INTRODUCTION
There is a long history of health claims concerning living microorganisms in food, particularly lactic acid bacteria. In a Persian version of the Old Testament (Genesis 18:8) it states that "Abraham owed his longevity to the consumption of sour milk." In 76 BC the Roman historian Plinius recommended the administration of fermented milk products for treating gastroenteritis. (Bottazzi V., 1983) Since the advent of the microbiology era, some investigators (Carre C., 1887), (Tissier H., 1984), (Metchnikoff E., 1907) attributed such health effects to shifts of the intestinal microbial balance. Metchnikoff claimed that the intake of yogurt containing lactobacilli results in a reduction of toxin-producing bacteria in the gut and that this increases the longevity of the host. Tissier recommended the administration of *Bifidobacteria* to infants suffering from diarrhea, claiming that *Bifidobacteria* supersede the putrefactive bacteria that cause the disease. (Tissier H., 1984)

Rettger et al., (Rettger L.F., Cheplin H.A., 1921, Rettger L.F., et al., 1935) and Kopeloff (Kopeloff 1926) showed that *Lactobacillus acidophilus* may survive in the human gut but the "Bulgarian bacillus" did not.

The significant role of the intestinal microflora for resistance to disease was shown by Bohnhoff et al., (Bohnhoff N., et al., 1954), Freter (Freter R. 1954, 1955, 1956), and Collins and Carter. (Collins F.M., Carter P.B., 1978) Oral administration of antibiotics to mice rendered the animals more susceptible to infection with *Salmonella typhimurium*, *Shigella flexneri*, and *Vibrio cholerae*. Thus, $\leq 1 \times 10^1$ *Salmonellae enteritidis* were sufficient to kill germ-free guinea pigs, whereas $1 \times 10^9$ bacteria were required to kill animals with complete intestinal microflora.

Thus it can be observed that microbial cultures have been used for thousands of years in food and alcoholic fermentations, and in the past century have undergone scientific scrutiny for their ability to prevent and cure a variety of diseases. Elie Metchnikoff in 1907 first introduced the probiotics concept when he observed the long life of Bulgarian persons who consumed fermented milk foods. He suggested that lactobacilli might counteract the putrefactive effects of gastrointestinal metabolism.
NORMAL INTESTINAL MICROFLORA

In a healthy animal, the internal tissues, e.g. blood, brain, muscle, etc., are normally free of microorganisms. However, the surface tissues, i.e., skin and mucous membranes are constantly in contact with environmental organisms and become readily colonized by various microbial species.

The mixture of organisms regularly found at any anatomical site is referred to as the normal flora, except by researchers in the field who prefer the term "indigenous microbiota".

- Resident Microbiota
- Transient Microbiota
- Opportunistic Microbiota

The normal flora of humans consists of a few eukaryotic fungi and protozoa, but bacteria are the most numerous and obvious microbial components of the normal flora. The human body is made up of about 10 trillion cells, but it hosts 100 trillion more microbes as normal flora. The vast majority of cells living on and in the body are bacteria and few are other microbes.

Intestinal colonization acts as an important antigenic stimulus for the maturation of the gut-associated lymphoid tissue. Capacity to generate IgA producing cells progressively increases in response to intestinal antigenic stimulation, particularly the establishment of the gut microflora, The range of non-digestible molecules (prebiotics) that beneficially affect the host by selectively stimulating the growth or activity of one or a limited number of bacterial species in the colon, and thus improve host health can be of great value.

ALTERATION OF NORMAL INTESTINAL MICROFLORA

Various factors affect the gut microflora during diarrhoea, chronic illness and treatment with antibiotics, chemotherapy, steroids and even after surgery. There is a potential loss of normal flora and live bacterial food supplement which beneficially affect the host by improving its intestinal microbial balances. This changes the balance of normal intestinal flora in favour of pathogen. (Collins M.D., et al., 1999) Microbial metabolites may possess genotoxic, mutagenic or carcinogenic activity and contribute subsequently to the development of cancer. (Fuller R., 1989)

A huge and diverse range of bacterial species colonize the human body. The microbiota extends from mouth to anus and into the vaginal tract of women. They also reside on the skin. Many lines of research have demonstrated the significant role of the microbiota...
in human physiology. The microbiota is involved in the healthy development of the immune system, prevention of infection from pathogenic or opportunistic microbes and maintenance of intestinal barrier function. For a variety of reasons, normal native bacteria may not always perform these functions optimally. Probiotics and prebiotics have been studied and used to improve these function.

