Chapter 1

Introduction

1.1 Background

In the late nineteenth century, microbiologists identified microflora in the gastrointestinal tract of healthy animals that differed from those found in diseased animals. As further research continued into the isolation and characterization of these microorganisms, it was revealed that ingestion of these bacteria could confer a wide range of therapeutic benefits to humans. These beneficial microflora were termed probiotics. Since then, the popularity of probiotics has been increasing rapidly worldwide (Benkouider, 2004 (a, b); Kotilainen et al., 2006).

The most commonly studied probiotic bacteria include members of the genus *Lactobacillus*, especially, *L. acidophilus* and *Bifidobacterium* spp. (Tannock, 2002). These bacteria were found to prevent diarrhea in children, suppress pathogens in the intestinal tract, alleviate symptoms of lactose indigestion and enhance the population of beneficial bacteria in the human gut (Rafter, 2004; Sanders, 2000, 2003).

The numerous perceived health benefits and the growing awareness about probiotics have caught the attention of the food industry (Saarela et al., 2002; Salminen and Gueimonde, 2004). Food companies are increasingly manufacturing foods with incorporated probiotic bacteria, which fall under the new category of foods called Functional Foods. Probiotic dairy products such as yogurts containing *L. acidophilus* and *Bifidobacterium* spp. constitute a significant amount among the commercially available probiotic foods (Reid et al., 2003). Functional foods have been developed in most food categories and by conservative estimates, the global market size is increasing. In addition to providing consumers options for improving their health and well-being, functional foods such as probiotics in dairy products are an attractive market sector, providing new economic opportunities.

The existence of probiotics has been known for over a century and the relationship between certain foods and health has been investigated for many years. A
A number of definitions have been proposed to describe probiotics and an appropriate one was suggested by Havenaar & Veld (1992) who defined probiotics as “mono- or mixed cultures of live microorganisms which, when applied to animal or man, beneficially affect the host by improving the properties of the indigenous microflora”. The most frequently used probiotics belong to the genera Bifidobacterium and Lactobacillus (isolauri, 2004). There is general agreement on the important role of the gastrointestinal microflora in the health status of not only humans, but also in animals. Thus, it is not surprising that there has been tremendous interest in the probiotic industry as probiotics have been classified as functional food ingredients (Roberfroid, 2003). In several universities and research centers across the world, currently there are many ongoing studies on various aspects of probiotics i.e. health effects, effects on food products and other.

Lactic acid bacteria including Lactobacillus spp. such as Lactobacillus casei, Lactobacillus acidophilus, and Bifidobacterium spp. are becoming very popular in dairy industries due to their therapeutic benefits. Some health benefits include improvement in intestinal disorders and lactose intolerance, altered vitamin content of milk, antagonism against various pathogenic organisms and antimutagenic and anti-carcinogenic activities. These bacteria are widely used in the production of fermented foods and beverages and contribute both sensory qualities of the food and the prevention of spoilage. These organisms have added a new dimension to the importance of fermented milks in human nutrition and health. Moreover, they are present in large numbers in the normal human and animal gastrointestinal flora (Sgouras et al., 2004).

Health promoting benefits of consumption of LAB (Lactic acid bacteria) have been known for several years, since Metchnikoff (1908) first attributed longevity of Bulgarian peasants to consumption of fermented milks. The term ‘probiotic’ was first described by Fuller (1989) as ‘a live microbial feed supplement that beneficially affects the host by improving its intestinal microbial balance’. Schrenzenmeir and de Verse (2001) redefined probiotics to help broaden the scope, while emphasizing the fact that other compartments in the body, besides the intestines, might be targets for probiotic organisms. They defined probiotics as ‘a preparation or product containing viable, defined micro-organisms in sufficient numbers that alter the microflora in a compartment of the host and by that exerts health effects in this host’.
Recent research has credited several health benefits to probiotic organisms that are indigenous to the gastrointestinal tract, as well as those consumed through probiotic products.

