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Helicobacter pylori infection: efficacy of probiotics and role of genome wide association studies

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Abstract

Helicobacter pylori is a Gram-negative human gastric pathogen which has a possible etiologic role in peptic and gastric ulcers, gastric adenocarcinoma and more rarely, lymphoma of the mucosa-associated lymphoid tissue. The pathogenicity of *H. pylori* is intimately associated with the expression of virulence factors such as urease, *cagA, iceA*, *vacA* and bacterial adhesion and maintenance factors. The *cagA, iceA* and *vacA* virulence factors of *H. pylori* show great deal of genetic diversity with respect to their ancestor strain, host and environment factors that collectively influence the pattern and severity of the disease. Antimicrobials (triple-therapy regimen) noted to be effective in empirical management of pathogen while emergence of drug resistance remains a major obstacle in eradication of this gastro-duodenal pathogen. In this regard probiotics have great potential of treating *H. pylori* infection because they exhibit antagonistic activities against many pathogens including *H. pylori*. Focus of this review is to highlight importance of probiotic micro-organism in improving host defense mechanism. Review also highlights the need of further investigation involving genome wide association studies to unravel the interactions between host, probiotics and bacterial pathogen that would determine fate of pathogen in a complex environment of gastro-duodenal region.

Key words: *Helicobacter pylori*, Peptic ulcers, Probiotics, Bacteriocins, GWAS

Introduction to gastric pathogen *Helicobacter pylori*

Helicobacter pylori is of major concern today because of its causal relationship with gastroduodenal diseases. In 1984, this Gram-negative, spiral-shaped, microaerophilic bacterium that colonizes the mucosal layer of the gastric epithelium was isolated [1]. *H. pylori* bacteria was originally called *Campylobacter pyloridis*, renamed as *Campylobacter pylori* and then later reclassified to *Helicobacter pylori* because its morphological, structural and genetic characteristics has indicated that it should be placed in a new genus [2]. The bacteria are prevalent worldwide and more than half of the world population is infected with *H. pylori* [3-4]. *H. pylori* infection is common in the Indian subcontinent. Chances of exposure are widespread and infection occurs early in life under the age of five. About 79-83% of the population is exposed to *H. pylori* during the first two decades of life [5-6]. Serological surveys indicate a sero-prevalence of 22-57% in children under the age of five, increasing to 80-90% by the age of 20 and remaining constant thereafter [7-8]. Gender preference was not seen in case of *H. pylori* infection. Data of both developing and developed countries depicts direct relation of disease prevalence with age. High age-specific prevalence of *H. pylori* infection in developing countries reflects the lower socioeconomic level of those areas. Infection with *H. pylori* is highly associated with chronic active gastritis, peptic ulcers, gastric adeno-carcinoma and more rarely, lymphoma of the mucosa-associated lymphoid tissue (MALT) [9-10]. In 1994, the International Agency for Cancer Research, an arm of the World Health Organization, classified *H. pylori* as a potential human carcinogen [11].

Pathogenicity of *H. pylori*

H. pylori has a very strong affinity for epithelial lining of stomach and duodenum, where it attaches and subsequently disrupts microvilli and tight junctions between adjacent cells. Eroded epithelial lining allows acid and bacteria to get through it and establishes pathogen in the mucous layer. Almost all *H. pylori* strains produce urease that decomposes urea and forms ammonia, which is harmful to gastric mucosa [12]. Moreover, low acidity is beneficial for organisms to colonize the stomach. *H. pylori* urease is an important virulence factor that consists of four subunits, A, B, C and D. Subunit B, a peptide with 569 amino acids and encoded by the *ureB* gene, shows the strongest antigenicity and protection among all *H. pylori* proteins [13]. Interestingly, the fragment E of *ureB* (*ureBE*) expressed in *E. coli* is also able to elicit effective immune response in mice [14]. *H. pylori* also secretes proteases and phospholipases that damages mucosal lining. Lipopolysaccharide of the pathogen origin attracts inflammmatory cells to the mucosa. Neutrophils of the host release myeloperoxide in response to pathogen attack. A bacterial platelet-activating factor promotes thrombotic occlusion of surface capillaries. Taken together, these changes damage the protective mucosal layer in gastro-duodenal region. Exposed epithelial cells are highly prone to damaging effect of acid-peptic digestion and ultimately, inflammation of the gastric mucosa occurs which may lead to peptic ulceration [12].

