CHAPTER 1

Introduction
1. INTRODUCTION:

1.1 Probiotics

The concept behind probiotics was introduced in the early 20th century, when Nobel laureate Elie Metchnikoff, known as the "father of probiotics," proposed in *The Prolongation of Life: Optimistic Studies* that ingesting microorganisms could have substantial health benefits for humans. Scientists continued to investigate the concept, and the term "probiotics"—meaning "for life"—eventually came into use.

There have been numerous definitions of probiotics all of which boil down to two broad definitions;

i) The definition of probiotic bacteria adopted by the joint Food and Agricultural Organisation/World Health Organisation (FAO/WHO) working group, -- "live microorganisms which when administered in adequate amounts confer a health benefit on the host" (FAO/WHO., 2002).

ii) Guarner and Schaafsma defined probiotic -- "living microorganisms that, upon ingestion in certain numbers, exert health benefits beyond inherent basic nutrition" (Guarner et al., 1998).

Specific strains of a probiotic species confer specific benefits and are hence used accordingly for treatment. Several probiotic supplements are available as single strain products, as well as products with multiple species and strains. In addition, probiotic supplements are generally available for use in specific doses of each species/strain or a combination of several species or strains or both so that the requirement of a
clinically relevant dose is achieved. Probiotics generally are the strains of
*Bifidobacterium* or *Lactobacillus* species. These are derived from the microbiota of
the healthy human intestines as well as there are nonhuman strains isolated from the
fermented dairy products (Tsilingiri et. al, 2012; Robert *et al.*, 2006).

1.2 *Lactobacillus* as probiotics:

The genus *Lactobacilli* is one of the most common probiotic genera. *Lactobacilli*
refer to a genus of lactic acid-producing, friendly bacteria that make up many of the
400 normal probiotic species in the human body. The most important of these are
probably *L. acidophilus, L. rhamnosus, L. casei* and *L. plantarum*. *Lactobacillus*
strains isolated from the human intestinal tract and fermented dairy products are
generally regarded as safe (GRAS) and have been extensively exploited for their
probiotic properties. *Lactobacilli* among bacteria have emerged as the most
commonly used probiotics in animal feeds and human foods (Cross *et al.*, 2002;
Puertollano *et al.*, 2008; Tsilingiri et. al, 2012). *L. plantarum* for example is widely
found in a range of food such as dairy, meat, and vegetable products. It is commonly
found in the human gastrointestinal tract as a consequence of its proven ability to
survive gastric transit and colonize the gut. *L. plantarum* is considered a food-grade
microorganism because of its long and documented history of safe use in fermented
foods (de Vries *et al.*, 2006).

WHO and FAO recommended intake of a specific dose of vegetable and fruits in
daily food to prevent chronic pathologies such as hypertension, coronary heart
problems, and risk of strokes. The consumers tend to prefer the foods and beverages
which is fresh, highly nutritional, health promoting and ready to eat or ready to drink. Lactic acid (LA) fermentation of vegetables and fruits is a common practice to maintain and improve the nutritional and sensory features of food commodities. A great number of potential lactic acid bacteria (LAB) were isolated from various traditional naturally fermented foods. The genus *Lactobacillus* is a heterogeneous group of LAB with important implications in food and feed fermentation. *Lactobacilli* are currently used as probiotics, silage inoculants, and as starters in fermented food. Asian traditional fermented foods have been found to be generally fermented by LAB such as *Lactobacillus plantarum*, *L. pentosus*, *L. brevis*, *L. fermentum*, *L. casei*, *Leuconostoc mesenteroides*, *L. kimchi*, *L. fallax*, *Weissella confusa*, *W. koreenis*, *W. cibaria*, and *Pediococcus pentosaceus*, which are considered as the probiotic source of the food practice. Availability of certain specific nutrients such as vitamins, minerals, and acidic nature of fruits and vegetables provides conducive medium for fermentation by LAB (Manas *et al.*, 2014).

