CHAPTER 1
INTRODUCTION
1.1 Probiotic lactic acid bacteria (LAB) and human health

The mammalian gastro-intestinal tract contains a complex and diverse society of both pathogenic and nonpathogenic (probiotic) bacteria. Probiotics are thought to supplement the microbial gut community, maintain epithelial barrier function and promote general immune homeostasis (Schaible and Kaufmann, 2005). Lactic acid bacteria (LAB) have an important role as probiotic starter culture due to increasing consumer awareness of the potential risks derived not only from food borne pathogens, but also the artificial chemical preservatives used to control them (Hamilton-Miller, 2003a). LABs include a group of Gram-positive bacteria from the genera *Bifidobacterium*, *Streptococcus*, *Lactococcus*, *Lactobacillus*, *Carnobacterium*, *Enterococcus*, *Pediococcus* and *Weisella*; non-spore forming cocci or rods which produce lactic acid as the major end product of carbohydrates fermentation (Rodriguez et al., 2000). Lactic acid bacteria are used in the food industry because they have got “GRAS-Generally Recognised as Safe” status (Jack et al., 1995). Their growth lowers the pH that inhibits the growth of most of the other microorganisms, the biochemical conversions involved in growth, enhances the flavor, improves organoleptic and nutritional properties (Scillinger and Lucke, 1989) and many strains produce antagonistic compounds such as organic acids, hydrogen peroxide, diacetyl and bacteriocins (Ray and Daeschel, 1994). More recent commercial efforts focus on food supplementation with live probiotic cultures in the form of fermented milk products.

Bacterial species such as *Bifidobacterium breve* (Yakult), *B. bifidum* (Bb-12), *B. esselnsis* (Danone {Bio Activia}), *B. infantis* (Shirota, Immunitass, 744, 01), *B. lactis* (Bb-02), *B. longum* (BB536, SBT-2928), *Lactobacillus acidophilus* (La2, La5, *johnsonii*, NCFM, DDS-1, SBT-2062), *L. bulgaricus* (Lb12), *L. fermentum* (RC-14), *L.
lactis (La1, A164, BH5), L. plantarum (299v, Lp01), L. rhamnosus (GG, GR-1, 271, LB21), L. reuteri (SD2112) are widely used in the development of probiotic drinks (Sharma and Mishra, 2013), pills, tooth paste, chewing gums, poultry, food supplements, infant formula meals (Krishnakumar and Gordon, 2001). The stimulatory capacity of probiotics have been tested in farm animals where their contribution to improve overall health status, immune system functions, reduce risk of infection and improve yield of poultry and meat products is highly appreciated (Reuter, 2001). Evidences support the stimulatory capacity of the probiotic microorganisms, but the final verdict is not out yet. Questions as to which species, strains or mixtures thereof are most beneficial, and the molecular basis for these effects require more detailed studies (Scheinbach, 1998; Gibson and Rastall, 2004).

A series of review articles have been published in the past year outlining the efficacy of probiotics in human health (Marteau et al., 2002; Teitelbaum and Walker, 2002; Bengmark, 2003; Steidler, 2003; Tuohy et al., 2003; Fedorak and Madsen, 2004). Some of these are enlisted below:

- Probiotic LAB strains help to restore a healthy microbial balance in the digestive tract (Karen Collins, 2007).
- They reduce the chance of infection by opportunistic pathogens like Bacillus cereus, Campylobacter jejuni, Clostridium botulinum, C. perfringens, C. sporogenes, Enterococcus faecalis, Escherichia coli, Leuconostoc mesenteroides, Listeria monocytogenes, Neisseria mucosa, Pseudomonas putida, P. aeruginosa, Staphylococcus aureus, Shigella, Streptococcus mutans and Salmonella typhimurium. These are only a few to name them (Kaur et al., 2012a).
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They are used to treat ulcerative and gastrointestinal illness (McFarland, 2007; Kumar et al., 2012).