Recent management of infectious disease and research work has focused on the gastrointestinal tract. The human intestinal microflora is an important constituent in the intestine’s mucosal barrier and has therapeutic role. The first known microbes used for this purpose was specifically known as probiotics called lactobacillus. (Gibson G.R., Roberfroid M.B., 1995)

To generalize, it is possible to categorize the gut microbiota components on the basis of whether they exert potentially pathogenic or health promoting aspects. Lactic acid producing genera such as the Bifidobacteria or lactobacilli have a long standing association with health. These bacteria can be increased either by feeding appropriate strains as a probiotic or through the provision of prebiotic growth substrates. The rapidly expanding research support and product availability for probiotics and prebiotics is evidence for their growing popularity.

The history of probiotics began with the history of man by consuming fermented foods that is well known. (Gismondo, et al., 1999, Guarner et al., 2005) In 1908 a Russian researcher Ellie Metchnikoff, who won a Nobel Prize, firstly proposed the beneficial effects of probiotic microorganisms on human health. Metchnikoff hypothesized that Bulgarians are healthy and long lived people because of the consumption of fermented milk products which consists of rod shaped bacteria (Lactobacillus spp.). Therefore, these bacteria affect the gut microflora positively and decrease the microbial toxic activity. (Gismondo, et al., 1999, Çakır 2003, Chuayana, et al., 2003 )

Microbial cultures have been used for thousands of years in food and alcoholic fermentations, and in the past century have undergone scientific scrutiny for their ability to prevent and cure a variety of diseases. Elie Metchnikoff in 1907 first introduced the probiotics concept in 1908, when he observed the long life of Bulgarian peasants who consumed fermented milk foods. He suggested that lactobacilli might counteract the putrefactive effects of gastrointestinal metabolism. The concept of probiotics has evolved from the work of Metchnikoff (1908) although the term was probably first used by Lilly & Stillwell (1965).
Introduction

The term *probiotic*, meaning "for life," is derived from the Greek language. It was first used by Lilly and Stillwell (Lilly D.M, Stillwell R.H., 1965) in 1965 to describe "substances secreted by one microorganism which stimulates the growth of another" and thus was contrasted with the term *antibiotic*. It may be because of this positive and general claim of definition that the term *probiotic* was subsequently applied to other subjects and gained a more general meaning. In 1971 Sperti (Sperti G.S., 1971) applied the term to tissue extracts that stimulate microbial growth. Parker (Parker R.B., 1974) was the first to use the term *probiotic* in the sense that it is used today. He defined probiotics as "organisms and substances which contribute to intestinal microbial balance." Retaining the word *substances* in Parker's definition of probiotics resulted in a wide connotation that included antibiotics. In 1989 Fuller (Fuller R., 1989) attempted to improve Parker's definition of probiotic with the following distinction: "A live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance." This revised definition emphasizes the requirement of viability for probiotics and introduces the aspect of a beneficial effect on the host, which was, according to his definition, an animal. In 1992 Havenaar et al., (Havenaar R., Huis In't Veld MJH., 1992) broadened the definition of probiotics with respect to host and habitat of the microflora as follows: "A viable mono- or mixed culture of microorganisms which applied to animal or man, beneficially affects the host by improving the properties of the indigenous microflora." Salminen (Salminen S., 1996) and Schaafsma (Schaafsma G., 1996) broadened the definition of probiotics even further by no longer limiting the proposed health effects to influences on the indigenous microflora. According to Salminen, a probiotic is "a live microbial culture or cultured dairy product which beneficially influences the health and nutrition of the host." According to Schaafsma, "Oral probiotics are living microorganisms which upon ingestion in certain numbers, exert health effects beyond inherent basic nutrition."