These include their ability to relieve symptoms of lactose intolerance (DeVerse et al., 1992), increase immune function, cholesterol lowering potential (Noh et al., 1997), antimutagenic activity (Lankaputhra and Shah, 1998) and treatment of diarrhea (Guandilini et al., 2000) to name a few. The ‘natural’ target of ingested probiotics is the intestine, its microflora and the associated immune system, and therefore investigations and clinical studies of non-intestinal infections are rather scarce (de Verse and Schrenzenmeir, 2002). Therapeutic activity of probiotic bacteria can be due to competition with pathogens for nutrients and mucosal adherence, production of antimicrobial substances, and modulation of mucosal immune functions (O’Sullivan et al., 2005). However in recent times, the increasing popularity of probiotic dairy products, especially probiotic yogurt has led to the creation of a multibillion dollar market for probiotic yogurt and other foods where probiotics can be incorporated (Playne, 2002).

As the popularity of yogurt and yogurt drinks grew, various factors related to the growth and survival of probiotics in yogurt and yogurt drinks became more apparent. Although several of the yogurt products contain either L. acidophilus or Bifidobacterium spp., there are only few reports of yogurt and yogurt drink containing only Bifidobacterium spp (Godward et al., 2000).

For probiotics to be effective, scientists have suggested that there should be a minimum number of $10^6$ - $10^7$ cfu of probiotic bacteria /gram of product at the time of consumption (IDF, 1992; Lourens-Hattingh et al., 2000; Lourens-Hattingh and Viljoen, 2001). While some reports have shown probiotic growth and survival numbers to be stable during the shelf life of the product (Dinakar and Mistry, 1994), others have cited a rapid decline in the number of viable probiotic bacteria over the shelf life (Stanton et al., 2003). Studies have shown that a number of factors can affect the growth and the survival of Bifidobacterium spp. in dairy products. These factors include strains of probiotic bacteria, pH of milk, presence of lactic and acetic acids, interactions with other microorganisms, storage temperature and manufacturing conditions (Shah, 2000 (a, b); Boylstonetal2004).

As matter of using prebiotic or hydrolyzed milk to enhance the growth and survival of probiotic bacteria and specially Bifidobacteria, the result showed a
variety of effects depending on the strain of probiotic used in the dairy products. The combination of probiotic and prebiotics has been used on several aspects of health benefit, which have also been documented (Gibson and Roberfroid, 1995; Niness, 1999). However, very little attempt has been made to develop a synbiotic product with combination of probiotic and prebiotics (Gibson and Roberfroid, 1995).

Moreover, very little information is available on the synergistic effects of prebiotics and hydrolyzed milk on the growth and survival of Bifidobacteria spp., which could lead to new products for the dairy market.

Reports also claim a correlation between anti-hypersensitivity and consumption of fermented milks containing L. casei. The human gastrointestinal tract constitutes a complex microbial ecosystem (Simon & Gorbach, 1986). More than 400 different species have been identified in feces of a single subject (Finegold et al., 1977; Moore et al., 1974). The equilibrium that exists in the large intestine is dynamic and is affected by age, diet composition and other environmental factors. Although these factors are significant, the continued maintenance of the intestinal microflora, which predominantly contains beneficial species such as probiotic bacteria, can improve our well being. The human gastrointestinal tract is known to possess active clearance mechanisms for microorganisms, and it has proven difficult to introduce new bacterial strains into this ecosystem (Savage, 1979). Constituents of the normal flora and some pathogenic bacteria possess the ability to colonize the mucosal surface of gastrointestinal tract and carry out a process of fermentation consuming substrates and producing end products that significantly influence our health (Haenel, 1961).

Some of the commonly known probiotics belong to the lactobacilli and bifidobacteria genus. Strains of Lactobacillus casei, L. acidophilus, Bifidobacterium bifidum, B. longum and B. infantis have been identified as possessing probiotic properties, and many have been used to treat gastrointestinal diseases. They have shown to exert either bacteriostatic or bactericidal activity against several pathogens. Many probiotics are now widely marketed through food products such as yoghurt, fermented milks, fermented juices and freeze dried supplements.

