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REVIEW ARTICLE

Probiotics Role in Food Processing

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ABSTRACT
Probiotics are defined as live microbial food ingredients that have a beneficial effect on human health. The term "probiotics" refers to the products containing live microorganisms, which increase the population of friendly intestinal bacteria upon ingestion. US FDA has recommended that the minimum probiotic count in a probiotic food should be at least 10^6 CFU/ml. Lactic acid bacteria (LAB) and bifidobacteria are the most common types of microbes used as probiotics. These are also called as "friendly bacteria" or "good bacteria", as they maintain natural balance of the microorganisms by reducing the growth of harmful bacteria and promote the healthy digestive system. The global functional food market is thriving with a $50 billion annual share in that the world probiotic market alone is estimated at $15 billion. Therefore, there is a huge scope of development of functional foods incorporated with different probiotic microorganisms.
Keywords: *Functional foods, Lactobacillus. casei, Probiotics, Prebiotics, Yakult.*

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INTRODUCTION

The term "probiotics" (meaning "for life" in Greek) refers to the products containing live microorganisms, which increase the population of friendly intestinal bacteria upon ingestion. Probiotics are defined as live microbial food ingredients that have a beneficial effect on human health (25). The FAO/WHO defines probiotics as 'live microorganisms which when administered in adequate amounts confer a health benefit on the host' (12). Probiotics are viable microbial dietary supplements that, when introduced in sufficient quantities, positively influence our health mainly by improving the composition of intestinal microbiota. There are a large number of probiotics currently used and available in dairy fermented foods, especially in yogurts.

Some selected strains of *Lactobacillus, Bifidobacterium, Streptococcus, Lactococcus* and *Saccharomyces* have been promoted in food products because of their reputed health benefits. The physiological effects related to probiotic bacteria include the reduction of gut pH, production of some digestive enzymes and vitamins, production of antibacterial substances, e.g., organic acids, bacteriocins, hydrogen peroxide, diacetyl, acetaldehyde, lactoperoxidase system, lactones and other substances, reconstruction of normal intestinal microflora disorders caused by diarrhoeas, antibiotic therapy and radiotherapy, stimulation of immune functions, suppression of bacterial infections, removal of carcinogens, improvement of calcium absorption as well as the reduction of faecal enzyme activity.

Lactobacillus and *Bifidobacterium* are most commonly used probiotics in food and feed (Table.1) which have been suggested to be associated with alleviation of lactose intolerance; prevention and cure of viral, bacterial and antibiotic or radiotherapy induced diarrhoeas; immunomodulation; antimutagenic and anticarcinogenic effects and even blood cholesterol reduction. Other microorganisms such as yeast *Saccharomyces cerevisiae* and some *Escherichia coli* and *Bacillus* species are also used as probiotics. Lactic acid bacteria (LAB) which have been used for food fermentation since the ancient time, can serve a dual function by acting as food fermenting agent and potentially health benefits provider. LAB is recognized as GRAS with no pathogenic or virulence properties have been reported. For the use of LAB as probiotics,

some desirable characteristics such as low cost, maintaining its viability during the processing and storage, facility of the application in the products, resistance to the physicochemical processing must be considered.

Table 1: Probiotic microorganisms [24, 19]

Lactobacillus **Species**	***Bifidobacterium*** **Species**	**Others**
L. acidophilus	*B. adolescentis*	*Bacillus cereus*
L. brevis	*B. lactis*	*Enterococcus faecalis*
L. casei	*B. longum*	*Clostridium butyricum*
L. rhamnosus	*B. infantis*	*Enterococcus faecium*
L. fermentum	*B. bifidum*	*Escherichia coli*
L. lactis	*B. breve*	*Pediococcus acidilactici*
L. gallinarum		*Lactococcus lactis subsp. cremoris*

The term "Prebiotics" was first coined by Gibson and Roberfroid and they define it as, "a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon". "Synbiotics" applies to product that has a mixture of both prebiotics and probiotics. The prefix 'syn' implies the synergism between probiotics and prebiotics *i.e.,* they work well together (27).