They can be useful for development of personal health care products for treating vaginal and urinary infections (Reid et al., 2001; Reid and Bruce, 2006; Falagas et al., 2006; Kaur et al., 2013a).

They inhibit growth and translocation of peptic ulcer causing Helicobacter pylori (Hamilton-Miller, 2003b; Kaur et al., 2012a).

They promote recovery from antibiotic associated diarrhea, constipation, diarrhea and dysentery (Szajewska and Mrukowicz, 2005).

Probiotic LAB can increase the bioavailability of protein and fats in the diet by breaking down these nutrients in the digestive tract. This is particularly important for infants, toddlers and patients who need building up during and after illness (Pravez et al., 2006).

They contribute to health by enhancing specific and nonspecific immunity (Schiffrin et al., 1997).

Probiotic bacteria produce interferon gamma (IFN-\(\gamma\)) that stimulates immune system of the host by improving phagocytic cell functioning (Schiffrin et al., 1997; Schaible and Kaufmann, 2005). IFN-\(\gamma\)-activated macrophages inhibit growth of pathogenic bacteria like Mycobacterium as a result of Tfr downregulation (Schaible and Kaufmann, 2004; 2005).

They improve immune functioning by increasing number of IgA-producing plasma cells, monocytes, macrophages, T lymphocytes and natural killer cells that collectively check pathogen growth (Reid, 2001; Hakansson and Molin, 2011).
They stimulate gastrointestinal immunity by interacting with Payer’s patches (Falk et al., 2002; Szajewska et al., 2005).

They have been proposed to optimize the effects of vaccines like rotavirus vaccine, typhoid fever vaccine (Goyal et al., 2012).

They are widely recommended to treat milk allergies caused primarily by lactose content (Sanders, 2000; Kirjavainen et al., 2003).

They are frequently used to treat allergies such as atopic eczema in pregnant women and newborns (Kukkonen et al., 2007).

They synthesize vital nutrients such as folic acid, niacin, riboflavin, vitamins B6 and B12 (LeBlane et al., 2007).

Due to synthesis of β-galactosidase, they increase lactose tolerance and are therefore prescribed to treat milk allergies (Kirjavainen et al., 2003).

They also improve mineral absorption property of the gut (Famularo et al., 2005).

Probiotics may help to prevent liver damage caused by excessive alcohol intake (Kirpich et al., 2008).

They reduce risk of certain cancers (Kulkarni and Reddy, 1994).

They also detoxify carcinogens (Wollowski et al., 2001).

They suppress growth of certain tumors (Sekine et al., 1994).

Oral doses of probiotic also help in controlling Halitosis (bad breath) caused by Fusobacterium nucleatum due to production of hydrogen peroxide (Kang et al., 2006).

They significantly reduce serum cholesterol concentrations and normalize lipid profiles with more of the HDL component (Simons et al., 2006).
They prevent tooth decay as LAB establishes a cariostatic effect by adhering to
dental tissues where they fight against the cariogenic bacteria (Koll et al., 2008).
They tend to reduce blood pressure in hypertensives (Sanders, 2000).

Thus, probiotic microorganisms in the gut compete with pathogenic
microorganisms, thereby preventing pathogenic colonization and invasion. Although
most microorganisms are able to synthesize organic molecules required for their
survival and maintenance, some molecules e.g. amino acids, fatty acids, nucleotides,
enzyme cofactors etc. are used directly or metabolized from nutrients available in the
host gut. Abundance of such nutrients within distinct host microenvironments led to
loss of genes required for their biosynthesis in many microorganisms. This dependency
on essential host nutrients represents a major force for pathogen selection of distinct
host habitats. An instructive example for nutritive host-pathogen competition is
represented by the mutual requirement for iron. Iron is an essential micronutrient for
growth, basic metabolism and maintenance of most of the living organisms. Probiotic
bacteria stimulates the immune system of the host by improving phagocytic cell
functioning and inhibition of pathogenic bacterial growth as a result of TfR
downregulation (Kaur et al., 2010).

