Chapter 1

Introduction
1. INTRODUCTION

A healthy human gastrointestinal tract (GIT) is a kinetic microbial ecosystem that facilitates normal physiological functions of the host unless harmful and potentially pathogenic bacteria dominate it. In the human GIT, there exists variability in bacterial numbers and populations between the stomach, small intestine and colon. In comparison to other regions of GIT, the human large intestine is a complex, heavily populated and diverse microbial ecosystem. Bacterial numbers in the colon are in the range of $10^{11}-10^{12}$ for every gram of gut contents (Cummings and Macfarlane, 1991). In that order for the intestine to function optimally, the ‘balance’ of the bacteria must be maintained, that seems to be increasingly difficult due to the changing in life styles of human beings. The large-gut microflora is acquired at birth and initially facultative anaerobic strains dominate. Normal healthy adults also possess other anaerobes including clostridia, veillonellae, coliforms, streptococci and facultative anaerobic lactobacilli (Mitsuoka, 1982).

A delicate balance exists between the human colon microflora and its host. A number of factors may shift the balance of the gut microflora away from potentially beneficial or health promoting bacteria such as lactobacilli and bifidobacteria, towards a predominance of potentially harmful or pathogenic microorganisms, like clostridia, sulphate reducers and certain Bacteroides species. Predominance of these harmful organisms may pre-dispose to a number of clinical disorders. Distress of this system may lead toward the symptoms of acute gastroenteritis and there is also the possibility of chronic disorders like inflammatory bowel disease, colonic cancer, etc. It is, therefore, important that gut microflora interactions be managed and sustained in an optimal manner. Understanding of the gut microflora and its interactions lead to the development of dietary
strategies that improve normal GIT microflora. Thereafter, microflora modulation can occur through food stuffs that contain sufficient level of growth supplements (prebiotics) that target the (potentially) health promoting flora. Impressing progress in functional food science has intended enhancing native (indigenous) lactobacilli populations, as be against to oral administration of exogenous strains. Additionally, the application of probiotics and the potential of prebiotics have developed a more class of functional foods; synbiotics, the combination of probiotic(s) and prebiotic(s). Dietary modulation studies have clearly established the ability to modify the gut microflora (Kurien et al., 2005; Nagpal et al., 2007; 2008).

In fact, a beneficial association of “lactic acid producing” microorganisms with the human host has been suggested more than 100 years ago Doderlein (1982) and in ruminants (Puniya et al., 2008). The LAB associated with fermented milk products, were advocated by Metchnikoff (1908) for their health benefits. It was probably Vergio (1954) who first introduced the term “probiotic”. Probiotic is a “live microbial feed supplements that have beneficial effects on the host by improving its intestinal microbial balance” (Fuller, 1997). Probiotics, most notably lactobacilli and bifidobacteria, are marketed as capsules, powders, enriched yoghurts, yoghurt-like products and milks. Lactobacilli are Gram-positive, nonsporing, catalase-negative organisms that are devoid of cytochromes and of nonaerobic habit but are aerotolerant, fastidious, acid-tolerant and strictly fermentative; lactic acid is the major end product of sugar fermentation. Lactobacilli are members of the LAB, whose primary fermentation end product is lactic acid. Due to wide importance, Lactobacilli species are widely used as probiotic (Ouwehand et al., 2002).

Development of a probiotic product is dependent on strains that fulfill the strict criteria of: human origin, possession of generally regarded as safe (GRAS) status, production of antibacterial factors
against invasive gram negative pathogens, desirable metabolic activity, technological suitability, nonpathogenic (Fuller and Gibson 1997; Drisko et al., 2003). Bacteria used as probiotic adjuncts are commonly delivered in food system therefore; they begin their journey to the lower intestinal tract via the mouth. In order to exert health-promoting probiotic effects, it is important for bacterial strain to survive inhospitable environment of human GIT. Therefore, probiotic organisms should survive in human GIT after ingestion, reach colon and should get established there. As such, probiotic bacteria should have the ability to resist the digestion process in the stomach and the intestinal tract. During their passageway through GIT, probiotic organisms are encountered by numerous harsh physicochemical pressures i.e. acid, bile salts and other hydrolytic enzymes. Berrada et al. (1991) reported the time from entrance to release from the stomach to be 90min. However, further digestive processes have longer residence times; therefore, there is need for the bacteria to be resistant the stressful conditions of the stomach and upper intestine, which contain bile. Cellular stress begins in the stomach, which has pH as low as 1.5 (Lankaputhara and Shah, 1995).

