Chapter 1: Introduction
1. Introduction

Around the world nutraceuticals are becoming a part of daily diet of consumers in order to overcome inadequate nutrition due to current lifestyle. An increased awareness about health and incidences of lifestyle diseases underscore the need for nutraceuticals. Nutraceuticals could complement drugs and reduce the over-dependence on antibiotics for treatment of diseases and disorders.

![Figure 1](https://www.frost.com) The classification of the different products under the term Nutraceuticals.

Source: Frost, Sullivan & Ficci [www.frost.com](http://www.frost.com)

Indian nutraceutical market is emerging with strong growth potential and looks promising for the manufacturers and consumers. The Indian market is in a need of developing customized products, affordable pricing and well designed distribution strategy. The Food Safety and Standards Authority of India (FSSAI) is the regulatory authority for special dietary use of food (nutraceuticals) in India. For the first time in the Indian regulatory system, the Food Safety and Standards Act, 2006 has formally recognized nutraceuticals.
According to the Frost and Sullivan analysis the functional food and beverages (67%) will capture the nutraceutical market in India in next five years. The probiotic fortified food will acquire (9%) of share of US $ 250 million market size. As the demand is growing, many pharmaceutical companies like Ranbaxy Laboratories, Cipla, Aristo, Lupin, Glenmark, Alkem Laboratories, Tablet India and Dr. Reddy’s Laboratories (DRL) etc. are investing in probiotic production in India (Sharma et al., 2013).

Most popular probiotic products are available from Amul, Yakult, Danone, Nestle and Mother Dairy. Probiotics are mainly available as ice creams, Lassi (sweetened curd) and Dahi (Indian yoghurt). Largest selling product is Dahi and Lassi (25%) followed by probiotic ice-cream (10%). Yakult (Japan) and Danone (France) collaboratively introduced Yakult in functional beverage category in 2007. Yakult is the fermented milk containing Lactobacillus casei strain shirota which has many health applications.

In India, Nestle acquired 30% share in dairy products such as fermented milk, yoghurt etc. Few more popular brands of probiotic products are Nesvita and Actiplus. Mother Dairy also provides active probiotic products like dahi, lassi, Nutrifit etc. Although the probiotic products are introduced in Indian market before ten years but it still lacks the proper regulatory guidelines to ensure the safety for all kinds of probiotic products. Most of the current products available in the market are glorified to attract the consumer but exact benefits and/or side effects are not highlighted in the advertisements. The commercial strains currently used in the marketed probiotic products are of foreign origin. The use of such strains in the Indian population need to be carefully evaluated for their safety and efficacy assessments.

New species and more specific indigenous strains are in more demand for the Indian population as the lineage is an important factor besides others like age, dietary habits, socio-economic status, geographic location and genetic makeup of individuals which affect the human microbiome. The comparison of gut microbiome of Indian population with other populations like American, Korean, Spanish and Bangla (Bhute et al., 2016) reveals striking differences between the microbiome. These differences in the microbiome composition drives the search of new indigenous probiotics that can have better adaptability with Indian population hence may confer more health benefits as compared to exotic probiotics.

According to FSSAI and ICMR–DBT guidelines, Probiotics are defined as “live microorganisms which when administered in adequate amounts confer a health benefit on the host”. Probiotics supplementations positively modulate the gut environment which
shows other beneficial effects on the host health. Probiotics help in lactose intolerance, they have curative effects on all types of diarrhea specially paediatric diarrheal conditions, and they help in reduction of cholesterol and obesity (Linares et al., 2016). Probiotics are helpful in the prevention of colorectal cancer, gut inflammation and related disorders (Lee, 2014). Dysbiosis in gut microbiota is observed in many illnesses, and probiotics are helpful to restore normal microbiota and gut ecosystem (Martín et al., 2013).

**Figure 2** The benefits of probiotics to human health
Source: ICMR DBT guidelines for evaluation of probiotics in food.
The health benefits conferred by probiotics are shown in Figure 2. Lactic acid bacteria (LAB) and Bifidobacteria are potential candidates for probiotics. *Lactobacillus* is one of the important genera that is used for the production of many fermented foods and beverages. LAB include nearly 20 major genera namely *Lactobacillus*, *Lactococcus*, *Streptococcus*, *Leuconostoc*, *Pediococcus*, *Aerococcus*, *Carnobacterium*, *Enterococcus*, *Oenococcus*, *Tetragenococcus*, *Vagococcus*, *Weisella* and others. *Lactobacillus* is the largest of these genera, comprising around 80 recognized species. *Lactobacillus* sp. carries “Generally Recognized As Safe (GRAS)” status. The LAB produce bacteriocins, exopolysaccharides, acetic acid, ethanol, aroma compounds and several enzymes. They are used in different dairy industries to improve texture and contribute to the pleasant sensory profile of the end product. They cause rapid acidification with the production of different organic acids mainly lactic acid which is helpful for the enhancement of the shelf life of the end product (Vuyst, 2004). LAB synthesize other important metabolites of medicinal and cosmetic value like B group vitamins, folic acid, exopolysaccharides, and short chain fatty acids (SCFA) such as butyrate, propionate and acetate. Figure 3 gives an overview of conventional and unconventional sources used for isolation of probiotic bacteria.