HISTORY

The probiotic concept was first introduced by Nobel Laureate Elie Metchnikoff, who hypothesized that fermented dairy products, rich in lactic acid cultures, improved longevity (21). He 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 micro flora of the colon, decreasing toxic microbial activities. The actual introduction of the concept belongs to Lilly and Stillwell in 1965, after which probiotics are characterized as "microorganisms that promote growth of other microorganisms" (20). In 1974, Parker talks about a food supplement for livestock and improved name of probiotics as "organisms and substances that helps the microbial ecosystem" (23). Their importance was highlighted by Fuller (13) who described probiotics as live microorganisms with beneficial effects on host body, improving intestinal microbial balance (13). Today the universal meaning of the term "probiotic" was established by the *World Health Organization* and the *Food and Agriculture Organization* of the United States. These two organizations defined probiotics as "live microorganisms which when administered in adequate amounts, have a beneficial effect on health of the host organism". In 2008, Yakult-Danone India (a joint venture of Japanese global probiotics leader, Yakult, with the French food major, Group Danone) entered the Indian probiotic market by launching their first product 'Yakult', a probiotic curd in December 2008.

PROBIOTIC NOMENCLATURE AND DOSING

Probiotic microbes are categorized by genus and species using standard taxonomy, with an alphanumeric designation to identify specific probiotic microbes to the strain level (15). For example, with the strain *Lactobacillus acidophilus* ATCC4356, the genus is *Lactobacillus*, the species is *acidophilus* and the strain designation is ATCC4356. Maintaining the strain designation is important since strains even of the same species can have different effects. The majority of probiotics are strains of the genera *Lactobacillus* and *Bifidobacterium*, but other bacteria are also used (e.g. *Streptococcus thermophilus*, *Enterococcus faecium*, *Propionibacterium freudenreichii* and *Bacillus coagulans)*. Dosing for probiotics is calculated in colony forming units (CFU), which indicate the number of viable microorganisms able to form a colony on an agar plate and this is generally expressed in a specified amount of the product (e.g. 10^9 CFU/capsule or 1 billion CFU/gram). Because recommended doses vary depending on the strain or product, it is not possible to state a "general dose" for probiotics; the dosage used in clinical practice should be based on human studies supporting the intended health benefit. Table 2 shows the recommended dose for the *Lactobacillus* species.

Table 2: Recommended dose of *Lactobacilli* (5)

Strains	Effective dose in CFU/day
L. casei shirota	6.5×10^9
L. rhamnosus GG	109×10^{10}
L. plantarum 299 v	5×10^8
L. acidophilus NCFB 1748	3×10^{11}
L. rhamnosus DSM 6594	16×10^9

US Food and Drug Administration have recommended that the minimum probiotic count in a probiotic food should be at least 10^6 CFU/ml. Table 3 gives some of the probiotic strains and their sources that are regarded as safe for consumption for humans by US FDA.

Table 3: US FDA recommended probiotic strains and their sources (26)

Strains	Sources
L. acidophilus NCFM®	Danisco (Madison WI)
B. infantis 35264	Procter & Gamble (Mason OH)
L. fermentum VRI003 (PCC)	Probiomics, Eveleigh, Australia
L. rhamnosus R0011, *L. acidophilus R0052*	Institute Rosell (Montreal, Canada)
L. casei strain Shirota*, B. breve* strain Yakult	Yakult (Tokyo, Japan)
L. johnsonii Lj-1 (same as NCC533 and formerly *L. acidophilus La-1)*	Nestle (Lausanne, Switzerland)
L. rhamnosus GG ("LGG")	Valio Dairy (Helsinki, Finland)
B. longum BB536	Morinaga Milk Industry Co., Ltd. (Zama-City, Japan)
L. rhamnosus HN001 (DR20)	Fonterra (Wellington, New Zealand)

WORLD SCENARIO OF PROBIOTICS FOODS

The global functional food market is thriving with recent estimates indicating up to a $50 billion annual share (30). The world probiotic market is estimated at $15 billion. The world probiotic market was estimated to 10% of the lactic bacteria drink market. Today, this market is growing at a pace of 5 to 30% depending on the country and product type (3). In Europe, the largest segment of this market comprises foods prepared with probiotics, prebiotics or synbiotics. The probiotic market, especially dairy products such as yogurts and fermented milks, has experienced rapid growth as functional foods in Europe. Probiotics are available in the United States in foods, dietary supplements and medical foods. More than 100 companies in the United States market probiotic products in supplement form. A few of these are listed in Table 4 (26).