1.2  Bacteriocins as microbial warfare agents of probiotic LAB

Many probiotic strains exhibit their antimicrobial property by synthesizing
proteinaceous toxins that inhibit the growth of similar or closely related bacterial
strain(s). Gratia in 1925 first discovered a colicine ‘colicines’. Pediocins are produced
by several strains of Pediococci including P. acidilactici, P. pentosaceous, P. damnosus
(Kaur and Balgir, 2007). Carnocin is produced by a strain of Carnobacterium and nisin
by Lactococcus lactis (Hurst, 1981).
LABs commonly harbour plasmid-borne genetic determinants for bacteriocin production and for maintaining immunity of the producer cells to their bacteriocins (Klaenhammer, 1993). Yet, there have been reports suggesting that chromosomal determinants may be involved as well in the bacteriocin production (Barefoot and Klaenhammer, 1983; Jaerger and Klaenhammer, 1986; Kawai et al., 1998; 2000). Most commonly the structural and immunity genes are in a cluster with two other genes that produce dedicated proteins for export of bacteriocin from the cell. Lactococcins A, B and M are all encoded on one plasmid with separate secretion protein (Belkumm et al., 1991; 1992). *Carnobacterium piscicola* LV17 produces at least three class II bacteriocins: carnobacteriocins A and B2, which are encoded on separate plasmids with separate secretion proteins, and carnobacteriocin BM1, with its structural and immunity genes located on the chromosome (Quadri et al., 1997; Ahn et al., 2003).

Bacteriocin activity is usually lethal to the bacteria. Mode of action of bacteriocin was extensively reviewed by Jack et al. (1995) and Moll et al. (1999). Various mechanisms have been proposed to describe the bactericidal action of bacteriocins. Bactericidal activities include formation of selective or nonselective anion carrier pores, inhibition of outgrowth of spores and modulation of enzyme activity (Edward and Morwood, 1993). Pore formation is the best described mechanism. The relatively small action spectrum of some bacteriocins suggests the presence of molecular receptors in the membrane of the target cell, although this has not been demonstrated (Van Belkum and Stiles, 2000). Class II peptides have a helical amphiphilic structure that allows them to be inserted into the target cell membrane, leading to depolarization and death (Cotter et al., 2005; Drider et al., 2006). The initial interaction with the heads of anionic membrane phospholipids takes place at the
hydrophilic N-terminus of peptides. The C-terminus of peptide is more hydrophobic than the N-terminal and is thought to be involved in hydrophobic interactions with the membrane.

1.3 Peptic ulcer disease

‘Peptic ulcer’, refers to an ulcer of lower oesophagus, stomach or duodenum. It is defined as mucosal erosions equal to or greater than 0.5 cm and penetrates the muscularis mucosae layer. An ulcer forms when the lining of the digestive system is corroded by acidic digestive juices (Malfertheiner et al., 2009). According to Medilexicon's medical dictionary, a peptic ulcer is "an ulcer of the alimentary mucosa, usually in the stomach or duodenum, exposed to acid gastric secretion". The English word ‘peptic’ comes from the Latin word ‘pepticus’ which comes from the Greek word ‘peptikus’ which comes from the Latin word ‘peptein’, meaning ‘to digest’. The English word ‘ulcer’ comes from the Latin word ‘ulcus’ (genitive: ulceris), meaning ‘a sore, a wound, an ulcer’ (McColl, 2010). Peptic ulcers are produced by an imbalance between the gastro-duodenal mucosal defense mechanisms and damaging forces of gastric acid and pepsin combined with superimposed injury from environmental or immunologic agent or due to bacterial infection. Peptic ulcers can be acute or chronic. Chronic ulcers are associated with widespread necrosis with fibrosis in the ulcer base, whereas acute ulcer is inflammation of the mucosa with neutrophilic infiltration. It is estimated that globally 5% and 10% of the adults are affected by peptic ulcers at least once in their lifetimes (Chan and Lau, 2010).