**Figure 3** Overview of the sources for the isolation of potential probiotic bacteria.
The probiotics which are commercially available and routinely used for human consumption are obtained from the conventional sources such as dairy products, human feces and human breast milk. Non-intestinal and non-dairy sources can offer better health advantages to lactose-intolerant individuals. Individuals with lactose-intolerance, vegans and those prescribed a cholesterol-restricted diet need alternative non-dairy products with equivalent nutritive value. Thus they mainly depend on fruits, vegetables, and cereals for their dietary needs. Probiotics originating from fruits and vegetables may suit their gut environment more than those from different sources. LAB have been earlier isolated from different vegetables and fruits (Sathe et al., 2007; Swain et al., 2014) and non-dairy fermented products (Anandharaj et al., 2015). Probiotics enhance the gut epithelial barrier, increase adhesion of bacteria to intestinal mucosa, inhibit pathogen adhesion to intestinal mucosa, synthesize and secrete antimicrobial substances and positively modulate the host immune system.

The Indian regulatory authority guidelines for the selection of bacterial strains as a probiotic suitable for human consumption include successful passage and survival through gastric and intestinal conditions, ability to adhere to intestinal surfaces, temporary colonisation and antagonism against pathogens.

The criteria for selection of the probiotics according to ICMR-DBT guidelines include the following points:

- Strain identification
- In vitro testing of probiotic potential
- In vivo safety studies in animal models
- Evaluation of safety of probiotics for human use
- Effective dosage of probiotic strain/strains
- Labeling criteria

Well documented reports are there in literature for use of Lactobacillus spp. in different probiotic products. Lactobacillus spp. proved their potential as probiotics in the improvement of the gut health by reducing the pathogenic infections (Shokryazdan et al., 2014), by healing inflammatory bowel disorders (IBD) (Hemarajata & Versalovic, 2013; Shadnoush et al., 2015), modulation of the immune system and they have a probable role in the treatment of colon cancer (Gao et al., 2015).

Enterococcus is another important genus of lactic acid bacteria although it is not given a GRAS status. Enterococcus is Gram positive, facultative anaerobic bacterium, a commensal in gastrointestinal tract (GI tract) of humans and animals and it is also
commonly found in large numbers on vegetables and in plants, in soil and dairy products. *Enterococcus* sp. are reported to have anticancer potential (Nami *et al.*, 2014), immunomodulatory properties (Bhute *et al.*, 2016), antibacterial and anti-inflammatory properties (Arokiyaraj *et al.*, 2014). Different species found in GI tract are *Enterococcus faecalis*, *Enterococcus faecium*, *Enterococcus avium* and *Enterococcus hirae*.

The term Nutraceutical is a combination of words ‘nutrition’ and ‘pharmaceutical’ which is defined as any substance that is a food or a part of food which carries potential to give health benefits, including prevention and treatment of disease. LAB is most acceptable and commonly used starter culture (Saffa *et al.* 2017). LAB is leading in use as bacterial nutraceutical with basic contribution in food preservation through rapid acidification of product with formation of lactic acid. LAB also contribute in nutraceutical properties by synthesizing different molecules such as vitamins, antioxidant molecule, exopolysachharide, antimicrobial peptides, different sugars and different enzymes having positive health applications (Sirilum *et al.*, 2017).

The bacterial nutraceuticals are also used in food preservation by secretion of inhibitory metabolites such as bacteriocin, ethanol and organic acid such as lactic acid, acetic acid, formic acid and propionic acid (Sharma and Manisha, 2012). Overall LAB contribute to the one or multiple unique properties of food stuff especially in regard to taste, flavor, colour, texture, safety, preservation, nutritional value, wholesomeness and/or health benefits.

LAB were isolated from selected vegetable plants from three different regions; Junnar, Ambegaon and Khed of Pune district. Strains were evaluated for probiotic potential and assessed for safety by *in vitro* and *in vivo* safety tests. The effect of metabolites from *Lactobacillus* and *Enterococcus* on differential expression of genes in Caco-2 cells was studied using human genome array. Caco-2 were selected to study the effect these probiotic bacteria are likely to have on colorectal cancer. These genera were also checked for their ability to overcome inflammation induced in rats. Present study provides evidence for the usefulness of *Lactobacillus* and *Enterococcus* isolated from fresh vegetable sources as probiotic. The bioactive metabolites produced by these bacteria could be useful in prevention of colorectal cancer and inflammatory diseases of the gastrointestinal tract. Besides, these bacteria are also useful in post harvest preservation and in preventing the spoilage of ready-to-eat fresh-cut fruits and vegetables.