In eastern Himalayan regions of India a wide range of fermented vegetable products are prepared for bioprocessing the perishable vegetable for storage and further consumption. Lactic acid fermentation has been widely used across this region and vegetables such as gundruk, sinki, and khalpi are fermented vegetable product of Nepal, Sikkim, and Bhutan. *Lactobacillus brevis*, *L. plantarum*, *Pediococcus pentosaceus*, *P. acidilactici*, and *Leuconostoc fallax* are the predominant LAB involved in ethnic fermented vegetables. Predominant functional LAB strains associated with the ethnic fermented tender bamboo shoot products, mesu, soidon,
soibum, and soijim of the Himalayas, were identified as *L. brevis*, *L. plantarum*, *L. curvatus*, *P. pentosaceus*, *L. mesenteroides* subsp. *mesenteroides*, *L. fallax*, *L. lactis*, *L. citreum*, and *Enterococcus durans*. Some of the LAB strains may also possess protective and functional properties that render them as interesting candidates for use as starter culture(s) for controlled and optimized production of fermented vegetable products. Several other ethnically different fermented food products are available in this region varying according to the traditional practices. But most of them are lactic acid fermented with LAB being the predominant fermenters in almost all of them (Tamang *et al.*, 2005 and 2009).

1.3 General health benefits of probiotics:

Probiotics are now accepted as being useful in the prevention and/or treatment of certain pathological conditions, mainly (but by no means exclusively) infections of the small and large intestine. Controlled clinical trials have shown beneficial outcomes for the use of probiotics in such diverse conditions as rotavirus infections, antibiotic-associated diarrhoea, irritable bowel syndrome, inflammatory bowel disease, atopy in at-risk infants and chronic sinusitis (Ouwehand *et al.*, 2002; de Vrese *et al.*, 2002; Habermann *et al.*, 2002).

In addition to the above-mentioned, some of the health benefits which have been claimed from probiotics include the improvement of the normal microflora, prevention of infectious diseases and food allergies, reduction of serum cholesterol, anticarcinogenic activity, stabilization of the gut mucosal barrier, immune adjuvant
Introduction

properties, alleviation of intestinal bowel disease symptoms, and improvement in the digestion of lactose in intolerant hosts. Effects can also be seen in mucosal sites distant from the gut such as the bronchus, mammary glands, and the urogenital tract. De Vrese and Schrezenmeir in a tantalisingly incomplete report, found that heat-killed probiotic organisms were just as effective as viable ones (de Vrese et al., 2000 and 2002; Ouwehand et al., 2002).

Probiotics in dietary supplements or foods may be live, heat-treated, irradiated, spray-dried, or freeze-dried. Inactivated probiotics may be as effective as live probiotics in certain conditions and may be more favourable because of lower infectious risk (especially in infants whose gut defense barrier is immature) (Tengfei et al., 1998).

It is postulated that the probiotic organisms colonise the intestine and then exert several effects. These include competing for nutrients, competitive exclusion (occupying sites on the gut wall to which pathogens would otherwise bind), production of inhibitory compounds (such as lactate, hydrogen peroxide, short-chain fatty acids and bacteriocin-like substances) and immunomodulatory stimuli. Temporary colonisation of the gut with an appropriate probiotic strain not only promotes the state of ‘eubiosis’ (favourable balance of the gut flora) but also can have a favourable immunomodulatory effect (Hamilton-Miller, 2003).
1.4 Immunomodulation by probiotics:

Immunomodulation is a therapeutic approach in which there is an effort to intervene in auto regulating processes of the defense system. It encompasses any intervention directed at modifying immune response with therapeutic intent (Juan C. Gea-Banacloche, 2006).

Gut colonization by probiotics have shown evidence of a role in promoting and maintaining a balanced immune response in early life. A changed or less varied gut microbiota composition has been associated with atopic diseases and/or obesity. Moreover, certain gut microbial strain or strains have been shown to inhibit or attenuate immune responses associated with chronic inflammation in experimental models. However, there has been no fully adequate longitudinal study of the relation between the neonatal gut microbiota and the development of allergic diseases (e.g., atopic asthma) and obesity. The emergence of promising experimental studies has led to several clinical trials of probiotics (live bacteria given orally that allow for intestinal colonization) in humans (Grand et al., 1976).