When a peptic ulcer affects the stomach mucosa, it is called a ‘gastric ulcer’, one in the duodenal epithelial lining is called a ‘duodenal ulcer’, while an ‘oesophageal ulcer’ is an ulcer in the lower one-third of oesophagus (Aldoori et al., 1997). When the
lining of these organs is corroded by acidic digestive juices secreted by stomach cells, peptic ulcers form. Most gastric ulcers are usually single, mostly associated with hyperacidic environment, with most common complication being hemorrhage. In contrast, most duodenal ulcers are associated with bacterial infection i.e. *Helicobacter pylori* infection, with most common complication being rupture and peritonitis. Peptic ulcer disease affects millions of Americans each year at an annual cost for the country estimated to run in the billions of dollars (Stack *et al.*, 2002).

1.4 Causes of peptic ulcer disease

1.4.1 Bacterial infection by *Helicobacter pylori*

A major causative factor (60% of gastric and up to 90% of duodenal ulcers) is chronic inflammation due to *H. pylori* that colonizes the antral mucosa. Dr. Barry Marshall and Dr. J. Robin Warren were awarded the 2005 Nobel Prize in Medicine for this discovery. *H. pylori* can produce urease which neutralizes the gastric acidity and hence escapes destruction by gastric acid and can colonize gastric mucosa. *H. pylori* can cause chronic active gastritis (type B gastritis), resulting in a defect in the regulation of gastrin production by the infected part of the stomach and gastrin secretion can either be increased, or as in most cases, decreased, resulting in hypo- or achlorhydria (Wu *et al.*, 2009). Gastrin stimulates the production of gastric acid by parietal cell that contributes to the erosion of the mucosa and therefore ulcer formation. *H. pylori* infection with an imbalanced acid production in gastric mucosa and exposure to environmental and dietary factors eventually leads to ulcer formation (Figure 1.1). Studies on the varying occurrence of ulcers in third world countries suggest that dietary factors play a major role in the pathogenesis of the disease (Kurata and Nogawa, 1997; Stack *et al.*, 2002; Salih *et al.*, 2007; McColl, 2010).
1.4.2 Chronic use of NSAIDs

Another major cause is the long term use of NSAIDs (Non-Steroidal Anti-Inflammatory Drugs), commonly referred to as ‘pain-killers’ for headaches, period pains and other minor pains. Examples include aspirin and ibuprofen. Many NSAIDs are OTC (Over the Counter) medications i.e. can be bought from any medical store without a valid prescription, while others, such as diclofenac, naproxen and meloxicam can only be acquired with a doctor's prescription (Stack *et al.*, 2002). The gastric mucosa protects itself from gastric acid and drugs like aspirin, diclofenac disrupt this protective mucosal layer, which leads to formation of peptic ulcer (*Figure 1.1*). The secretion of mucosal layer is stimulated by certain prostaglandins (Kurata and Nogawa, 1997). NSAIDs block the function of enzyme cyclooxygenase 1 (COX-1), which is essential for the production of these prostaglandins. COX-2 selective anti-inflammatory drugs (such as celecoxib or a recently withdrawn drug rofecoxib) preferentially inhibit COX-2, which is less essential in the synthesis of the protective mucosal layer over the gastric epithelium and roughly has lower risk of NSAID-related gastric ulceration. Non-steroidal anti-inflammatory drugs lower the stomach's ability to make a protective layer of mucus, making it more susceptible to damage by stomach acids. NSAIDs can also affect the blood flow to stomach, undermining the body's ability to repair cells (Salih *et al.*, 2007; Lanza *et al.*, 2009).