Table 4: Probiotic products with targeted health benefits available in the US (26)

Indication	Strains	Products
Infant diarrhoea	*L. rhamnosus GG*	Culturelle (capsule) Danimals (drikable yogurt)
Antibiotic associated diarrhoea; *C. difficile*	*S. boulardii* *L. rhamnosus GG* *L. casei DN114001*	Florastor (powder) Culturelle (capsule) Danimals (drikable yogurt) DanActive (fermented milk)
Immune support	*L. rhamnosus GG*	Culturelle (capsule) Danimals (drikable yogurt)
Irritable bowel syndrome symptoms	*B. infantis 35264 (aka* "Bifantis™")	Align (capsules)

Although the worldwide market for probiotics is growing fast, however, in India, the market has just started to conceive with leading companies like Amul, Nestle and Mother Dairy making their first move (6). In India, these companies have come up with their probiotic products, which are very popular. Some of these are presented in Table 5. Yakult-Danone India, a 50:50 joint venture of Japanese global probiotics leader, Yakult, with the French food major, Group Danone, has entered the Indian probiotics market with launching of their product 'Yakult', a probiotic curd in December 2008. Yakult is the only probiotic drink

that contains more than 6.5 billion beneficial bacteria (*Lactobacillus casei* strain Shirota, named after its founder Dr Minoru Shirota). Yakult-Danone India have already invested Rs 136 crore in India and going to invest another Rs 100 crore in 2-3 years to develop the market nationally (6).

Table 5: Probiotic products marketed in India (2)

Probiotic Products	Company
Probiotic curd	Heritage Foods (India) Ltd.
'b-Activ' probiotic curd (*L. acidophilus* and *B. lactis* strain *BB12)*	Mother Dairy
'Nesvita' probiotic yoghurt	Nestle
Probiotic ice creams, 'Amul Prolife', 'Prolite' and 'Amul Sugarfree'	Amul (Brand of Gujarat Cooperative Milk Marketing Federation Ltd.)
Yakult, Probiotic curd with *L. casei* strain Shirota	Yakult Danone India (YDI) Private Limited
Probiotic drugs	Ranbaxy (Binifit) Dr. Reddy's Laboratories Zydus Cadila; Unichem JB Chem; Glaxo Smith Kline
Fructo-Oligo Saccharides, Probiotic drugs	Glenmark Alkem Labs

Major players in the probiotics drug market in India include companies like Ranbaxy (Binifit), Dr. Reddy's Laboratories, which has four probiotic brands, Zydus Cadila, Unichem, JB Chem and Glaxo SmithKline (2). National Research Development Corporation, a Govt. of India enterprise under Ministry of Science and Technology, has also taken active role in commercialization of the probiotic technologies developed in various Indian research institutes and laboratories for popularization of this new concept. Many Indian research institutes that are working on the probiotic technologies are tabulated in Table 6.

Table 6: Indian research institutes involved in Probiotic technologies (7)

Sl. No.	Institute	Technology
1.	Punjab Agricultural University, Ludhiana, Punjab	Probiotic curd
2.	National Dairy Research Institute (NDRI), Karnal, Haryana	Probiotic ice cream
3.	Haryana Agricultural University, Haryana	Cereal based probiotic foods

PROBIOTIC INTERVENTION

Probiotic foods are those foods which carry live mono or mixed culture of microorganisms which when consumed by humans, beneficially affects the host by improving the properties of intestinal microflora by selectively stimulating the growth and/or activity of one or a limited number of naturally present or introduced bacterial species in the colon, leading to improved host health (1).

