Synopsis

*Helicobacter pylori* is one of the most genetically diverse bacterial species that successfully colonizes at least 50% of the world population. It has been associated with humans for thousands of years and most probably evolved from ancestral gastric *Helicobacter* species in early mammals. One of the important characteristics of this pathogen is the degree of allelic diversity and genetic variability which helps it to adapt and colonize. Phase variation is one of the mechanisms used by *H. pylori* to generate variation. The presence of homopolymeric nucleotide or dinucleotide repeats in an ORF make it prone to frequent length changes as a consequence of slipped strand mispairing mediated mutagenesis.

Interestingly, R-M genes comprise a significant percentage of *H. pylori* strain-specific genes and are more prevalent in *H. pylori* than in other bacterial species whose genomes have been fully sequenced. R-M systems in *H. pylori* have been identified on the basis of sequence similarity to known restriction endonucleases and methyltransferases, genetic organization, and specific enzyme isolation and characterization. Analysis of genome sequences of *H. pylori* strains 26695, J99, HPAGI and 26 others has revealed the presence of more than 20 R-M systems in each strain, which are far more than detected in any other bacterial genome sequence till date.

*hp1369* and *hp1370* are two ORFs in stain 26695 coding for hypothetical proteins. *hp1369* has a stretch of poly-G repeats, thus making *hp1369-hp1370*, a candidate of phase variation. *hpag1_1313* is homolog of *hp1369-hp1370* which got up-regulated, in a person suffering from acute gastritis, thus making these genes an interesting subject of investigation.

This study was therefore initiated with the following objectives:

1. Cloning, over-expression and purification of Type III MTase (ORF- *hp1369-hp1370*) and its cognate restriction enzyme (*hp1371*).

2. Biochemical characterization of MTase (HP1369-HP1370): Determination of oligomeric status, kinetic properties, binding affinities for AdoMet and DNA.

3. Study the role of polynucleotide repeat sequence in HP1369-HP1370 MTase of *H. pylori* strain 26695, and investigate the phenomenon of “phase-variation”.

Sequence analysis shows the presence of a poly-G track (10 Gs) at 3’-end of *hp1369* which is a signature sequence for phase variation. Addition of a single nucleotide can place both
hp1369 and hp1370 in-frame, which could code for a single polypeptide. hp1369 and hp1370 in H. pylori strain 26695 alone do not code for any functional protein but with the fusion of hp1369 and hp1370 can code for a protein with all the nine motifs of a DNA MTase. Interestingly, on the basis of arrangement of Motifs, it is probably the first example of ε type of methyltransferase. By site-directed mutagenesis a single G nucleotide was inserted in the poly-G track and both the ORFs (hp1369 and hp1370) became in-frame, coding for fully functional HP1369-HP1370 MTase. Kinetic parameters for functional HP1369-HP1370 MTase were determined, and has shown that there was substrate inhibition in methylation reaction at higher concentrations of AdoMets. When preincubation studies were done, enzyme-DNA complex was found to be more competent than enzyme-AdoMet complex. HP1369-HP1370 MTase exists as dimer in solution, having affinity for duplex DNA and does not bind to single-stranded DNA. Binding affinity for ligand (AdoMet) was determined by Isothermal Titration Calorimetry method.

H. pylori has evolving restriction-modification systems. It is capable of taking new R-M systems from the environment in the form of DNA released from other bacteria or other Helicobacter strains. H. pylori genome is dynamic with high mutation rates. Random mutations in R-M genes can result in a non-functional R-M systems or R-M systems with new properties. The dynamics of R-M system plays a vital role in shaping up the genome.