

Industrial Production and Health Benefits of Probiotics

Sakib Ahmed¹, Apurva Koul²

^{1,2}University Institute of Biotechnology, Chandigarh University, Gharuan

Abstract

The probiotics means the form of live micro-organisms present in human dietary components. There are many types of bacterial strains used in the probiotic production but *lactobacillus* sp. are most common among them. Production of these agents is done by fermentation process. Gastro-intestinal health is ensured by these probiotics themselves. They kills pathogenic bacteria and start growing a healthy neighbor which in return ensures health of an individual.

INTRODUCTION

Probiotics are the live microorganisms which are used to alter the gastrointestinal flora of the human to provide many health benefits. They are often called the good bacteria. They act as the immunity enhancing materials by competing with the bad bacteria to give better digestion and immunity. The most likely used probiotics are lactobacilli, bifidobacteria, bacilli and yeast.

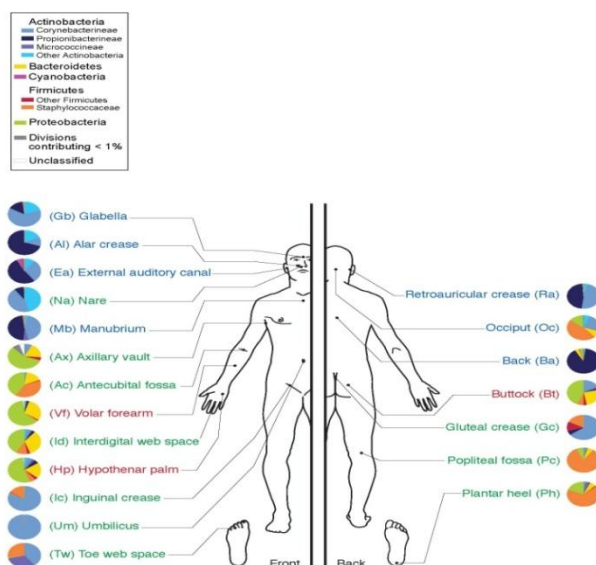


Fig.2: Human Microbiota

The association of probiotics with the healthy life is far of history. A long time has been gone since Tissier concluded that the gut microbiota of breast fed infant has been dominated by the rod bacteria with a bifid shape (bifidobacteria) which were not present in the formula based breast fed infants that were suffering from diarrhea. This concept concluded that gut microflora played a role in maintaining good health.

After this concept, a number of studies were conducted but they were initially failed due to the strain specificity and the slow growth of probiotics in different hosts. This was a very big challenge for researchers to identify and to solve the problems of specificity and substrate. Now that the industries are capable of producing huge quantities of probiotics, the world is going in a very different direction to be health conscious and to consider probiotics in their daily routine. Fermentation is one of the crucial process which allows the industries to produce these healthy probiotics. The most commonly used species of bacteria is *Lactobacillus* sp., which can be characterized as the gram-positive, non-motile, non-sporulating bacteria that produces the lactic acid as their main bioproduct during their fermentation.

