Chapter 1

Introduction

Aim & Objectives
1.1 *Helicobacter pylori* (*H. pylori*)

*Helicobacter pylori* (*H. pylori*) are a Gram-negative microaerophilic, spiral shape ε-proteobacteria functioning as pathogen responsible for Gastro Intestinal (GI) tract diseases in human host. *H. pylori* is the most common bacterial infection worldwide, it is typically acquired during childhood and the infection can persist in the gastric ecosystem throughout the life span of the host, if untreated. *H. pylori* specifically colonize on the gastric mucosa as they require basic pH for survival (Everhart 2000). Due to presence of its pathogenic island, it results in to chronic persistent. Studies have indicated that infected individuals have 2 - 6 fold increased risk of developing gastric cancer and mucosal associated lymphoid tissue lymphoma compared to their uninfected counterparts (Dutta et al. 2006; Cendron and Zanotti 2011).

1.2 The major virulence factors of *H. pylori*

I. The Vacuolating toxin (VacA)

VacA is initially synthesized in the bacteria as a single polypeptide with molecular weight of about 140 kDa (Cover et al. 1994; Telford et al. 1994; Schmitt and Haas 1994; Phadnis et al. 1994). The protein VacA is subsequently released by type V-secretion system. In this context, the N-terminal leader sequence of 33 amino acids and the C-terminal auto transporter domain with molecular weight of about 33 kDa are cleaved, yielding a mature toxic protein with molecular weight of 88.2 kDa (Nguyen et al. 2001). The polypeptide can be further cleaved into an N-terminal fragment of 33.4 kDa molecular weight (named p34 or p37, residues 1-311) and a C-terminal fragment of 54.8 kDa molecular weight (named p55 or p58, comprising residues 320-821). Many data have shown that the N-terminal domain is essential in the toxic activity of VacA while the C-terminal domain is essential in binding to target membranes (Montecucco and Rappuoli 2001;
Cover and Blanke 2005). But certain literature reports that the cleavage is not required for VacA activity.

II. The Cytotoxin-associated gene product (CagA)
The Cytotoxin-associated gene A product CagA is, of approximately 125–140 kDa of molecular mass (Covacci et al. 1993; Tummuru et al. 1993) and has been designated as a bacterial onco protein (Ohnishi et al. 2008) profoundly altering host cellular functions (Hatakeyama 2009; Lu et al. 2008). *H. pylori* have a type IV secretion system (t4ss), formed by cag pathogenicity island (cagPAI) proteins. After *H. pylori* has adhered to a host cell, a t4ss translocates CagA into the host cell (Olbermann et al. 2010), All Type I strains of *H. pylori* (Enroth et al. 2000) that possess a functional cagPAI are particularly frequently associated with severe, gastric atrophy and cancer (Wiedemann et al. 2009; Figueiredo et al. 2002; Amieva and El Omar 2008; Hatakeyama 2009). The cagPAI is, 37 kb long genomic fragment containing 28 genes (Labigne and Reuse 1996), encode multiple structural components of a bacterial t4ss as well as the 128 kDa effector protein, CagA (Hatakeyama 2009). Which suggest that t4ss is a mainly important component indirectly responsible for pathogenic nature of *H. pylori*.

III. The Type IV secreting system
Type IV secreting system is found in many strains of *H. pylori* and according to an important finding the cag-t4ss apparently does not inject its effector protein CagA randomly into target cells, but uses the α5β1 integrin as a cellular receptor for the pilus-associated adhesin CagL (Kwok et al. 2007). The type IV secreting system injects protein CagA into the stomach’s epithelial cells of host, where it disrupts the host cell cytoskeleton, its adherence of adjacent cells, intracellular signaling, and other cellular activities. However, the mechanism of translocation and the requirements on the host cell for targeting are not well understood. The t4ss consists of inner and outer membrane-spanning Cag
protein complexes and a surface-located pilus (Jimenez-Soto et al. 2009). Till date many molecular studies are carried out to identify the internal arrangements of t4ss and its interaction with host receptors (Zhong et al. 2007; Kutter et al. 2008). The hypothetical structure of t4ss is shown in Figure 1.2.1. However the mechanism of interacting cag proteins to build outer membrane structure of t4ss is not known. There are many Cag proteins which are still unknown.