The concept of the group name ‘lactic acid bacteria’ was created for bacteria causing fermentation and coagulation in milk, and defined as those which produce lactic acid from lactose. The family name Lactobacteriaceae was applied by Orla-Jensen (1919) to a physiological group of Gram-positive rods and cocci. Today, same physiological group is
now subdivided into four genera Streptococcus, Leuconostoc, Pediococcus, and Lactobacillus. Members of Lactobacillus casei are Gram-positive, facultatively anaerobic, catalase-negative, facultatively heterofermentative, non-spore-forming rods and are isolated from many habitats (e.g., meats, milks, dairy products, sour dough, silage, and sewage). Strains of Lactobacillus are important for many food fermentations and are normal constituents of intestinal microflora. Some Lactobacillus strains have desirable and functional characteristics (Saxelin et al., 1996). The Lactobacillus casei group contains a number of well-known probiotics strains including *L. casei shirota* (Sugita & Togawa, 1994) and *L. rhamnosus* GG (Saxelin, 1997). The identification of Lactobacillus strains within this group (*L. casei, L. paracasei, L. rhamnosus* and *L. zeae*) is important for both basic studies and applications in food industries. Most of these strains have very similar physiological properties and nutritional requirements and grow under similar environmental conditions. Traditional phenotypical tests for Lactobacillus identification can be difficult to interpret. The techniques are also time consuming and results are often ambiguous (Charteris et al., 1997; Hammes et al., 1992). While this group of Lactobacillus can be readily distinguished from other members of the Lactobacillus genus by fermentation profiles (Hammes et al., 1992), it is not possible to unequivocally distinguish between these four species on the basis of fermentation patterns. They form a closely related taxonomic group. In recent years, the taxonomy of this group has changed considerably with increasing knowledge of genomic structure and phylogenetic relationships between Lactobacillus species (Klein et al., 1998; Tisala-Timisjarvi et al., 1999). As commercially available probiotic strains are being produced and marketed for human consumption on a large scale, it is important to identify them to species and strain levels.

The bacterial populations of the human gastrointestinal tract constitute a complex ecosystem (Simon & Gorbach, 1986). More than 400 bacterial species have been identified in feces of a single subject (Finegold et al., 1977). Gastrointestinal tract is known to possess active clearance mechanisms for microorganisms, and it has proven difficult to introduce new bacterial strains into this ecosystem (Paul & Hoskins, 1972). There is, however, host specificity in colonization by individual species; for example, *Lactobacillus acidophilus, L. fermentum* and *L. plantarum* are commonly found in the feces of humans; whereas *L. delb. spp. bulgaricus*, the organism used in combination with *Streptococcus thermophilus* to make yoghurt, is unable to colonise the bowl and is not isolated in the feces (Finegold et al., 1977). In a number
of different circumstances it can be beneficial to alter the intestinal microflora by introducing lactobacilli. The nutritional and therapeutic benefits derived from this approach have been discussed in the review article by Gorbach (1990). The claimed benefits of bacterial supplementation include increased nutrient utilization, alleviation of lactose intolerance, treatment of hepatic encephalopathy and intestinal infections, and inhibition of bacterially derived generation of carcinogens in the intestinal tract (Finegold et al., 1977). Metchnikoff (1908) was the first to establish that microorganisms were responsible for the beneficial effects associated with lactobacilli fermented foods. He attributed the longevity of the Bulgarians to consumption of milk products fermented with Lactobacillus bulgaricus. Lactic acid bacteria have been widely used in making variety of cultured dairy foods for desirable flavor or other characteristic, and they have been known to produce the lactic acid and others, which influence growth or survival of other bacteria as well as themselves (Kim et al., 1980). The harmful effects of the undesired bacteria can be overcome by establishing a new balance between intestinal flora, through ingestion of lactic organisms present in cultured dairy products. Bacterial modification of primary bile acids is catabolism of bile acids by microorganisms and deconjugation of conjugated bile acids (Aries & Hill, 1970). There is scant information available about survival of \( L. \) casei in acidic conditions and varying concentrations of bile and bile are normally encountered in the gastrointestinal tract and their deconjugation ability.