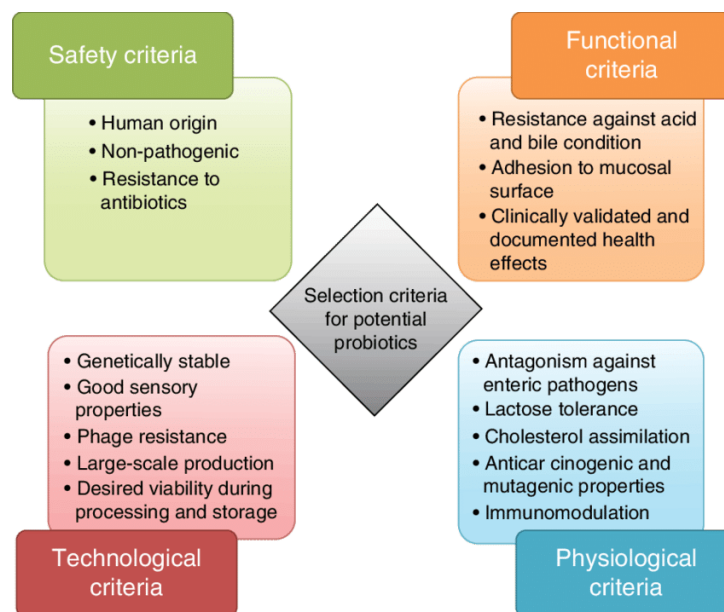


Fig.1: Selection Criteria for probiotics

History of Probiotics

A long time ago, Russian scientist, Nobel laureate and professor Elie Metchnikoff stated that "lactic acid bacteria (LAB) gives health benefits which provides the longevity of life." He suggested that "intestinal auto-intoxication" and the aging factor could be repressed by modification of gut microflora and replacing proteolytic microbes such as *Clostridium*, which produce toxic substances like ammonia, phenols and indoles from digestion of proteins. Elie Metchnikoff developed fermented milk with bacterium "*Bulgarian bacillus*".

In 1917, a German professor Alfred Nissle had isolated a non-pathogenic bacteria from the feces of a World War soldier who did not develop enterocolitis during a severe outbreak of shigellosis. Different disorders of intestinal tract were treated with that respective non-pathogenic strain. The "*Escherichia coli* Nissle 1917" is an example of non-LAB probiotic. In the Pasteur Institute, Henry Tissier isolated the first *bifidobacterium* from a breast-fed infant and he gave the name "*Bacillus bifidus communis*" to that strain. He sentenced that bifidobacteria would replace the diarrhea causing bacteria.

Production of Probiotics

Strain Selection and Isolation

The process of producing the probiotics starts with strain selection and their isolation. This is first most important step for the production of probiotics. The individual strain of *lactobacillus*, *bifidobacteria*, *saccharomyces boulardii* etc are taken and purified and these are also validated for its capabilities, health benefits and safety.

Media Preparation

In order to produce a viable probiotic, the scientists will have to make a suitable media that can proliferate and enhance the ability of probiotics. The media contains nitrogen sources, water, salts, carbohydrates and other micronutrients that are necessary for the bacterial growth. MRS Broth is used to cultivate the LAB or bifidobacteria. This process is carried out in the large scale fermentor.

Higher cell count of bifidobacteria is produced in the ultra skimmed milk of different protein concentration and milk with the nitrogenous substrates

Fermentation

Once the selection and isolation of strain is done, the substrate along with strain enters in large scale fermentation which is the initial step for commercial production of probiotics. Initially all the materials and equipments would be properly sterilized before providing to bacteria. Fermentation starts by multiplying the bacteria with their favorable conditions and after the desired cell count is reached, the process will be terminated. The fermentation technologies like immobilized cell system and continuous culture are not generally used in the food industry but they can be used to enhance probiotics values.

Separation and Filtration

After the fermentation is done, the product would be separated and filtered through several procedures. The centrifuge is mainly used to separate the probiotic strains from other materials. During this process, the material is kept in the centrifuge and gets rotated at an appropriate rpm which helps to separate probiotics from the other by products and un-wanted metabolites.

For the centrifugation of large scale fermented products, the large scale centrifugation is required which is quite expensive and every industry could not use it hence they opt for filtration.

Cryoprotection

Once the desired strains got separated, they now need to be stored for a long time and this requires a good cryoprotecting agent. The probiotics cells should not get any injury. To fulfill this demand, the cryoprotectants or/and the lyoprotectants added to probiotics to protect from injury during freezing and freeze-drying.

Cryoprotectants stop the rate of ice formation by increasing the viscosity of solution and protect the amorphous structure of ice in close proximity of cell whereas lyoprotectants stabilize the cellular lipid bilayer membrane when there is no water present.

The most commonly used cryoprotectant and lyoprotectant are peptides and carbohydrates whereas, skim milk powder is used in dairy industry. Once the probiotics got protected, they are ready to perform different freezing processes.

Pelletization

Received 15 December 2020; Accepted 05 January 2021.

Pelletizing is the process of making pellets by compressing the desired material. The pellets which are 4-5 mm in diameter are taken from a bath of liquid nitrogen and packed in bags which are stored and shipped at -45 to -55 degree Celsius.

Packaging

The probiotics that are formed in dosage form are carefully packaged and ready to ship. Packaging and shipment are very crucial steps in maintaining the viability. These products can best perform when protected from such conditions:

Light- Probiotics give best results when protected from direct sunlight therefore the black and opaque bottle glass are more appropriate to block the harmful UV rays.