Health claims of lactic acid bacteria (LAB) used in functional foods and pharmaceutical preparations are based on the capacity of these microorganisms to stimulate the host immune system and most of these effects were dose dependent. *L. casei, L. delbrueckii ssp. bulgaricus* and *L. acidophilus* have shown to enhance the IgG1 response favouring Th2 balance, while *L. acidophilus* also increased the IgG2a response inducing Th1 balance. Studies also show that lactic acid bacteria induce
distinct mucosal cytokine profiles showing different adjuvant capacity among them (Savini et al., 2010).

Since the bacterial properties required for the different applications are clearly distinct, strain selection is very important for a more rational use of these microorganisms. The precise mechanisms by which different probiotics impact the mammalian immune system have yet to be discovered. It is likely that extracellular bacterial factors play a pivotal role, as these molecules establish the first interactions between the bacteria and host cells. For instance, the lactic acid bacterium (LAB) *Lactobacillus plantarum* WCFS1 which is a single colony isolate of the strain NCIM8826 has been shown to demonstrate its immunomodulatory properties *in vitro* depending on the presence of specific cell-envelope molecules. Even subtle differences in the composition of these molecules can induce large differences in the host cell immune response. The exact role of these molecules and the type of host response they generate *in vivo* remains to be identified (Smelt et al., 2013).

In recent years, the beneficial effects of probiotics on immune-mediated diseases, such as allergies and asthma, have been documented (Di Giacinto et al. 2005). One explanation for the beneficial effect of probiotics on allergic responses is their inhibition of the production of IgE. Furthermore, many studies have proposed that probiotics, even as inactivated cells, are able to turn a Th2 response into a Th1 or Th0/Treg response. Inactivated probiotic preparations were shown not only to have the capacity to bias primary immune responses towards a Treg/Th0-type profile but also to modulate the development of Th2-biased responses. Heat-
inactivated *Lactobacillus casei* Shirota was able to induce IL-10 in the same pattern as the viable cells. The immunomodulatory properties of probiotic *Lactobacilli* in a study against *H. pylori* showed that this was not a result of adhesion to epithelial cells or bacteriocin production (Majamaa and Isolauri 1997; Cross *et al.* 2004; Ryan *et al.* 2008; Valentina Taverniti and Simone Guglielmetti, 2011). Hence the probiotic potential to modulate immune response therapeutically requires further understanding for which we have taken this study for particular potential probiotic strains of *Lactobacilli*.

There has been an alarming increase in the number of antibiotic resistant pathogenic microbes because of indiscriminate use of the same. Hence use of probiotics is an increasing trend to mitigate these effects by the use of strains of GRAS (Generally Regarded As Safe) microbes particularly those of the genus *Lactobacillus*. Studies in the disease state hardly reflect and predict the immunomodulatory effects of the bacteria in the healthy intestine, e.g. during immune homeostasis. Knowledge on how different bacterial strains affect the local and systemic immune system in the absence of disease will gain mechanistic insights in bacterial-host interactions and will help clarify the magnitude of their effects in non-diseased individuals. Also intraperitoneal mode of administration has been chosen to elicit a direct and immediate immune response to the antigens of the potential probiotic strains. This study aims and intends to search, identify, infer and understand the isolates of the genus *Lactobacillus* as potential candidates for probiotics. Keeping the abovementioned facts and understandings we have put forward the following aims and objectives of our study.
1.5 Aims and objectives:

1.5.1 Hypothesis:

Probiotics are being increasingly used as an alternative to indiscriminate use of antibiotics. There have been a large number of reports of positive effects on disease amelioration through antipathogenic activity and several immunogenic factors. Keeping in view the various research reports we intend to isolate Lactic Acid Bacteria particularly *Lactobacillus* species for probiotic potential and hence have hypothesized the following:

"High bile and low pH resistant Lactic Acid Bacteria are commensurate with probiotic properties being retained within the testing environment of the gastrointestinal tract amongst the varying microbiota, both averse and amiable with respect to properties including immunomodulation."

1.5.2 Objectives:

1. Isolation and identification of *Lactobacillus* species from the fermented food products of Assam.


3. Characterization of probiotic properties of *Lactobacillus* species

   a) growth at low pH

   b) growth at high bile concentration

   c) antipathogenic activity
d) antibiogram profiling

4. Study of immunomodulation by potential probiotic *Lactobacilli* in mice