L. Acidophilus	L. Casei	L. Plantarum	L. Brevis	L. Salivarius
Cellular morphology	Rods	Coccobacilli	Rods	Rods	Rods	Rods
Gram reaction	+	+	+	+	+	+
Growth at 45C°	+	+	_	_	_	+
Growth at 15C°	_	_	+	+	+	_
homo/hetero fermentation	HE	HM	HE	HM	HE	HM
Gas from glucose	+	_	_	+	+	_
Catalase	_	_	_	_	_	_
Motility test	_	_	_	_	_	_
Glucose	+	+	+	+	+	+
Mannitol	_	_	+	+	+	+

3.2. DISCUSSION

- While discussing the efficacy of the LAB probiotics with all their different sizes and shapes of strains it was found that they show effective safety profile on treating certain gastrointestinal disorders.
- With developing of manufacturing techniques took a big steps so the probiotics different dosage form like tablets, capsules and, powders were enhanced and evaluated, started from selection of suitable strains as first step for amplification and to ferment it till final packing in suitable container.
- The body has an array of defense mechanisms. The small intestine is a very active part of the body. “It secretes about six to ten liters of fluid a day Most of the time acid flushes the small intestine and keeps it sterile. as any other pharmacutical products LAB probiotics show minimum side effects and contraindcation the we already mentioned up so It's necessary to heed on these side effects of probiotics and the problems encountered and giving more attention especially in developing countries.
- Probiotics are not all alike. For example, if a specific kind of Lactobacillus helps prevent an illness, that doesn’t necessarily mean that another kind of Lactobacillus would have the same effect or that any of the Bifidobacterium probiotics would do the same thing.
- Although some probiotics have shown promise in research studies, strong scientific evidence to support specific uses of probiotics for most health conditions is lacking. The Food and Drug Administration (FDA) has not approved any probiotics for preventing or treating any health problem. Some experts have cautioned that the rapid growth in marketing and use of probiotics may have outpaced scientific research for many of their proposed uses and benefits.
- Whether probiotics are likely to be safe for you depends on the state of your health.
- In people who are generally healthy, probiotics have a good safety record. Side effects, if they occur at all, usually consist only of mild digestive symptoms such as gas. On the other hand, there have been reports linking probiotics to severe side effects, such as dangerous infections, in people with serious underlying medical problems. The people who are most at risk of severe side effects include critically ill patients, those who have had surgery, very sick infants, and people with weakened immune systems
- Even for healthy people, there are uncertainties about the safety of probiotics. Because many research studies on probiotics haven’t looked closely at safety, there isn’t enough information right now to answer some safety questions. Most of our knowledge about safety comes from studies of Lactobacillus and Bifidobacterium; less is known about other probiotics. Information on the long-term safety of probiotics is limited, and safety may differ from one type of probiotic to another.
- We don’t know how much of the probiotic people would have to take or who would most likely benefit from taking probiotics. Even for the conditions that have been studied the most, researchers are still working toward finding the answers to these questions.
- There must be biochemical and quality control tests of probiotic traditionally used to confirm lactobacillus sp bioavailability and to ensure that an adequate number of viable bacteria are present in the products throughout their shelf lives and to differentiate biovariants of species based on their analysis of glucose fermentation response. Viability is an important factor, but not the only criterion for quality assurance.
- the common quality-control parameter of colony-forming units per gram may not be the only parameter indicative of the efficacy of the final product. Other factors, such as probiotic growth during product manufacture, enteric coating, preservation technology, metabolic state of the probiotic, and the presence of other functional ingredients in the final product, may play a role in the effectiveness of a product. More research is needed to understand how much influence such factors have on in vivo efficacy.

3.3. CONCLUSION

- In the present study we conclude the general background, including history of probiotics, its use and its applications in various products with the food industry. Probiotics have been found to be useful in treating specific medical conditions. Several mechanisms include the following: competitive adherence to the mucosa, secretion of antimicrobial substances, strengthening of the gut, and modulation of the immune system.
- The criteria for the selection of probiotics include the tolerance to gastrointestinal conditions (gastric and bile), ability to adhere to the gastrointestinal mucosa.
- Lactobacillus species are divided into two: homofermentative and heterofermentative. There are 6 common different types of lactobacillus species each one has its own molecular and biological characteristics according to their morphology and shape.
- Regarding safety, side effect, contraindication and drug interaction it was found that lactic acid bacteria have a good safety profile.
- From a production point of view the different types of dosage forms were introduced based on their development, selection criteria, quality control tests and certain methods.
- When someone is trying to live a healthier life it's easy to overlook for his/her gut. It's important for all people to understand the importance of taking probiotics and taking care of their gut which has a huge impact on their mental health.
- The Sudan drug regulatory commission should conduct further investigations being in continuous evolution and the need for further studies should be conducted.

3.3 Recommendation

- There is a need for further studies on probiotics which are basically as mentioned earlier in this research in a continuous evolution when compared the first form of probiotics to the one in which there is currently, studying new types of beneficial bacteria to benefit as much as possible in improving the humans health is necessary. In some countries, probiotics are not considered as pharmacological therapy, or treated as a one of medications perhaps with increased scientific research in the future changing their consideration and giving probiotics more attention.
- We suggest to the Sudan Drug Regulatory Commission that to give more heeding to probiotics and provide it in local pharmacies as appropriate and conduct further investigations to ensure its effectiveness and safety to the patient's.
- Increase Pharmacists' awareness about Probiotics, as a new product and provide reliable sources and adequate research's to learn more.
- Utilizing the expertise of the advanced countries in the area of research's, production and manufacturing of probiotics, to be produced and developed locally in the near future.

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