CHAPTER 1

INTRODUCTION

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The aggregation of all microbiota of humans are known as human microbiome which resides on or within tissues as well as in the corresponding anatomical regions such as, placenta, mammary gland, seminal fluids, ovarian follicle, lungs, saliva, skin, and oral mucosa. Probiotics are health benefiting micro-organisms that can modulate the different internal and external parameters of the host's environment. These are the bacteria which always show a close association with normal flora of human external body surface as well as internal surfaces. But the colonization of predominant beneficial bacteria is mostly on the gastrointestinal tract which is known as Probiotic [Ducatelle *et al.* 2015].

The meaning of the probiotic is "for life". Based on their activities on the host body they are defined as living but non-pathogenic bacteria which has a significant beneficial role on the gastrointestinal tract. The term was first introduced by Vergin (1954) during his studies on gut microbes and its antagonistic mechanism on ant-microbial components [Kavita *et al.*, 2015]. Later, Lilly and Stillwell (1965) [Amarna, 2016; Seppo, *et al.*, 1999] redefined the probiotic as a product which can stimulate the growth of the other micro-organism. Then further this probiotic is considered as non-pathogenic by Fuller (1992). And finally they were described as a microbe that when administered on host show some beneficial role it by FDA and WHO and these are the bacteria which generally mediate important immunological and physiological processes [Hardy *et al.* 2013; He and Shi, 2017].

Prebiotics are generally referred to those food ingredients that generally include different carbohydrates which are non-digestible and they are responsible for promoting host health by

enhancing the proliferation of the good bacteria i.e., probiotics. [DeVrese and Schrezenmeir, 2008] These are the organisms that are basically resistant to bile, stomach acid and some of the hydrolytic enzymes present in the intestine. Prebiotics are the compounds which cannot be absorbed by upper GI tract. They are digested and absorbed by these beneficial bacteria in the intestine which is initiated by some specific carbohydrate modifying enzymes [Kuo 2013]. From the several studies, it was observed that prebiotics like inulin and pectin can reduce the prominence of some gastrointestinal disease, such as; bowel disorders, intestinal inflammation, colon infections etc [Pena 2007]. In recent times, several plants are considered as a prime source of prebiotics such as, dragon fruit, yacon root etc. [Saulnier *et al.*, 2009].

The concept of the synbiotic was introduced by Gibson. He speculated that the effect of probiotic as a health modulator and will be enhanced by the presence of prebiotic and symbiotic (probiotic+prebiotic) [Vrese and Schrezenmeir, 2008]. The synbiotic affect the gastrointestinal tract's microbial environment by promoting the proliferation of probiotics in a selective manner [Cencic and Chingwaru, 2010].

Several factors such as, antibiotic abusing, stressful life leading, non-maintained diet and different pathogenic infections may lead to dysbiosis which again causes lack of immune response and may even develops low grade chronic inflammation [Vieira *et al.* 2013].

The important and preliminary effects of probiotics that are documented till date are many, which include prevention of different gastrointestinal diseases, attributing anti-oxidant activity, anti-bacterial activity, anti-inflammatory as well as reduction in cholesterol assimilation [Harish and Varghese, 2006].

The species used as probiotic generally belong to LAB group. The probiotics most extensively studied for their health promotion are *Lactobacillus, Streptococcus* and *Bifidobacterium*. The different strains of *Lactobacillus* are utilized as probiotic including *Lactobacillu. acidophilus*, along with *L. reuteri, L. bulgaricus, L.casei, L.fermentum, and L. rhamnosus* etc. The other group of bacteria which is not included in the LAB are *Streptococcus*, *Bacillus*, *Enterococcus*, *Escherichia coli*, and *Propionibacterium sp*. etc. [Rastogi *et al.*, 2011].

The health enhancing criteria of the beneficial bacteria followed several mechanisms to modulate the intestinal immunity as well as its ecosystem along with its prevention to the disorders. The role of the probiotics for maintaining the beneficial role in the gut is as follows:

[1] Prevention to the lactose intolerance.

[2] Reduction of the cholesterol level.

[3] Improvement of the intestinal tract health.

[4] Declination of the allergy level.