A number of studies conducted in different populations of the World indicated the involvement of *cagA*, *iceA, vacA, babA*-blood group antigen binding adhesion factor *[15-21], hp0169 and com*B4 [22] virulence

factors in establishing gastroduodenal *H. pylori* infection (Table 1). The pathogenicity of the organisms is also attributed to maintenance factors such as motility, and adaptive enzymes [23]. *H. pylori* attaches to inner surface of the gastric epithelium and occasionally inside epithelial cells [24]. It produces adhesins which bind to membrane-associated lipids and carbohydrates that help in adherence to epithelial cells (Figure 1 and 2). For example, the adhesin *babA* binds to the Lewis b antigen displayed on the surface of stomach epithelial cells [25].

H. pylori strains possess a gene called *cagA*, which is a marker for the *cag* region, a pathogenicity island of about 35 kb. The *cag* pathogenicity island (PAI) has about 30 genes, part of which code for a complex type IV secretion system (Figure 3) that facilitate translocation of bacterial factors such as urease, lipopolysaccharides (LPS), broken fragments of peptidoglycan and porins through epithelial layer and subsequent activation of macrophages of gastric mucosa. Stimulated macrophages secrete IL-1β, IL-8, IL-12, TNF-α and induce expression of INF-γ by TH_1 cells. IL-8 and INF-γ disrupt epithelial barrier function. Antigen presenting cells (macrophages) with exposed bacterial antigens attached to class II MHC's induce clonal expansion in B-cells for production of anti *H. pylori* antibodies and activate Tc cells through TH_2 derived IL-2 that ultimately lead to destruction of infected cells. Apoptotic pathway was activated when macrophage derived immune effector molecules viz. TNF-α and IL-1β stimulated *fas* expression in sensitized cells. The

TABLE 1. Virulence and maintenance factors of *Helicobacter pylori*.

Factors	Function	References
	Virulence Factors	
vacA	Encodes a protein cytotoxin that induces vacuolation in eukaryotic cells	11, 15-21
cagA	Stimulates the production of interleukin-8; a part of it also code for type IV secretion system.	15-21, 26-29, 34
babA	Binds to Lewis b antigen displayed on the surface of stomach epithelial cell	15-21, 25
iceA	Up-regulated upon contact of H. pylori with the gastric epithelium	15-21
oipA (hp0638)	Induces IL-8 secretion by epithelial cell	200
picB	induces IL-8 expression in gastric epithelial cells	36
Urease	Neutralizes acid	12, 13
	Adhesion and Maintenance Factors	
hp0169	Essential for H. pylori stomach colonization as it encodes for a collagenase	22
comB4	Essential for colonization, as it encodes a putative ATPase which is a part of DNA transformation associated type-IV transport system	22
rocF	Encodes arginase that facilitates production of ammonia and favours NO production in stimulated macrophages	35
MUC5AC	Primary receptor for H. pylori in human stomach	151