Temperature- The shelf life of probiotics is sensitive to heat. High temperature will lead to destruction of viable cells. So the temperature should be kept low as much as possible.

Moisture- The bacteria present in powdered form, are hibernating and they are dormant until the consumption. Moisture will cause the activation of these bacteria therefore; no moisture should be present in the place during storage and shipment.

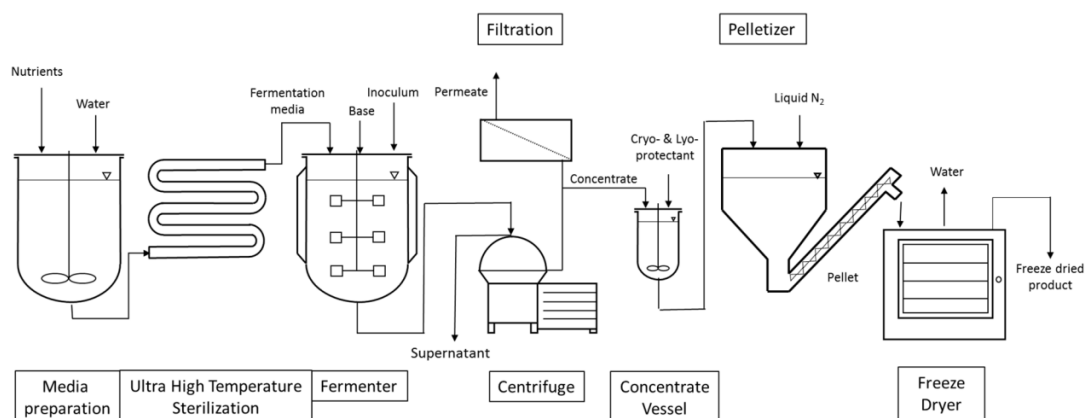


Fig.3: Production of probiotics

Quality Assurance

The quality assurance team sets strict rules to avoid contamination during all the processes so that proper hygiene and sanitization can be kept constant. The quality assurance team is responsible to ensure everything from equipment to process for the production of pure probiotics.

Quality Control

This verification is done during all steps and processes. The probiotic are tested on different parameters to ensure that they should meet rigorous probiotic testing standards.

After inspecting all the terms, the probiotic company can confirm that the respective probiotics are safe for human use.

Health Benefits of Probiotics

Probiotics have the ability to intend the body's natural gut microbiota. Many probiotic preparation have been used to treat diarrhea caused by antibiotics. There are various studies concluded positive probiotic effects on different types of extraintestinal and gastrointestinal disorders including Irritable Bowel Syndrome, Inflammatory Bowel Disease, Vaginal Infections and immune enhancement.

BENEFICIAL PROBIOTIC STRAINS			
BIFIDOBACTERIUM BIFIDUM the most dominant probiotic in infants and in the large intestine. Supports production of vitamins in gut, inhibits harmful bacteria, supports immune system response and prevent diarrhea.	LACTOBACILLUS ACIDOPHILUS relieves gas, bloating, improves lactose intolerance. Shown 61% reduction in <i>e. coli</i> , lower cholesterol levels, and creating of vitamin K. Also, important in GALT immune strength.	BACILLUS COAGULANS an endospore probiotic that is heat resistant and improves nutrient absorption. Also has been shown to reduce inflammation and symptoms of arthritis.	BIFIDOBACTERIUM LONGUM supports liver function, reduces inflammation, removes lead and heavy metals.
LACTOBACILLUS CASEI supports immunity, inhibits <i>h. pylori</i> , and helps fight infections.	BIFIDOBACTERIUM INFANTIS alleviates IBS symptoms, diarrhea, and constipation.	LACTOBACILLUS BREVIS shown to survive the GI tract, boost cellular immunity, enhanced natural T-killer cells, and kill <i>h. pylori</i> bacteria.	BIFIDOBACTERIUM BREVE helps colonize healthy gut community and crowd out bad bacteria.
BACILLUS SUBTILIS an endospore probiotic that is heat resistant. Elicits a potent immune response and supports GALT. Suppresses growth of bad bacteria like salmonella and other pathogens.	LACTOBACILLUS BULGARICUS a powerful probiotic strain that has been shown to fight harmful bacteria that invades your digestive system and is stable enough to withstand the acidic digestive juices of the stomach. It also neutralizes toxins and naturally produces its own antibiotics.	LACTOBACILLUS RHAMNOSUS supports bacterial balance and supports healthy skin. Helps fight urinary tract infections, respiratory infections, and reduce anxiety by reducing stress hormones and GABA neurotransmitter receptors. Also, survives GI tract.	SACCHAROMYCES BOULARDII a yeast probiotic strain that restores natural flora in the large and small intestine and improves intestinal cell growth. It has proven effective in treating inflammatory bowel disease like Crohn's disease. It's been shown to have anti-toxin effects, be antimicrobial, and reduce inflammation.