Figure 1.2.1 Hypothetical structure of t4ss
1.3 Gastro Intestinal (GI) track diseases, Gastric cancer & its prevalence

Ideally referring Gastro Intestinal (GI) track diseases due to *H. pylori* is related to inflammation, irritations ulcers and cancer cause in any section of GI track, which includes entire digestive track from mouth to anus. Technically most prominent GI track diseases caused by *H. pylori* infection is gastritis as initial infection and it can evolve into more severe diseases, such as atrophic gastritis, peptic ulcer, lymphoma of the mucosa-associated lymphoid tissue or gastric adenocarcinoma (Zarrilli *et al.* 1999). **Gastric cancer is 14th most common cause of death overall worldwide**, which kills > 700 000 people every year (Herszenyi and Tulassay 2010; Herrera and Parsonnet 2009). Epidemiological studies have determined that the attributed risk for gastric cancer conferred by *H. pylori* is approximately 75% (Peek and Blaser 2002). **Gastric cancer**, despite its declining incidence rate, **remains the fourth most common cancer**, the **second leading cause of cancer-related death**. Early stages of the disease are often clinically silent, with patients having advanced stage disease at the time of diagnosis, and reported 5-year survival rates are approximately only 20% (Correa *et al.* 2004). *H. pylori* infection is the strongest known risk factor for gastrointestinal malignancies that arise within the stomach. There are two types of gastric cancer diffuse and intestinal cancer. *H. pylori* infection increases the risk of developing both types of gastric cancer (i.e. diffuse and intestinal), chronic inflammation is not a prerequisite for development of diffuse-type cancer, thus suggesting that different mechanisms underlie the ability of *H. pylori* to induce gastric malignancies. Also, it is likely that *H. pylori* influences early stages in gastric carcinogenesis, as suggested by Wong *et al.* in 2004 **eradication of the infection significantly decreases the incidence of gastric cancer** only in patients without premalignant lesions at the time of diagnosis.
Almost 50% of the world’s population is *H. pylori* positive and in some countries 100% population is found to be *H. pylori* positive (Matysiak-Budnik and Megraud 1997). *Helicobacter pylori* is a highly recombining pathogen (Ahmed et al. 2009) and there are therapies present to control the infection. One of such therapy is treatment based on Triple therapy, including two antibiotics, amoxicillin and clarithromycin, and a proton pump inhibitor taken simultaneously for 7-10 days. However, this treatment may fail for several reasons and the main reasons for failure are compliance, side effects and *H. pylori* resistance to the antibiotics like clarithromycin, metronidazole, tetracycline, fluoroquinolones, and rifamycins (Megraud 2004). Considering the Probiotic treatment of *H. pylori* infection, a medicine named A. pylori is available to control the infection of *H. pylori*. But according to the local survey analysis medicine A. pylori is not efficient enough to be considered as first line of treatment and thus, it is given with other antibiotics as second line of treatment. Thus, it can be considered that *H. pylori* resistance to the antibiotics has become an emerging issue and have arise a need for better medication options.

Therefore the present study focuses on studying the cagPAI protein members using available protein analysis software. The structure of typeIV secreting system can give insight about its function of CagA translocation mechanism. An attempt for predicting the outer membrane structure of typeIV secreting system is done, as proteins of outer membrane are comparatively easy to target. The Protein interaction study between various cagPAI protein members, elucidate the outer membrane structure and order of assembly of cagPAI protein members to form typeIV secreting system. Further in silico attempts were made to find Potential therapeutic target proteins from *Helicobacter pylori*, whose activity could be inhibited or elevated to control proliferation of *H. pylori* in host which in-turn decelerate the rate of disease expansion and can reduce risk of cancer or ulcer.
1.4 Aim and Objectives

The most widely used drug for treatment of Gastric Cancer/Ulcer has evident many side effects. However, due to severe allergic reactions an anti-acidic agent, Bismuth (E.g. Bismuth-subsalicylate, Ranitidine Bismuth Citrate) from the current line of treatment was soon withdrawn from the markets. Moreover, Helicobacter pylori have tendency to generate antibiotic resistance. Therefore, there arises the need of understanding the pathogenic mechanism of bacterium and to find way to control its proliferation or to eradicate it; and we have focused the same in our study.

To fulfill this Aim, following are the objectives:

- Literature study and primary analysis of all known and unkown Cag Pathogenic Island Proteins (cagPAI), of Helicobacter pylori (H. pylori).
- To determine the Outer membrane structure of typeIV secreting system (t4ss) formed by assembly of protein members of cagPAI; by means of software used in structural bioinformatics.
- In silico identification of Potential therapeutic target proteins from Helicobacter pylori, whose activity could be inhibited or elevated to control proliferation of H. pylori in host.
- In silico proteomic analysis and protein-protein or protein-ligand interaction studies for Potential therapeutic target proteins.
- Confirmations of the results obtain by in silico studies using Wet-lab techniques.