The main targets of probiotic intervention have been (9, 4)

a) Increasing natural resistance to infectious disease in the gastrointestinal tract and a first line of defence against disease;
b) Prevention of dangerous fungal overgrowth and some allergic reactions;
c) Reducing putrefactive/toxic microbial metabolism in the gut;
d) Promoting optimized digestive processes, allowing maximum nutritional benefit from food;
e) Improved resistance to toxic bowel problems and diarrhoea;
f) Stimulation of the immune system;
g) Improving lactose intolerance conditions;
h) Reduction of cholesterol levels;
i) Act as antioxidants;
j) Controlling diseases where components of the intestinal microbiota have been implicated in aetiology.

SOURCES OF PROBIOTIC STRAINS

The various sources of probiotic strains includes human origin *i.e.* human large intestine, small intestine and breast milk and animal origin includes food sources like raw milk or fermented foods. Some of the important roles to be exhibited by bacterial strains:

1) Colonization in the intestinal, respiratory and urogenital tract. 2) Inhibiting the carcinogenesis, directly or indirectly, by stimulating the immune system. 3) The metabolism of lactose, the absorption of calcium and the synthesis of vitamins (11).

SELECTION CRITERIA FOR PROBIOTICS (12)

According to the WHO/FAO definition, the main criteria for a probiotic strain should be the fact that it confers a health benefit on the host. Several selection criteria for probiotics have been formulated. It should, however, be stressed that probiotic strain characteristics such as survival of the passage through the upper gastrointestinal tract, thus resisting the action of gastric juice, bile salts and digestive enzymes, are not enough to call a certain microbial strain probiotic.

a) Survival of the passage of the upper gastrointestinal tract (resistance to gastric acid, bile salts and
b) proteolytic enzymes; activity during transit from stomach to colon)
c) Interaction with the resident microbiota (ability to transiently adhere to the intestinal epithelium and colonize the colon; no significant effect on the dominant microbiota; effect on the numbers and diversity of the endogenous *Lactobacillus* and *Bifidobacterium* species)
d) Beneficial effect on the function of the host (probiotic-host interactions; prevention of the risk of
e) disease)
f) Resistance towards technological processing (e.g. effects of food matrix, food ingredients, and
g) mechanical and heat treatments) and storage (e.g. refrigeration and duration)
h) High safety profile and excellent tolerance (no risk group II LAB species, no D-lactic acid)

CHARACTERISTICS OF PROBIOTICS

It will determine their ability to survive the upper digestive tract and to colonize in the intestinal lumen and colon for an undefined time period. Some probiotics produce antimicrobial substances like bacteriocins. Therefore, the potential health benefits will depend on the characteristic profile of the probiotics. The most common probiotics are *Lactobacillus* and *Bifidobacterium*. In general most probiotics are gram-positive, usually catalase-negative, rods with rounded ends and occur in pairs, short, or long chains. They are non-flagellated, non-motile and non-spore-forming and are intolerant to salt. Optimum growth temperature for most probiotics is 37 ^{o}C but some strains such as *L. casei* prefer 30 ^{o}C and the optimum pH for initial growth is 6.5-7.0. Probiotics produce a variety of beneficial compounds such as antimicrobials, lactic acid, hydrogen peroxide and variety of bacteriocins. Probiotics should have the ability to interact with the host microflora and competitive with microbial pathogens, bacterial, viral and fungal.

Fuller (13) listed the following features of a good probiotic:

a) It should be a strain, which is capable of exerting a beneficial effect on the host animal (e.g. increased growth or resistance to disease).
b) It should be non-pathogenic and non-toxic.
c) It should be present as viable cells, preferably in large numbers.
d) It should be capable of surviving and metabolizing in the gut environment (e.g. it should be resistant to low pH and organic acids).
e) It should be stable and capable of remaining viable for periods under storage conditions.

MECHANISM OF ACTION OF PROBIOTICS

The mechanism of action of probiotics in humans on its consumption includes (29):

a) Adherence and colonization of gut,
b) Suppression of growth of pathogenic bacteria,
c) Production of antimicrobial compounds,
d) Improved intestinal barrier function,
e) Stimulation of mucosal and systemic host immunity.