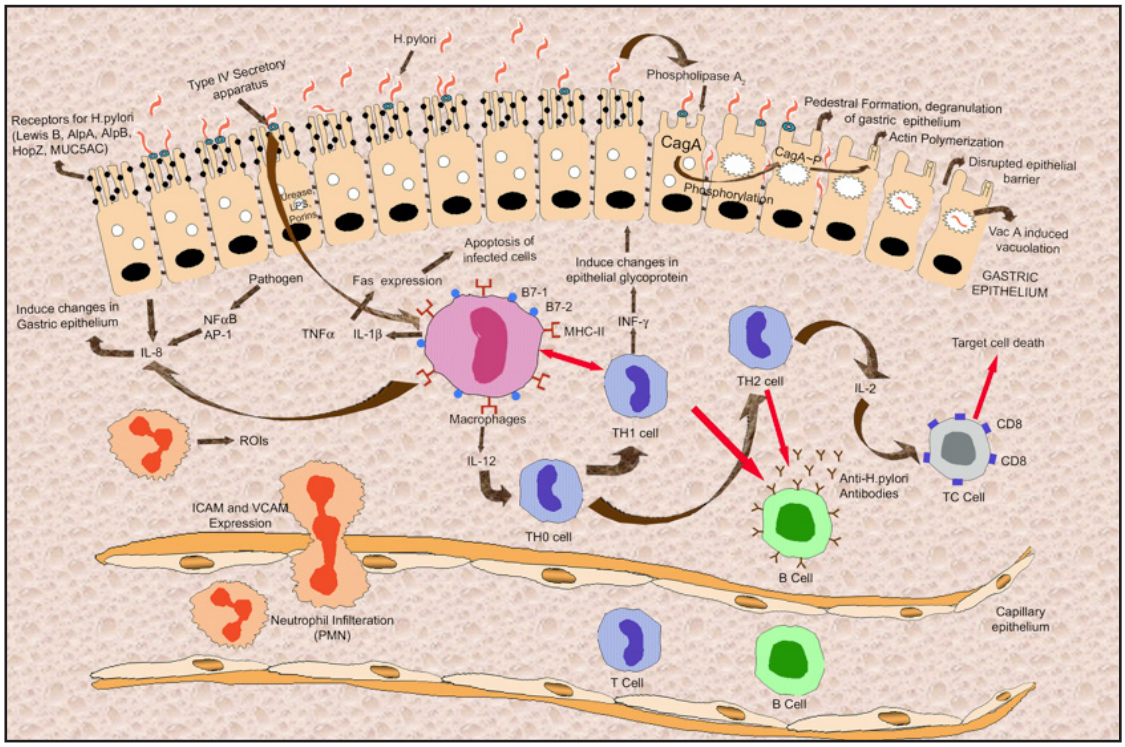

FIGURE 1. Pathological changes in gastric epithelium upon *H. pylori* infection.