Fuller (1989) has redefined a probiotic as ‘a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance’. A prebiotic is a nondigestible 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, and thus improves host health (Gibson & Roberfroid, 1995). The majority of simple sugars and oligosaccharides ingested and digested by humans are absorbed in the small intestine (Bond et al., 1980). However some prebiotic such as lactose, raffinose, stachyose and fructooligosaccharides (such as oligofructose or inulin) are able to reach the colon intact (Roberfroid et al., 1993).

Prebiotics have been selectively manufactured to contain several or all of the following attributes; active at low dosages, varying viscosity, lack of side effects, varying sweetness, control of microflora modulation, persistence through the colon, good storage and processing stability and inhibiting
pathogen adhesion. Established and possible effects of prebiotics include nondigestibility and low energy value, stool bulking effect and modulation of the gut flora, promotion of bifidobacteria and repression of clostridia. Oligosaccharides are mostly a group of short chain non-digestible polysaccharides that occur naturally in foods. They are typically defined as glycosides that contain between 3 to 10 sugar moieties and are characterized by the type and sequence of the monosaccharide moieties present (Crittenden, 1999). In the last 10 years, there has been an increasing interest in the consumption of probiotics and functional foods in Western diets (O’sullivan, 1996). Probiotic bacteria are able to suppress potentially pathogenic microorganism in the gastrointestinal tract and enhance the population of beneficial microorganisms (Yaeshima et al., 1997).

The health benefits derived by the consumption of foods containing probiotic bacteria are well documented and more than 90 probiotic products are available worldwide (Shah, 2000). To provide health benefits, the suggested concentration for probiotic bacteria is 10^6 cfu/g of a product (Shah, 2000). However, studies have shown low viability of probiotics in market preparations (Shah et al., 1995). A number of factors have been claimed to affect the viability of probiotic bacteria in fermented food including acid and hydrogen peroxide produced by bacteria, oxygen content in the product, and oxygen permeation through the package (Shah, 2000). Viability of probiotic bacteria in fermented products declines over time because of the acidity of the product, storage temperature, storage time, and depletion of nutrients (Dave & Shah, 1997a). Loss of viability of probiotic bacteria occurs in fermented products, and these products have limited shelf life (Dave & Shah, 1996). However, in order to achieve maximum viability in a product, in the gut and maximum health benefits, there is a need to have a better understanding of probiotic.

Proteolysis in milk and dairy products has both beneficial and undesirable effects in the dairy industry. The process of cheese ripening results from activities of natural milk proteinase, rennet, and proteinases of the starter culture (Thomas & Mills, 1981). Lactobacillus casei is recognized to be an important component of the micorflora in several kinds of hard and semi-hard cheeses, predominating over other mesophilic lactobacilli (Taylor & Sharpe, 1958). Robertson (1964) examined the flora of cheddar cheese from several countries and found L. casei to be the most frequently occurring species in each case.
Helicobacter pylori is a spiral-shaped, Gram-negative rod that has developed sophisticated strategies to colonise epithelial cells lining the antrum of the stomach and to survive in acidic environments. Bacterial interference between normal flora and pathogenic organisms has been described since the late 19th century (Reid et al., 1990). Recently, attention has been paid to the interactions between H. pylori and probiotic lactobacilli. H. pylori colonization was inhibited in L. salivarius fed mice, and H. pylori antibody titers became marginal, while H. pylori colonized in large numbers and caused active gastritis in Lactobacillus-free mice (Kabir et al., 1997). In another study, probiotics including L. acidophilus has been reported to have antagonistic action against ulcer causing bacterium, H. pylori (Lorca et al., 2001). Cocconier et al. (1998) have reported the oral administration of a spent culture supernatant of L. acidophilus resulted in the suppression of H. felis in a murine infection model. In addition, an L. acidophilus culture supernatant was effective in vitro and had a partial, long-term suppressive effect on H. pylori in humans (Michetti et al., 1999). There is substantial evidence that the intestinal ecoflora can protect against infections and that the disruption of this microbial balance can increase susceptibility to infections. Many in vitro and in vivo studies have been shown that the normal intestinal flora is an extremely effective barrier against both pathogenic and opportunistically pathogenic microorganisms (Fuller, 1991).