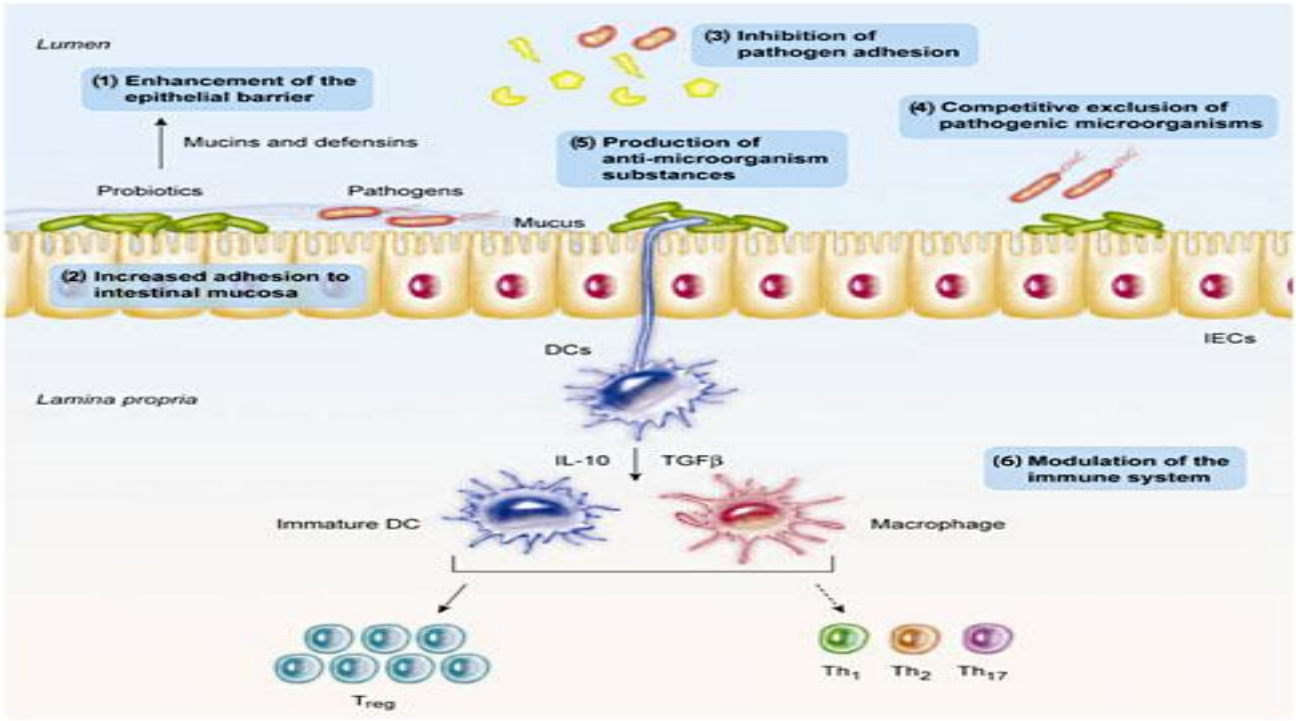
[5] Synthesis of different biomolecules as well as anti-microbial compounds.

[6] Reduction of the carcinogenesis (to a certain extent).

[7] Prevention of the inflammation.

[8] Prevention of the pathogenic bacteria.

Based on the above role mentioned, the probiotics are considered as a health promoting agents on administrating to the host act as a food supplement. The food which contains the beneficial living bacteria is generally milk and different milk products.



(Adapted from Bermudez *et al.* 2012)

The action of the probiotic organism for enhancing the health promotion is as follows:

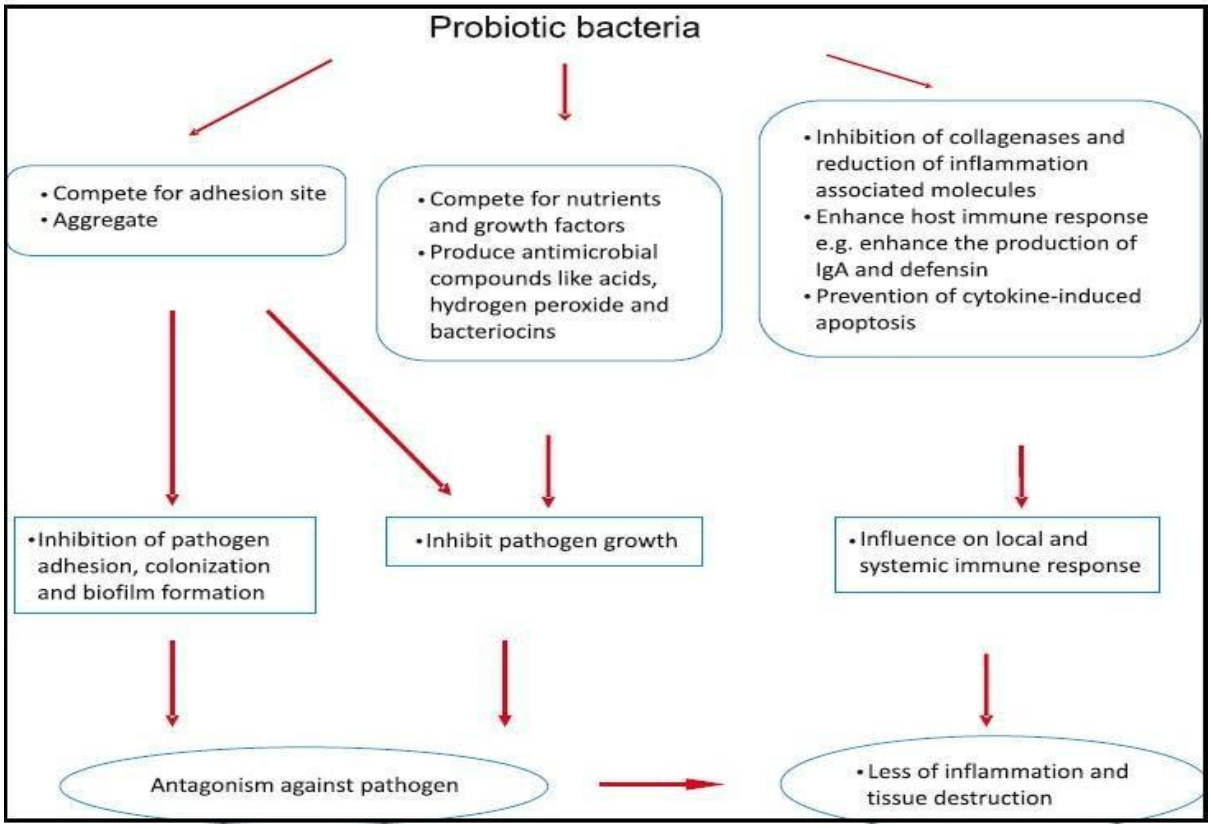
[1] The competitive adhesive property of the probiotic organisms leads to the inhibition of the attachment of the pathogenic bacteria. It gives a tough competition for the nutrients, shows enhanced expression of the intestinal mucin genes, aid in production of anti-microbial components [Dsouza *et al.*,2002].

[2] Several study also suggest that probiotics produce certain compounds which have the ability to neutralize the endotoxins which are generally produced by different disease making organisms that in turn increases the plasma level [Fioramonti *et al.*, 2003].

[3] The restriction in the growth of the disease causing microbes has been achieved by manufacturing several compounds, such as; anti-microbial organic compounds, hydrogen peroxide and also lactic acid that lowers the intestinal pH which cannot allow any pathogenic bacteria to grow [Forestier *et al.*,2001].

[4] The production of the amines by the coliform bacteria which are pathogenic in nature and can be inhibited by the antagonistic effects of the beneficial microbes [Abhisingha *et al.*, 2018].

[5] The host immune system stimulates the production of the antibodies, WBC, and also elevates the number of the immune cells, like, macrophages, monocytes and natural killer cells. This entire innate mechanism can be highly affected by the presence of the probiotics in it [Gill and Guarner,2004].