INHIBITION OF PATHOGENIC BACTERIA

The gastrointestinal environment contains a wide range of contents ranging from harmless beneficial dietary and microbial flora to harmful pathogenic bacteria. The mammalian organism fights against these pathogenic bacteria through various ways: blocking pathogenic bacteria effects by producing bactericidal

substances and competing with pathogens and toxins for adherence to the intestinal epithelium; regulation of the immune responses by enhancing the innate immunity and modulating pathogen-induced inflammation via toll-like receptor-regulated signalling pathways; regulate intestinal epithelial homeostasis by promoting intestinal epithelial cell survival, enhancing barrier function and stimulating protective responses (fig. 1) (8). The strategy is based on the ability of probiotic bacteria (B) to bind pathogens (C) in intestinal epithelial tissue (A). Anti-pathogenic action of probiotics consists in production of lactic acid (D) which decreases the pH, interacts with the toxins produced by pathogens (E), with the production of hydrogen peroxide (F) and synthesis bacteriocine (G).

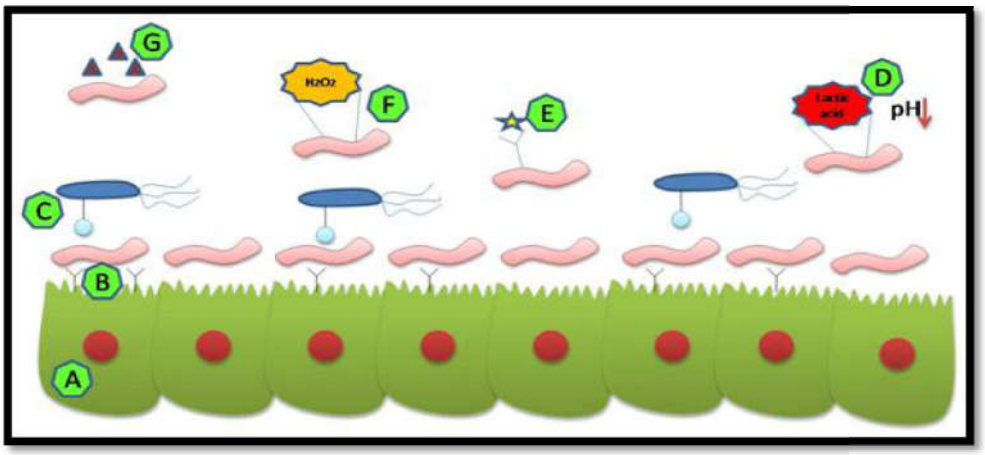


Fig. 1: Schematic representation of mode of action of probiotics in the intestines

PROBIOTICS OF DIFFERENT CATEGORIES

The probiotic products are not only available in the form of dairy formulated products but also available in other non-dairy based products. Some of them are listed in Table 7.

Table 7: Probiotics of different categories (14)

Category	Products
A) Dairy-based probiotics products	Drinkable fresh milk and fermented milks
	Yogurt, Cheese
	chocolate mousse
	frozen fermented dairy desserts
	sour cream
B) Non-dairy based probiotic products	
i) Soy based	Non-fermented soy based frozen desserts
	Fermented soymilk drink
	Soy-based stirred yogurt-like drinks
ii) Fruit and vegetable based	Vegetable-based drinks
	Fermented banana
	Beets-based drink
	Tomato-based drink
	Green coconut water
	Cranberry, pineapple and orange juices
	Cabbage, Carrot and Noni juice
iii) Cereal based	Cereal-based puddings
	Rice-based yogurt
	Yosa (oat-bran pudding)
	Mahewu (fermented maize beverage)
	Maize-based beverage
	Starch-saccharified probiotic drink
	Probiotic cassava-flour product
	Meat products
	Dosa (rice and Bengal gram)

HEALTH PROMOTING EFFECTS OF PROBIOTICS

A wide variety of beneficial health effects have been attributed to probiotics and shown to be strain dependent. Probiotics can influence human health on three levels, in a strain specific manner: (i) by interacting with other microorganisms present on the site of action (competition for nutrients, production of antimicrobial agents, competitive exclusion), (ii) by strengthening mucosal barriers, and/or (iii) by affecting the immune system of the host (22).