Fig.4: Some Diseases and related Probiotics

1. Colon Cancer Treatment:

The effect of oligofructose plus two probiotic strains in patients at risk of developing colonic cancer is tested by SYNCAN study resulting that a symbiotic preparation can suppress the expression of biomarkers for colorectal cancer.

2. *Helicobacter pylori* Infection:

The *Helicobacter pylori* is a Gram negative pathogen that is responsible for type B gastritis, peptic ulcer and gastric cancer. There is development of probiotics going on for such pathogens. According to the study, in vitro, the lactic acid bacteria can resist the growth of pathogen and decreases the urease enzyme activity that is necessary to remain in acidic condition in stomach to the pathogen. But before commercialization, these anti-*Helicobacter* probiotics needs to be controlled and checked for the safety and health as they can cause some side effects like fewer, acid flux and low risk of recurrent infection.

3. Inflammatory diseases and Bowel syndrome:

Some diseases like pouchitis and Crohn's disease and irritate bowel syndrome can be caused or supported by alteration in the gut microflora. Some studies shows that the role of probiotics in associate manner can have potential to control many diseases.

4. Constipation:

The ability of probiotic therapy to alleviate constipation (difficulty in passing stool, excessive hardness of stool, slow transit through the bowel) is debatable, but many may be feature of selected strains.

5. Lactose Intolerance:

It is a genetically identified beta-galactosidase deficiency that results in the hydrolysis of lactose into glucose and galactose. On reaching at the large bowel, the remaining lactose degraded by bacterial enzymes causing osmotic diarrhea. The cause of beta-galactosidase

deficiency include pelvic radiotherapy which damages mucosa and generates the infection of rotavirus which infects lactase producing cells and short bowel syndrome.

Lactase intolerant person develops diarrhea, discomfort in abdomen. Some yoghurt preparations, using *S. thermophilus* and *L. delbrueckii* sp. *Bulgaricus*, are more efficient in this because higher beta-galactocidase activity which increases the lactose metabolism and it claims as the health benefit due to probiotics.

6. Probiotics and Allergy:

Evidences exist that early exposure to bacteria can play a role in protecting against allergy, therefore the probiotics provide a better immune system in infants. In the same time, these probiotic sort of material can improve mucosal barrier function. The role of gut microflora in helping against allergies is infant and children in qualitative as well as quantitative differences.