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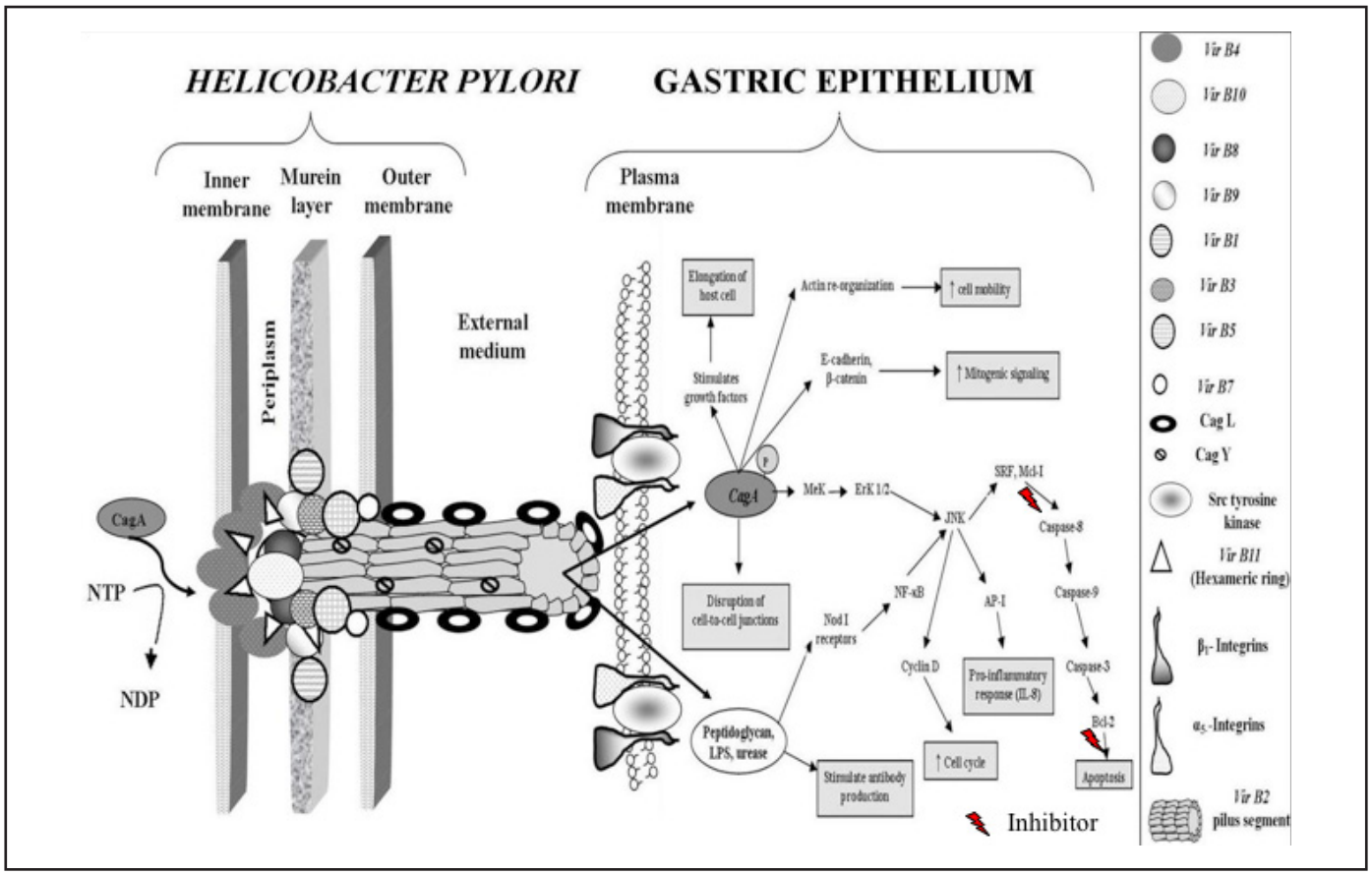

FIGURE 2. Constitution of type IV secretory apparatus and pathophysiology of *H. pylori* infection.

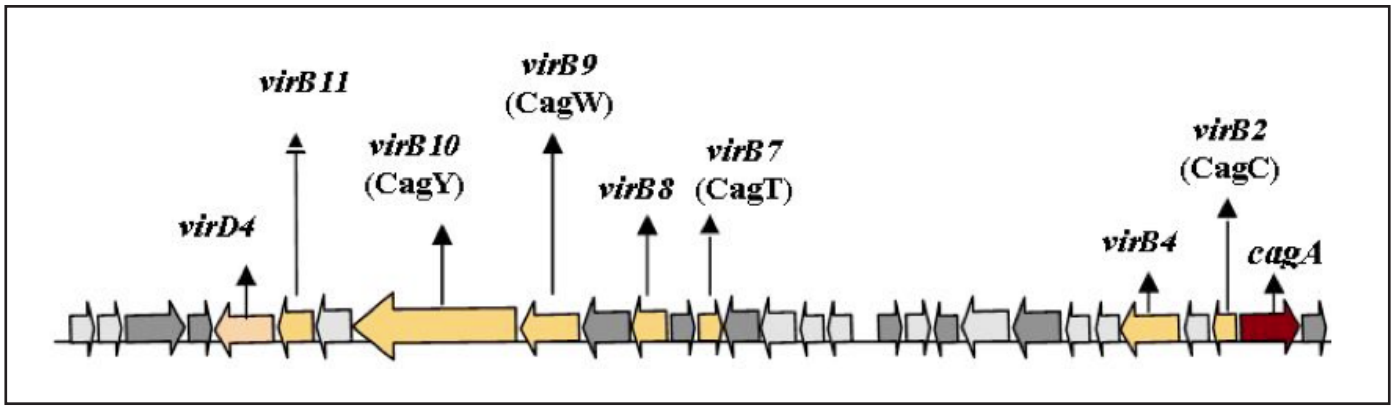

FIGURE 3. Genetic organization of cag pathogenicity island (PAI).