1.2 Problem Statement

Probiotics are live microorganisms that confer a health benefit on the host. In other words, probiotics are beneficial bacteria that are similar to other beneficial bacteria already in the body. Probiotics can restore the numbers of beneficial bacteria that have been destroyed by antibiotics or other influences, and in that way they can improve the balance of good and bad bacteria. Some evidence shows that probiotics are useful for preventing and treating diarrhea, urinary tract infections in women, irritable bowel syndrome, Crohn’s disease and ulcerative colitis in both sexes.

Cultured and fermented foods, such as some types of yogurt, sauerkraut, kefir (a fermented milk drink), pickles, aged cheese and miso (a Japanese fermented soybean paste) are natural sources of probiotics. Probiotics are also challenging for the industrial applications. The probiotic concept is open to lots of different applications in a large variety of
fields relevant for human and animal health. Probiotic products consist of different enzymes, vitamins, capsules or tablets and some fermented foods contain microorganisms which have beneficial effects on the health of host. They can contain one or several species of probiotic bacteria. Most of products which destine human consumption are produced in fermented milk or given in powders or tablets. These capsules and tablets do not used for medicinal applications. They are just used as health supporting products. The oral consumption of probiotic microorganisms produces a protective effect on the gut flora. Lots of studies suggest that probiotics have beneficial effects on microbial disorders of the gut, but it is really difficult to show the clinical effects of such products. The probiotic preparations use for traveller's diarrhea, antibiotic associated diarrhea and acute diarrhea which has showed that they have positive therapeutic effect (Gismondo, et al. 1999, Çakir 2003, Quwehand 1999). More than 400 bacterial species exit in human intestinal tract. It is an enormously complex ecosystem that includes both facultatively anaerobic and anaerobic microorganisms (Naidu, et al. 1999). The numbers of genera is nearly steady, because they each have their own growth niches (Fooks, et al. 1999).

The composition of the gut microflora is constant but can be affected by some factors such as; age, diet, environment, stress and medication (Albertellasic 2007). To have a healthy intestine the balance of the bacteria must be maintained but this is difficult as the lifestyles change. Lots of factors may change the balance away from potentially beneficial or health promoting bacteria like lactobacilli and bifidobacteria to potentially harmful or pathogenic microorganisms like clostridia, sulphate reducers and Bacteroides species. It makes the host more susceptible to the illnesses. In this case the prevalence of the beneficial bacteria must be supported. Using of probiotics help to protect the host from various intestinal diseases and disorders while increasing the number of beneficial bacteria and make the balance steady again (Fooks, et al. 1999). Probiotics are suggested as food to provide for the balance of intestinal flora (Holzapfel, et al. 1998). Probiotics are used for long times in food ingredients for humans and also to feed the animals without any side effects. Also probiotics are acceptable because of being naturally in intestinal tract of healthy human and in foods (Çakir 2003, Albertellasic 2007).

Probiotics are also available in the form of a dietary supplements, like tablets and capsules. According to an estimate, each capsule contains more than 20 billion
cultures. This potency is high enough to restrain growth of the harmful bacteria that can cause digestive problems and to improve digestion and absorption of important nutrients, vitamins and minerals. Looking at several benefits of probiotics now they have been incorporated in daily diet of normal persons also.