[**Adapted by Rastogi *et al.*, 2011]**

The types of gastro-intestinal disorder that generally takes place on the mammalian host, specifically on humans are described as follows;

[1] Diarrhea is described as loose or watery stools thrice during in a day as stipulated by WHO. [Narayan *et al.*, 2010] It is of three types;

a) Diarrhea caused by rotavirus known as acute infantile diarrhea is the gastro-intestinal abnormalities. Rehydration therapy is given along with probiotic as a treatment dose [Szymański *et al.*, 2006].

b) The disturbance/destruction of intestinal microflora by abusing the antibiotics causes diarrhea known as antibiotic associated diarrhea. Due to excess use of the

antibiotic on host it diminishes the normal microflora of the gut too, which in turn affect the normal condition of the intestine [Bartlett, 2002].

c) The traveller's diarrhea is a kind of diarrhea which when occurs on human is due to the presence of some of the harmful bacteria, such as, *E. coli* and in some cases *Salmonella* sp. that occurs in maximum number of travelers of the world. And this can be also controlled by the antagonism with probiotic bacteria [Lewis *et al.* 2014].

[2] Inflammatory Bowel Syndrome (IBS) is the most common disease and is generally characterized by bloating, fluctuating bowel habit and different abdominal discomfort along with flatulence. This is a kind of disease which can be easily experienced and can be treated by probiotics. Several study suggested the elimination of such infection by Lab group of probiotics [Fuyuno *et al.* 2016].

[3] The disease which is categorized by the absence or low level of β-galactosidase enzyme activity and leads to a condition where host can't even tolerate lactose digestion. This particular type of condition is known as lactose intolerance which is a very common disease in recent days can also be treated with certain strain of probiotics [Vonk *et al.* 2012].

[4] IBD is chronic disorder that can cause in repeated manner which initiates irritation in the GI tract. The symptoms include watery diarrhea along with occasional bleeding [Matsumoto *et al*. 2005].

Ulcerative colitis: (UC): This disease occurs in the GI tract and causes serious damages on the intestinal and rectal linings. If it lasts for long there is a risk remained for the ulcer

formation in colon that may also cause cancer, in which Lactobacillis is a suggested treatment for it [Kelesidis and Pothoulakis, 2012].

Crohn's disease: (CD): In this disease intestine inflammation takes place, and it can also cause damages or inflammation in the entire route of the gastrointestinal tract. It generally makes the body unable to digest food which gives best result on probiotic treatment [Chermesh *et al*., 2007].

Probiotics are also known as potent modulators of the innate as well as adaptive immunity. The mechanisms on which this modulation depends are many which also lead to activate different immune cells [Tsai *et al.* 2012]. Among many other probiotic, LAB are primarily responsible for maintaining the immune response towards all the antigens including pathogens [Menard *et al.* 2004 and Feng *et al*. 2016]. The key role of the LAB is to modulate our immune system by regulating the anti- and pro-inflammatory response. Based on this anti-inflammatory response probiotic is proposed for effective therapeutic agent [Gomes *et al.* 2014]. The decrease level of the endotoxin is initiated by *Bifidobacteria* which improves normal modulation like enhancing barrier function etc. [Cani *et al.* 2008] *Lactobacillus* reportedly enhances the immunomodulatory effects [Santos Rocha *et al.* 2012].

Several probiotics potentially acts as an immunomodulator which can interact with monocytes, macrophages epithelium and lymphocytes [Borruel *et al.*, 2002]. Several researchers working on animal models reframed the fact that the significance of cytokines depends on the specific attachment to the receptors on cell membrane as well as inhibiting those cytokine-regulated genes [Biswas *et al.*, 2013]. IL 10, an anti-inflammatory cytokine, produced by T and B cells, macrophages, monocytes & NK cells which inhibit the pro-inflammatory cytokines, chemokines, and its receptors that may cause inflammation. An immunostimulatory and immunoregulatory

mechanism of probiotics is very predominat in many of the recent studies. They showed ability to act antagonistically against pathogenic infection and carcinogenesis. It thereby induces the production of the IL-12 which may activate natural killer cells. Similarly, immune-regulatory effects of the probiotics have been categorized by the stimulation of IL-10 and also Treg cells [Chiba *et al.*, 2010].

Thus, probiotic is growing its importance by incorporation of some robust native strain aimed with certain health benefits to promote the scientific research moving forward into a new dimension to develop an alternative therapeutics against some selective diseases including GI diseases. Thus, the aim of this study was to isolate the bacterial strains based on their potent probiotic abilities from different milk and milk products, and to evaluate their *in vitro* and *in vivo* characteristics to propose them as an effective therapeutic tool against gastro-intestinal diseases.