Brand/trade name	Food type	Sources/strains	Manufacturer company	Country
Aciforce	Freeze-dried product	<i>Lactococcus lactis</i> , <i>Lactobacillus acidophilus</i> , <i>Enterococcus faecium</i> , <i>Bifidobacterium bifidum</i>	Biohorma	The Netherlands
Actimel	Probiotic yoghurt drink	<i>Lactobacillus casei</i> Immunitas	Danone	France
Activia	Creamy yoghurt	<i>Bifidus Actiregularis</i>	Danone	France
Bacilac	Freeze-dried product	<i>Lactobacillus acidophilus</i> , <i>Lactobacillus rhamnosus</i>	THT	Belgium
Bactisubtil	Freeze-dried product	<i>Bacillus</i> sp. strain IP5832	Synthelabo	Belgium
Bififlor	Freeze-dried product	<i>Lactobacillus acidophilus</i> , <i>Lactobacillus rhamnosus</i> , <i>Bifidobacterium bifidum</i>	Eko-Bio	The Netherlands
Hellus	Dairy product	<i>Lactobacillus fermentum</i> ME-3	Tallinna Piimatööstuse AS	Estonia
Jovita Probiotisch	Probiotic yoghurt	<i>Lactobacillus</i> strain	H & J Bruggen	Germany
Proflora	Freeze-dried product	<i>Lactobacillus acidophilus</i> , <i>Lactobacillus delbrueckii</i> subsp. <i>bulgaricus</i> , <i>Streptococcus thermophilus</i> , <i>Bifidobacterium</i>	Chefaro	Belgium
Provie	Fruit drink	<i>Lactobacillus plantarum</i>	Skanemejerier	Sweden
ProViva	Natural fruit drink and yoghurt	<i>Lactobacillus plantarum</i>	Skanemejerier	Sweden
Rela	Yoghurt, cultured milk and juice	<i>Lactobacillus reuteri</i>	Ingman Foods	Finland
Revital Active	Yoghurt and yoghurt drink	Probiotics	Olma	Czech Republic
Yakult	Milk drink	<i>Lactobacillus casei</i> Shirota	Yakult	Japan
Yosa	Yoghurt-like oat product	<i>Lactobacillus acidophilus</i> , <i>Bifidobacterium lactis</i>	Bioferme	Finland
Vifit	Yoghurt drink	<i>Lactobacillus</i> strain	Campina	The Netherlands
Vitamel	Dairy products	<i>Lactobacillus casei</i> GG, <i>Bifidobacterium bifidum</i> , <i>Lactobacillus acidophilus</i>	Campina	The Netherlands

Source: Siró et al. (2008), Vergari et al. (2010), Kaur and Das (2011)

CONCLUSIONS

Probiotics are not less than miracle in the improvement of health. There are health benefits of probiotics deeper than oceans but on the other side they can sink the person too. The organic material stands against pathogen in the gut of animals and reproduces there forming a large military of these fighting agents. Probiotics provides numerous benefits in resisting pathogenic bacteria. Single stain or/and multipl strains of these bacteriaacts acts as barrier and protects the body and its vital and significant parts.

The selection of these strains is very hard as prior probiotic production; each and every study should be conducted to know the limits of benefits to be gained.

REFERENCES

- [1]. Production of High-Quality Probiotics by Fermentation - Scientific Figure on ResearchGate. Available from: https://www.researchgate.net/figure/Desirable-selection-criteria-for-potential-probiotic-micro-organisms_fig1_280057159 [accessed 17 Dec, 2020]
- [2]. Advances in Probiotics, Prebiotics and Nutraceuticals - Scientific Figure on ResearchGate. Available from: https://www.researchgate.net/figure/1-List-of-probiotic-products-available-in-the-market_tbl1_324371121 [accessed 17 Dec, 2020]
- [3]. <https://www.mayoclinichealthsystem.org/hometown-health/speaking-of-health/an-introduction-to-probiotics>
- [4]. <https://www.google.co.in/amp/s/uaslabs.com/probiotic-production-explained/amp/>
- [5]. Zhou, J.S.; Shu, Q.; Rutherford, K.J.; Prasad, J.; Gopal, P.K.; Gill, H. Acute oral toxicity and bacterial translocation studies on potentially probiotic strains of lactic acid bacteria. *Food Chem. Toxicol.* 2000, 38, 153–161. [Google Scholar] [CrossRef]
- [6]. Morovic, W.; Roper, J.M.; Smith, A.B.; Mukerji, P.; Stahl, B.; Rae, J.C.; Ouwehand, A.C. Safety evaluation of HOWARU((R)) restore (*Lactobacillus acidophilus* NCFM, *Lactobacillus paracasei* Lpc-37, *Bifidobacterium animalis* subsp. *lactis* BI-04 and *B. lactis* BI-07) for antibiotic resistance, genomic risk factors, and acute toxicity. *Food Chem. Toxicol.* 2017, 110, 316–324. [Google Scholar] [CrossRef] [PubMed]
- [7]. Cremon, C.; Barbaro, M.R.; Ventura, M.; Barbara, G. Pre- and probiotic overview. *Curr. Opin. Pharmacol.* 2018, 43, 87–92. [Google Scholar] [CrossRef]
- [8]. <https://www.yakult.com.ph/production-process>
- [9]. Fernandez, M.; Hudson, J.A.; Korpela, R.; de los Reyes-Gavilan, C.G. Impact on human health of microorganisms present in fermented dairy products: An overview. *Biomed. Res. Int.* 2015, 2015, 412714.