1.4.3 Repeated use of corticosteroids in high doses

While the evidence for corticosteroids causing peptic ulceration is relatively poor except for high doses taken for over a month, the majority of doctors as of 2010 still believes that over dose of corticosteroids is the cause of peptic ulcer and would consider protective prophylaxis measures (Salih *et al.*, 2007). The mechanisms
responsible for peptic ulcer formation induced by corticosteroids include enhanced gastrin and parietal cell hyperplasia with increased acid secretion, diminished gastric mucus synthesis and suppressed arachidonic acid metabolism and prostaglandin (PG) synthesis (Bandyopadhyay et al., 1999; Luo et al., 2002; Hsiang et al., 2010).

1.4.4 Cigarette smoking

People who regularly smoke tobacco are more likely to develop peptic ulcers compared to non-smokers. Smoking has an inconsistent effect on gastric acid secretion, but it does have other effects on upper gastrointestinal function that could contribute to the pathogenesis of peptic ulcer disease. These include (a) interference with the action of histamine-2 antagonists, (b) acceleration of gastric emptying of liquids, (c) promotion of duodeno gastric reflux, (d) inhibition of pancreatic bicarbonate secretion, (e) reduction in mucosal blood flow, and (f) inhibition of mucosal prostaglandin production (Eastwood, 1988). Smoking slows down healing process and favors recurrence of the disease. Scientists are still not certain how this works, but it may be that cigarettes increase the amount of acid in the stomach (Aldoori et al., 1997; Kurata and Nogawa, 1997).

1.4.5 Alcoholic cirrhosis

Regular heavy drinkers of alcohol have a higher risk of developing peptic ulcers. Alcohol can irritate the lining of the stomach if people drink excessively. This leads to inflammation and increases the risk of an ulcer development. Drinking alcohol can also interfere with the healing process of ulcers that are already present, thus prolonging and exacerbating the symptoms. Moderate and occasional drinking does not lead to any problems associated with peptic ulcers (Aldoori et al., 1997; Brenner et al., 1997).
1.4.6 Excessive stress

Excessive stress has not been directly related to ulcers but it can exacerbate the symptoms and may increase the intensity of pain that people experience during the disease (Chey and Wong, 2007). Stress seems to have variable effects on gastric motility: delayed gastric emptying could increase the risk of gastric ulcer, while accelerated emptying could increase the net acid load delivered to the duodenum at any given level of gastric secretion, enhancing the risk of duodenal ulcer (Dubois and Castell, 1981). Psychological stress may also promote the growth of *H. pylori* in the duodenum as a consequence of increased duodenal acid load, since the *H. pylori*-inhibitory effects of bile seem to be reversed by acid (Graham, 1997; Levenstein et al., 1999).

1.4.7 Genetics

A significant number of individuals with peptic ulcers have close relatives with the same problem, suggesting that genetic factors may also be involved in development of infection (McColl, 2010). Genetic factors including blood group O and A; hyperpepsinogenemia (increase in pepsin and HCl secretion); hyperchlorhydria (increase in parietal cell number); Zollinger-ellison syndrome (increase in gastrin and HCl secretion) and Pernicious anemia (autoimmune distraction of parietal cells) were reported to be associated with peptic ulcer disease (Ohmann *et al.*, 2005).

1.4.8 Mucosal ischemia

During ischemia, the gastric mucosa may be injured as a result of several mechanisms acting alone or in concert. Ischemia may cause injury through reduced blood flow or oxygen supply to the affected part of stomach or duodenum. Additionally,
ischemia may cause reperfusion injury, which damages the mucosa and leads to problems related with ulcer formation (Haglund, 1994).

1.4.9 Excessive use of caffeinated food products

Excessive intake of caffeinated food products such as caffeine, coffee, cola drinks, carbonated beverages and tomato-based products are commonly thought to cause or exacerbate ulcers. Coffee should be avoided by the patients with peptic ulcer disease on the basis of its strong acid secretagogue property (Marotta and Floch, 1991). Coffee is linked to increased ulcer susceptibility. Both caffeinated and decaffeinated coffees have an acid-stimulating effect and therefore it is recommended that people with ulcers restrict not only caffeinated but also decaffeinated coffee intake (Marotta and Floch, 1991). Increased levels of cortisol and other stress hormones stimulated by caffeine consumption and coffee drinking suppress the activity of the immune system and raise stress levels which are associated with ulcer formation (Abu Farsakh, 2002).