There are two aspects in Salminen's definition that in our opinion need revision. First, the definition given by Salminen includes beneficial influences on "nutrition of the host" in addition to health effects. It is not clear what the term *nutrition* should imply in this context, which would not be covered by the term *health*. Major effects on nutrition also imply effects on health, whereas minor alterations are of no relevance to the definition "for life." Therefore, the term *nutrition* might be best omitted from the definition.
In contrast with previous definitions, Salminen's definition (Salminen S., 1996) considers cultured dairy products and microbial cultures to be probiotic. Indeed the matrix of a product may affect the activity of microbes and therefore the survival and effect of the microbes, and thus deserves consideration. However, because nondairy products, (eg, sauerkraut, fermented cereals and other plant-based foods, and salami) may contain viable probiotic microorganisms [eg, Lactobacillus plantarum (Molin G., 2001)], the limitation of the definition to dairy products is not justified. Furthermore, cultured dairy products include products that are cultured and then pasteurized or sterilized, which results in the loss of viable microorganisms. In fact there is evidence for health effects beyond nutritional value of such products, eg, anticarcinogenic and immunomodulating effects have been exerted by yogurt fractions and cell-wall components of lactobacilli and bifidobacteria. (Sekine K, et al., 1985, Farmer RE, Shahani KM, Reddy GV., 1987, Steward-Tull DES., 1980, Okutomi T, et al., 1990)

Abandoning the viability of microorganisms or omitting the survival of the microbes and their effects on the indigenous microflora as prerequisites for the claim probiotic has consequences for what may be called probiotic. The definitions given by Salminen (Salminen S., 1996) and Schaafsma (Schaafsma G., 1996) would include yogurt containing usual cultures (Streptococcus thermophilus and Lactobacillus delbrüecki, subsp. bulgaricus) because these cultures may compensate for lactase insufficiency in lactose maldigestion. (de Vrese M., et al., 2001) This substitution may be even more pronounced when bacteria that do not survive in the small bowel are ingested and release their ß-galactosidase into the upper intestine. This substitution may as well be achieved by bacteria that have been killed by irradiation, which leaves their cell walls intact and therefore enables protection during gastric transit.(de Vrese M., et al., 2001)

**World Health Organization:**

“**Live microorganisms which when administered in adequate amounts confer a health benefit on the host**.”
IDEAL PROPERTIES OF PROBIOTICS

- The probiotics must exert beneficial effect on the host
- The probiotic must be non-pathogenic and non-toxic
- The probiotics must contain a large number of viable cells
- The probiotic must be capable of surviving and metabolizing in the gut
- The probiotic must remain viable during storage and use (Rolfe, R. D., 2000)

HEALTH EFFECTS

The health effects attributed to the use of probiotics are numerous. The following outcomes are well documented: 1) lower frequency and duration of diarrhea associated with antibiotics (Clostridium difficile), rotavirus infection, chemotherapy, and, to a lesser extent, traveler's diarrhea; 2) stimulation of humoral and cellular immunity; and 3) decrease in unfavorable metabolites, eg, ammonium and procancerogenic enzymes in the colon. There is some evidence of health effects through the use of probiotics for the following:

1. reduction of Helicobacter pylori infection;
2. reduction of allergic symptoms;
3. relief from constipation;
4. relief from irritable bowel syndrome;
5. beneficial effects on mineral metabolism, particularly bone density and stability;
6. cancer prevention; and
7. reduction of cholesterol and triacylglycerol plasma concentrations (weak evidence).

Numerous effects can hardly be explained by a unifying hypothesis that is based on a single quality or mechanism and remains valid for all microorganisms exerting one or the other effect.

Antimicrobial activities of probiotics include the
(1) Production of bacteriocins/defensins,
(2) Competitive inhibition with pathogenic bacteria,
(3) Inhibition of bacterial adherence or translocation,and
(4) Reduction of luminal pH. Probiotic bacteria can also enhance intestinal barrier function by increasing mucus production.
Probiotics have several mechanisms of action to protect the host against intestinal disease it
given as follows:

1. Antimicrobial activity
2. Colonization resistance
3. Immune effects
4. Antimutagenic effects
5. Antigenotoxic effects
6. Influence on enzyme activity
7. Enzyme delivery.

**STRAIN CHARACTERISTICS AND HABITAT SPECIFICITIES**

Different strains of probiotic bacteria may exert different effects based on specific
capabilities and enzymatic activities, even within one species. *(Ouwehand A.C., 1999,
Bernet M.F., 1993)*

Different microorganisms express habitat preferences that may differ in various host
species. *(Freter R., 1992)* Lactobacilli are among the indigenous flora colonizing the
chicken's crop, the stomach of mice and rats, and the lower ileum in man. Bacteria colonizing
such high-transit-rate sites must adhere firmly to the mucosal epithelium *(Savage D.C., 1972,
Fuller R., 1973, Beachey EH., 1980)* and must adapt to the milieu of this adhesion site. The
competition for adhesion receptors between probiotic and pathogenic microorganisms,
therefore, is dependent on such habitat specifics.