Probiotics have been marketed as “Functional Foods” which are containing live microbes with health benefits. Various food related companies are now entering in the market of probiotics. Due to this extensively increasing demand of probiotic products their large scale production is required. But it has been observed that these functional foods are very expensive and are behind the reach of common man therefore to make them more popular and to give their positive effects to one and all their cost have to decreased.

This study suggests one possible approach for this. The study gives an idea to culture some selected probiotic organisms on economical growth media. Furthermore the suggestion is that the growth media should be designed with a waste material which is having maximum possible nutrients for the microbe and should be easily available in the locality. Since large scale production demands large amounts of waste materials therefore studies have been diverted towards industrial waste materials.

Still another problem which finds its solution through this study is the disposal of large amounts of industrial waste. These waste are having high organic content and when disposed as such they may lead to growth of pathogenic organisms on them thereby sometimes leading to serious diseases. Also when disposed without proper treatment these waste materials will lead to several environmental problems such as bad odour, change in colour, texture and other properties may change causing environmental pollution.

Food processing wastes are those end products of various food industries that have not been recycled or reused for other purposes. They are the useless product flows of raw materials whose economic values are less than the cost of collection and recovery for reuse; and therefore discarded as wastes. These wastes could be considered valuable by-products if there were appropriate technical means and if the value of the subsequent products were to exceed the cost of reprocessing.

With the increase in production of processed fruit products, the amount of fruit wastes generated is increasing enormously. Large amount of these wastes poses
the problem of disposal without causing environmental pollution. These wastes can be effectively disposed by manufacturing useful byproducts from them.

This study gives an idea to utilize these waste materials for beneficial purposes.

1.3 Aims and Objective:

The study is aimed at developing an economical media for probiotics without sacrificing their natural characteristics. The main objectives of the present study are:

- To study the growth of some selected probiotics (namely *Lactobacillus acidophilus*, *Bacillus subtilis*, *Bifidobacterium bifidium*, *Enterococcus faecium*, *Saccaromyces cerevisiae*, *E.coli*) on the waste materials such as fruit peels, agricultural waste, industrial waste etc.

- To study the chemical composition of the waste materials supporting the growth of probiotics.
- To study the characteristics of these probiotics after growth on the waste materials.
- To determine various factors affecting the growth of probiotics on the waste materials.
- To optimize the waste materials supporting the growth of probiotics to increase the yield.
- To compare the growth of probiotics on waste materials with that on the standard culture media available.
- To check for the contaminants on the optimized media.
- To study the impact of complete studies on the environment.

The study will lead to the development of a cheaper, economical media for the growth of probiotics, that can be used in the industries for their large scale production.

The study will also contribute towards the control of environmental pollution giving a suggestive measure to use the waste materials for some useful purpose.
1.4 Thesis Organization

The first chapter of the thesis gives a brief introduction of the topic highlighting the term “Probiotic”. The chapter gives an idea for the advantages and beneficial effects of probiotics on human as well as on animals. The contents of the chapter also deals with the aims and objectives of the research and shows the problem which leads to undertake the study.

The second chapter describes in detail the term probiotics and clears the history behind them. This chapter explains the causes of health effects of probiotics. The chapter also deals with different organisms regarded as probiotics, their taxonomic classification and properties. The chapter presents the criterion for an organism to be regarded as probiotic. The waste material to be used in the study and criterion for its selection has also been described in the chapter. The chapter gives brief knowledge for the use of waste materials for several useful purposes. It provides details of some of the materials which are directly or indirectly synthesized by using the waste materials.

The third chapter deals with the materials and methods used in the studies. The chapter gives detailed protocols and steps of various methods used. It also gives a list of chemicals, media and instruments used in the work.

The forth chapter carries all the results and outcomes of the studies. Results are shown in the form of tables and graphs. The respective figures are followed by the discussion related to the results. This helps in drawing the conclusion from results and leads for better understanding for the topic.

The fifth and the final chapter presents all the conclusions drawn from the study based on results.