1.4.10 Other factors

Some suggested risk factors such as diet and spice consumption (in particular black pepper, red pepper, chili powder, onions and garlic), were hypothesized as ulcerogens (helping cause ulcers) until late in the 20th century, but have been shown to be of relatively minor importance in the development of peptic ulcers (Chey and Wong, 2007).
1.5 Symptoms of peptic ulcer disease

Peptic ulcer disease is a chronic condition with a natural history of spontaneous relapses and remissions lasting for decades, if not for life. Although they are different diseases, duodenal and gastric ulcers share common symptoms. The most common
presentation is that of recurrent abdominal pain which has three notable characteristics: localization to the epigastrium, relationship to the food and episodic occurrence along with occasional vomiting in some patients. NSAIDs induced ulcers are more common in the elderly population who are on long term pain killers due to their joint pains (Silverstein et al., 1995).

Peptic ulcer disease is characterized by frequent occurrence of one or more symptoms (Chan and Lau, 2010; Kaur et al., 2010; McColl, 2010). Abdominal discomfort which is a dull, gnawing ache, comes and goes for several days or weeks, occurs 2 to 3 h after a meal, occurs in the middle of the night-when the stomach is empty, is relieved by antacid medications. This pain can appear anywhere from the navel up to the breastbone. Epigastric pain made worse (gastric ulcer) or relieved by eating (duodenal ulcer). Regurgitation of acidic contents of stomach in lower part of esophagus causes sensation of heartburn. Loss of weight with no obvious explanation (Kaur et al., 2010; McColl, 2010).

Complications of peptic ulcers include perforation through the ulcer, bleeding into the stomach or obstruction in the stomach. Peptic ulcers are more prone for bleeding into the stomach. When the ulcer continues to erode mucosal and submucosal lining, it eventually erodes into the blood vessel causing acute bleeding called as hematemesis. Chronic bleeding due to erosion of small capillaries, causes iron deficiency anemia, especially in elderly patients (McColl, 2010). Perforation of stomach wall, another life-threatening complication can lead to peritonitis and death within few hours (Ohmann et al., 2005). Long standing ulcers can lead to fibrosis in the stomach lining and shrinks the lumen of the stomach causing obstruction and vomiting (el-Omar
et al., 1995). Eventually, due to chronic irritation, mucosal epithelium can undergo dysplasia leading to development of gastric cancer (Silverstein et al., 1995).

1.6 **Helicobacter pylori: Etiologic agent of peptic ulcer disease**

*H. pylori* was first isolated by Marshall in 1984. Originally called *Campylobacter pyloridis*, the name was changed to *Campylobacter pylori* and then later to *Helicobacter pylori* as specific morphologic, structural and genetic features indicated that it should be placed in a new genus (Goodwin et al., 1989). The association of presence of this organism with histologic gastritis was established (Lee et al., 1997). Infection with *H. pylori* is now estimated to be 40-70% worldwide (Benson et al., 2004). *Helicobacter pylori* infection is common in the Indian subcontinent (Gill et al., 1994). Exposure occurs in childhood and approximately 80% of adults have been infected at some time (Graham et al., 1991). Sero-surveys indicate a seroprevalence of 22-57% in children under the age of five, increasing to 80-90% by the age of 20 and remaining constant thereafter (Kang et al., 1999; Jais and Baruna, 2004).