On the other hand, bacteria are found in much higher numbers in the colon,
particularly in the feces, than are lactobacilli. It is self-evident that effects bound to this
luminal site of action may be exerted even more efficiently by such microorganisms, which
do not necessarily need to adhere to the mucosa. Moreover, preferences for microhabitats
have to be considered. Four microhabitats in the gastrointestinal tract were outlined by Freter
*(Freter R., 1992)* as follows: 1) the surface of epithelium cells; 2) the crypts of the ileum,
cecum, and colon; 3) the mucus gel that overlays the epithelium; and 4) the lumen of the
intestine.
Introduction

Several indigenous, pathogenic, or probiotic microorganisms target the surface of the epithelium by specific adhesion, often mediated by special organelles, eg, fimbriae. (Beachey E.H., 1980, Gibbons R.J., 1975) The crypts are typically colonized by motile, spiral-shaped bacteria of the genera *Borellia*, *Treponema*, *Spirillum* (Lee A., 1980., Lee A., 1985) and others, eg, *H. Pylori*. (Blaser M.J., 1990) The mucus layer can form a microbial habitat and can protect the host against colonization in some circumstances. As a result of its complex and varying composition and for technical reasons, its function in this context is least clarified.

The luminal content of bacteria depends greatly on bowel transit. Therefore, the microbial density in the small bowel is low, whereas it is abundant in the lumen of the colon, which gives space to microorganisms without adhesion molecules.

When the great variety of species, strain characteristics, and the habitat specifics are considered, it becomes clear that a proven probiotic effect on a one strain or species can not be transferred to other strains or species.

The term *prebiotic* was introduced by Gibson and Roberfroid (Gibson G.R., 1995) who exchanged "pro" for "pre," which means "before" or "for." They defined prebiotics 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." This definition more or less overlaps with the definition of dietary fiber, with the exception of its selectivity for certain species. This selectivity was shown for bifidobacteria, which may be promoted by the ingestion of substances such as fructooligosaccharides and inulin (Gibson G.R., 1995, Hidaka H., 1986, Gibson G.R., 1995), transgalactosylated oligosaccharides (Tanaka R., 1983, Ito M., 1993, Rowland I.R., 1993), and soybean oligosaccharides. (Hayakawa K., 1990, Saito Y., 1992)

The term *synbiotic* is used when a product contains both probiotics and prebiotics. Because the word alludes to synergism, this term should be reserved for products in which the prebiotic compound selectively favors the probiotic compound. In this strict sense, a product containing oligofructose and probiotic bifidobacteria would fulfill the definition, whereas a product containing oligofructose and a probiotic *Lactobacillus casei* strain would not. However, one might argue that synergism is attained in vivo by ingestion of lactobacilli on the one hand and promotion of indigenous *Bifidobacteria* on the other hand.
MECHANISM OF PROBIOTICS ACTIVITY

Probiotics exert their effects on the host but mechanisms are still speculative. The mechanism of action of Probiotics strain seems to be the manufacture of specific chemicals and from existing evidence appears to be strain specific. Enhancement of colonization resistance and/or direct inhibitory effects against pathogen is likely to be important mode of action in situations in which probiotics have reduced the occurrence and duration of gastrointestinal They may antagonize pathogen directly through production of antimicrobial and antibacterial compounds such as Cytokines and Butyric acid (De Vuyst and Vandamme, 1994; Kailasapothy and Chin,2000), reduce the gut pH by stimulating the Lactic acid to improve micro flora (Langhendries et al., 1995), compete with pathogens for the binding to receptors at various sites (Fujiwara 1997, Kailasapathy and Chin 2000).


A Group of requirements have been identified for microorganisms to be defined as an effective probiotics. (Salminen et al., 1996) These are,

1) Adhere to cells
2) Exclude or reduce pathogenic adherence.
3) Persist and multiply.
4) Produce acids, peroxidases and bacteriocins antagonistic to pathogen growth.
5) Safe, non-invasic, non-carcinogenic and non-pathogenic.
6) Co- aggregate to form a normal balanced flora.