Some of the health benefits as suggested by FAO/WHO (12) are given below.

a) Improved food digestion (e.g. proteins, dietary polysaccharides)
b) Reduction of lactose maldigestion
c) Supply and bioavailability of nutrients and growth factors (e.g. vitamins, minerals)
d) Maintenance and balancing of the colon microbiota
e) Reduction of intestinal disturbances (gastrointestinal infections, constipation)
f) Reduction of the risk and duration of diarrhoea (rotavirus, acute infectious, antibiotic- associated, Clostridium difficile-associated, traveller's diarrhoea)
g) Inhibition of undesirable and pathogenic bacteria (orogastrointestinal infections caused by *Streptococcus mutans, Helicobacter pylori, E. coli, Salmonella Typhimurium, Clostridium difficile*; urinary tract infections and respiratory tract infections)
h) Modulation/stimulation of the immune system (cell-mediated and antibody mediated effects)
i) Reduction of the risk of atopic diseases and allergies (asthma, hay fever, food allergy, eczema, dermatitis)
j) Beneficial effects on functional bowel disorders (irritable bowel syndrome)
k) Beneficial effects on inflammatory bowel diseases (pouchitis, ulcerative colitis, Crohn's disease)
l) Anti-carcinogenic activities
m) Lowering of blood serum cholesterol levels

PROBIOTIC PRODUCTS

The fermented dairy products enriched with probiotic bacteria have developed into one of the most successful categories of functional foods that gave rise to the creation of a completely new category of probiotic products like the daily-dose drinks in small bottles, yoghurt, ice creams, milk based desserts, powdered milk for infants, butter, mayonnaise, cheese, products in the form of capsules or fermented food of vegetable origin. Some of them are listed in Table 8. These include strains of *L. casei, L. johnsonii, L. rhamnosus* and *L. plantarum* which are all of human origin and are known under defined brand names. It has been estimated that there were approximately 70 probiotic-containing products marketed in the world (28) and the list is continuously expanding.

Table 8: Commercial probiotic products in market (10, 31)

Product Name	Species	Company	Applications
Nesvita	*LAB*	Nestle, India	Gut Probiotic
Improval	*Saccharomyces cerevasie, Lactobacillus sporogenes*	Cadila, India	Vaginal infections
Amul	*Lactobacillus acidophilus,*	Amul, India	Diabetics
Binifit	*Streptococcus faecalis, Clostridium butyricum, Bacillus mesentricus LAB*	Ranbaxy, India	Antibiotic associated Diarrhoea (AAD)
Actimel®	*Lactobacillus casei DN 014001*	Danone, France	Immune booster
Yakult ®	*Lactobacillus casei*	Yakult Honsha Co., Ltd, Japan	Gastrointestinal health
Bifilac	*JPC, Clostridium butyricum, Bacillus mesentricus*	Tablets India Pvt. Ltd, India	Irritable bowel syndrome, Diarrhoea.
Biosporin®	*Bacillus subtilis Bacillus licheniformis*	Biofarm, Dniepropetrovsk, Ukraine Garars, Russia	Pyo-speticpost operational complications, bacterial vaginitis
PoultryStar®	*Enterococcus, Pediococcus, Lactobacillus, Bifidobacterium*	Biomin, Herzogenburg, Austria	Improved weight gain, decreased mortality, inhibition of enteric pathogens
Mitomax®	*Pediococcus acidilactici, Saccharomyces boulardii*	Imagilin Technology, USA	Supports digestive system, reduces stress

CHALLENGES DURING APPLICATION OF PROBIOTICS

The challenges during application of probiotics includes strain selection, inoculation, growth and survival during processing, viability and functionality during storage, assessment of viable counts of the probiotic strains (particularly-multiple probiotic strains) and effects on sensory properties. Probiotics are certainly very sensitive to many environmental stresses, such as acidity, oxygen and heat. Before a probiotic can benefit human health, it must fulfil several criteria related to the safety and stability (activity and viability in products; adherence; invasive potential; resistance to low pH, gastric juice, bile acid and pancreatic juice; colonisation/survival *in vivo*) and functional and physiological aspects (adherence to intestinal epithelium/tissue/virulence, antagonism to pathogens, antimicrobial activity, stimulation/suppression of immune response, selective stimulation of beneficial bacteria and clinical side effects in volunteers/patients). The viability of probiotics is a key parameter for developing probiotic foods. Several factors shown in Fig. 2 affect the viability of probiotic bacteria until they reach the target site of the host (18).