Taxonomic status of *H. pylori* is given in **table 1.1**. It belongs to domain “Eubacteria” because it has no nuclear membrane, no organelles (except for ribosomes) and its genetic material is found within a single strand of circular chromosome. *H. pylori* is considered under kingdom “Bacterium” because it is a unicellular microorganism that lacks a nucleus and membrane bound organelles. *H. pylori* falls under phylum “Proteobacteria” as it stains pink with gram stain indicating Gram-negative characteristic. Its outer membrane consists of lipopolysaccharides, rather than peptidoglycan as found in Gram-positive bacteria. As seen in *H. pylori*, many of these bacteria use flagella for movement. Most of the bacteria within the class “Epilonproteobacteria” inhabit intestinal tract of mammals. They can either be
symbionts (either benefiting or not affecting the host) or parasites such as *H. pylori*. Order “Campylobacterales” is composed of mesophiles, meaning they live in moderate temperatures (10-50 °C). The human stomach, which is the habitat for *H. pylori*, falls within this range. Family “Helicobacteraceae” is characterized by the helical shape of its members. They live in the acidic mammalian stomach by producing urease. They are all flagellated and can move quite fast. “*Helicobacter*” genus was once part of the *Campylobacter* genus but was later made into its own group. *H. pylori* is the most widely known species of the *Helicobacter* genus (Holt et al., 1994).

**Table 1.1: Taxonomic status of *H. pylori***

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<tr>
<th>Taxonomic hierarchy</th>
<th>Characteristic</th>
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<tr>
<td>Domain</td>
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<td>Bacteria</td>
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<td>Phylum</td>
<td>Proteobacteria</td>
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<td>Campylobacterales</td>
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<td><em>Helicobacter</em></td>
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<tr>
<td>Species</td>
<td><em>Helicobacter pylori</em></td>
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*H. pylori* is a Gram-negative, spiral-shaped, microaerophilic organism, that colonizes the mucosal layer of the gastric epithelium. In 1994, the International Agency for Cancer Research, an arm of the World Health Organization classified *H. pylori* as a potential human carcinogen (Dunn et al., 1994). *H. pylori* weakens the protective mucous coating of the stomach and duodenum, which allows acid to get through to the sensitive lining beneath. Both, the acid and the bacteria irritate the lining and cause peptic ulcer (*Figure 1.2*). *H. pylori* is able to survive in stomach acid because it secretes
enzymes (urease, protease and phospholipases) that neutralize the acid (Smoot, 1997) (Figure 1.3). This mechanism allows *H. pylori* to make its way to the "safe" area—the protective mucous lining. Once there, the bacterium's spiral shape helps it burrow through the lining.

Figure 1.2: Biopsy sample of colon ulcer showing occurrence of *H. pylori* (Hildreth *et al.*, 2008)
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1.7 Origin of problem

Prevalence of *H. pylori* related gastritis is quite high in India. Situation is aggravated due to emergence of drug resistance in *H. pylori* (Megraud, 1997; Mukhopadhyay *et al.*, 2000; Saha, 2004; Devi *et al.*, 2007; Ahmed, 2008). This gastric pathogen has induced resistance against many antibiotics might be due to emergence of point mutations in *H. pylori* genome and/or decreased binding of the antibiotics to the ribosomes. Very few studies have actually focused on the role of probiotic lactic acid bacteria and bacteriocins produced by them in treating *H. pylori* infection. Therefore, the present study was aimed to isolate and characterize anti-*H. pylori* bacteriocin producing lactic acid bacterial isolate.

**Figure 1.3: Virulence factors of *H. pylori* that aid in stomach infection**
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Objectives of the Study

1. Screening, isolation and identification of Anti-*Helicobacter pylori* bacteriocin producing lactic acid bacterial strain from faecal samples of healthy individuals.

2. Production and purification of the bacteriocin at flask level.

3. *In vitro* characterization of bacteriocin produced
   (a) Biochemical characterization
   (b) Antimicrobial spectrum
   (c) Gene localization

4. Mechanism of bacteriocin action on *Helicobacter pylori*.

5. Study of *in vivo* therapeutic potential of Anti-*Helicobacter pylori* bacteriocin producing lactic acid bacterial isolate in mice model.