The summarized mechanisms underlying Probiotics activity could be:

1) Inhibit growth of potential pathogens by producing lactic acid, bactriocin etc..
2) Competitive exclusion of enteric pathogen.
3) Triggers cytokine synthesis from enterocytes by attaching to their surface.
4) Neutralization of dilatory carcinogens by the production of butyric acid.
5) Restore the normal intestinal flora during antibiotic therapy.
6) Produce toxic metabolites e.g. H₂O₂
Probiotics are gaining importance because of the innumerable benefits e.g.

- Treating lactose intolerance.
- Hyper cholesterol problem.
- Cardiac diseases and managing cardiac problems like Atherosclerosis, Arteriosclerosis.

Systematic Investigations should be made on the relation of gut microbes to the prevention of aging and on the influence of diets which prevent intestinal putrefaction in prolonging life and maintaining the forces of the body.

Probiotics offer an alternative to be used in place of or concurrently with conventional antimicrobials. Because of their relative safety, ease of use and excellent tolerability to bile’s, the use of Probiotics should be considered, not just as adjunctive replacement after antibiotic administration but as possible first line therapy when clinically indicated. Correct uses of Probiotics have been successful to enhance treatment of bacterial vaginosis, recurrent UTI, diarrhoea, cancer, competition with Antibiotic therapy, Dysbacteriosis and dislipidemis. Use of Probiotics appears to be a promising answer to current challenges experienced due to excessive use and development of tolerance of Antibiotics.

GI tract harbours a complex ecology of microorganisms. A good balance in microbiota promotes good gut health while disturbance in the micro ecosystem results in various gastrointestinal disorders. Probiotics supports the intestinal flora balance and their beneficial effect on various GI disorders (Ulcers, Lesions. \textit{H.pylori} Infection, Inflammatory bowel diseases, irritable bowel syndrome, acute gastroenteritis, maldigestion, lactose intolerance and colon cancer)

ADVANTAGES OF PROBIOTICS

Probiotics have many of advantages. These are not just true to old people but for young ones as well. Among a list of benefits of probiotics are the following things to take note of:

- \textbf{Nourishment of the body}. The hydrocarbon in our system can be stopped by probiotic bacteria. This results to easy break up of foods into simple components which permits nearly complete assimilation by the digestive structure. Thus, probiotics significantly augment complete nourishment and boost fast cellular progress and improvement.
• **Clearing of the intestinal area.** Probiotics has the ability to clear up our intestinal area by removing the accrued corrosion beneath the coating of crud in your intestinal partitions. They flush out the garbage from our intestines fully.

• **Creation of essential enzymes.** Probiotics are also useful in creating numerous essential enzymes and intensify the accessibility of some forms of vitamins and nutrients like vitamin K, lactase, fatty acids and calcium.

• **Strengthens the immune system.** The helpful bacteria can also intensify the function of our immune system to alleviate the indications of allergies, recurring exhaustion condition and systemic candid.

• **Eliminates digestive disorders.** In addition to the mentioned benefits, problem with constipation is rapidly alleviated with probiotics giving us regular bowel movements which also denote that diarrhoea problem is lessened. The friendly bacteria established in probiotics develop particular proteins that work as antigens. The pools of imprecise antigens obtainable in support of immune system are then utilized in combating majority of the distinctive illnesses in our digestive area.

• **Defers bacteria production.** As soon as probiotics are linked up to our intestinal partitions, they start to develop a gentle acidic surrounding that defers the development of bacteria which cause illnesses. In this manner, probiotics also aid our immune system due to the fact that it is distributed inside the intestines while continuing its function on the different parts of our body.

• **Eliminates harmful toxins from the body.** The microorganisms in probiotics work in harmony with the different tissues and organ cells to metabolize proteins and aid to get rid of poisonous excesses from our body.

• **Invigorates antibody development.** Probiotics can also invigorate B-lymphocyte and other associated antibody development by producing large groups of additional antibodies all set to defend our body from toxic contamination.

• **Destruction of viruses.** Lastly, the very useful bacteria can destroy viruses and parasites that endanger your overall health.
OBJECTIVES

The following objectives were set to be achieved through research:

- Isolation of Probiotics from various natural samples and their characterization in terms of morphology and biochemical activities.
- Study of effect of various factors like salt, pH. Bile salt and acids on isolated probiotics.
- Study of Growth pattern of probiotics isolates.
- Study of antimicrobial susceptibility of probiotics & its antimicrobial activity on other indigenous micro flora.
- Study of Growth of Probiotics isolates with Prebiotics.
- Study of possible mechanism of probiotics and synbiotics and its significance in their therapeutic evaluation.