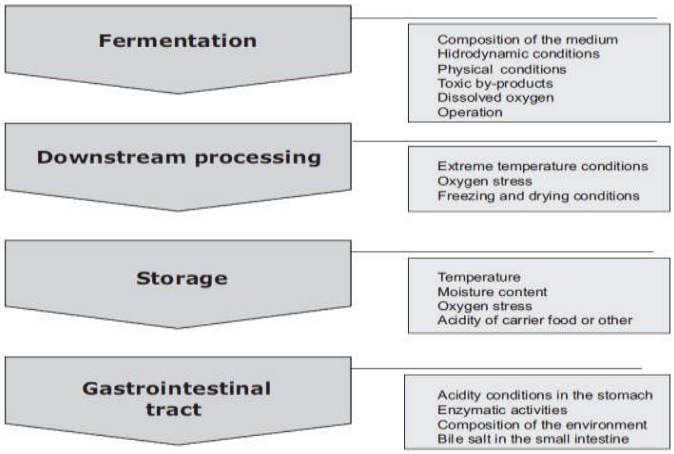
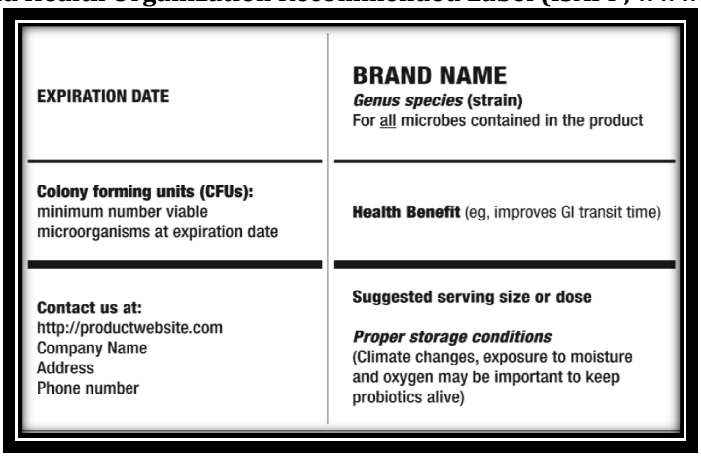


Fig. 2: Factors influencing the stability of probiotics during processing steps (18)

PROBIOTIC PRODUCT LABELS

The FDA defines a medical food as "a food which is formulated to be consumed or administered eternally under the supervision of a physician, and which is intended for the specific dietary management of a disease or condition for which distinctive nutritional requirements, based on recognized scientific principles, are established by medical evaluation". Medical foods are exempt from the labelling requirements for health claims and nutrient content claims. Figure 3 illustrates the type of information that experts from the *World Health Organization and International Scientific Association for Probiotics and Prebiotics (ISAPP)* (17) consider to be important on probiotic product labels. Patients should be advised to choose products that provide complete information.

Fig. 3: World Health Organization Recommended Label (ISAPP, www.isapp.net.)



LIMITATIONS

Probiotics are restricted to products that

1) Contain live microorganisms (e.g. as freeze-dried cells or in a fresh or fermented product),
2) Improve the health, growth and well-being of humans or animals, and
3) Can affect all host mucosal surfaces, including the mouth and gastrointestinal tract (e.g. applied in food, pill or capsule form), the upper respiratory tract (e.g. applied as an aerosol), or the urogenital tract (16). Though probiotics are "generally regarded as safe (GRAS)", but side effects such as septicemia and fungaemia have rarely been reported in high-risk situations (32).

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