After the bacteria pass through stomach, they enter the upper intestinal tract where bile is secreted into the gut. After traveling through this harsh environment, the organism colonizes the epithelium of the lower intestinal tract, where the bacteria must compete successfully with a complex and metabolically active indigenous flora. Thus, strains selected for use as probiotic bacteria should be able to tolerate acid for at least 90min, tolerate to bile salts, attach to the epithelium, exclusion of harmful enteric organism and grow in the lower intestinal tract before they can start providing any health benefits. Therefore, it is necessary to develop steps to rise above this problem in order to achieve beneficial health attributes from probiotics. The introduction of food with added probiotics, synbiotic put forwards a significant role to way out this problem
(Zimmer and Gibson, 1998), where the probiotic organism is administered in combination with a specific prebiotic.

A prebiotic is 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 that can improve host health”. Prebiotics are competent of modifying the composition of the colonic microflora by stimulating the potentially health promoting bacteria ultimately they become most dominant in numbers. A synbiotic is described as “a combination of a probiotic and prebiotic that beneficially affects the host by improving the survival and implantation of live microbial dietary supplements in the GIT. This mixture could improve the survival of the probiotic organisms as its specific substrate prebiotic is readily available for its fermentation (Nagpal et al., 2007). The concept of functional food has developed as a food, or food ingredient, with positive effects on host health and/or well-being beyond its nutritive value (Hugget and Verschuren, 1996). A particular food may be made more functional by increasing or adding a potential health promoting entity. Formulation of synbiotic preparation which promote host health can be applied, in both maintain the balance the balance of gut flora in healthy individuals and restoring the equilibrium in individuals whose GIT microbiota has been altered as a result of illness and/or disease, age, or diet.

Currently, a number of probiotic foods are available in several of forms. The fermented milk products are recognized as most suitable carrier medium for probiotics and prebiotics. The popular probiotic products already in market such as fermented milk and yoghurt are fresh products and normally must be consumed within a few days of manufacture. A major challenge associated with the application of probiotic cultures in a synbiotic preparation is retention of their viability during processing and subsequent storage. Different drying methodologies have been used for decades to increase the shelf-life of
probiotic food. Dried preparations have longer shelf-life and put forward definite reward over fermented products in terms of increased shelf-life, reductions in the costs of transport and storage and improvements in culture stability. In this connection, spray drying is more economical than freeze-drying; especially on large scale (Golker, 1993; Johnson and Etzel, 1993). However, most probiotic bacteria do not survive well during the temperature and osmotic extremes to which they are exposed during the spray drying process (Selmer-Olsen et al., 1999; Teixeira, 1979). Taken as a whole, freeze drying is the most convenient method for the production of dried probiotic/synbiotic preparations. Maintenance of good viability and stability of organisms during the development of probiotic foods is a most important confront. Therefore, the utilization of the synergistic effect of mixing a probiotic with a prebiotic might be beneficial to enhance the delivery of viable and metabolically active probiotics to the intestinal tract (Mattila-Sandholm et al., 2002). Because of their supposed health benefits Lactobacilli species are most widely used as probiotic preparations (Ouwehand et al., 2002; Collins et al., 1998).

Keeping above essentials in view, the present research has been carried out to develop a freeze-dried synbiotic formula employing a combination of selected probiotic and prebiotic with the following objectives:

1. To isolate and characterize lactobacilli of human and food origin having potential probiotic characteristics in vitro.
2. To study the effect of different concentrations of prebiotics on the growth of lactobacilli strain and to study the ecological interaction of probiotic strain in the presence of prebiotics and gut associated harmful microflora.
3. To develop a lyophilized synbiotic preparation using selected probiotic and to carry out its storage studies.