low GC content of the *cag* PAI relative to the rest of the *Helicobacter* genome suggests that the island was acquired from other bacterial species by horizontal gene transfer [26]. Infection with *cagA*$^+$ strains enhances the risk for development of duodenal ulcers and adeno-carcinoma of the distal stomach [27]. The *cag* PAI stimulates the production of interleukin 8 [28, 29] *via.* intracellular NOD1 receptor and the nuclear factor κB pathway. The elevated level of chemokines in host is responsible for an increased gastric inflammation which subsequently leads to disease development. Further, host chemokine response also enhances pathogen clearance by activation of apoptosis mechanism. Essentially, all *H. pylori* strains carry *vacA* gene that encode a protein cytotoxin that induces vacuolation in a wide variety of eukaryotic cells [11]. The gene encoding this cytotoxin is present in all strains but exhibits a mosaicism in the terminal(s) and median(m) regions. There are

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several alleles corresponding to varying amounts of toxin produced: *s1m1* corresponds to the highest production, followed by *s1m2*, while strains with the *s2m2* allele do not produce any toxin [30]. Two novel proteins; a secreted collagenase, encoded by *hp0169* and a putative ATPase encoded by *com*B4, being part of a DNA transformation-associated type IV transport system of *H. pylori* are found to be absolutely essential for colonization [22].

Patients infected with *cag*+ *H. pylori* strains have a stronger inflammatory response in the stomach and are at a greater risk of developing peptic ulcers or stomach cancer than those infected with strains lacking this island [31]. Pathological changes in gastric epithelium upon infestation with *H. pylori* are diagrammatically illustrated in Figure 1 and 2. After *H. pylori* attaches itself to gastric epithelium, the type IV secretion system expressed in part by the *cag* PAI injects its own peptidoglycan fragments as the inflammation inducing agent into the gastric epithelial cells. The injected peptidoglycan is recognized by Nod1 receptor, which then stimulates expression of cytokines that promote inflammation [32]. *H. pylori* also injects *cagA* protein into gastric epithelium, where it disrupts epithelial membrane barrier and other cellular activities [33]. Once inside the cell the *cagA* protein is phosphorylated on tyrosine residues by a host cell membrane-associated tyrosine kinase (src). Pathogen has been shown to activate the epidermal growth factor receptor (EGFR), a membrane protein with a tyrosine kinase domain. Activated EGFR alters signal transduction and gene expression in host epithelial cells that may contribute to pathogenesis. It has also been suggested that a C-terminal region of the *cagA* protein can regulate host cell gene transcription independent of protein tyrosine phosphorylation [27, 34].

iNOS expression and production of NO in macrophage are upregulated with *H. pylori* infection both under *invivo* and *invitro* conditions. Even major pathogenicity protein i.e. urease (*ureA*$^{+}$), activate iNOS and increase NO production. Urease is an essential survival factor for *H. pylori* during gastric colonization, is implicated on NO dependent damage and carcinogenesis [35]. Other virulence proteins including *vacA, cagPA*-1 and *picB* show a selective and significant decrease in stimulated iNOS mRNA, protein and NO_2^- production with the *ureA*$^{-}$ strain compared with wild type *H. pylori*. *H. pylori* induces a weak immune response which fails to eradicate the pathogen. Translation of iNOS mRNA and NO production by *H. pylori* stimulated macrophages is also inhibited by the polyamine spermine derived from ornithine decarboxylase (*ODC*) and is dependent on the availability of iNOS substrate i.e. L- arginine [36]. siRNA knockdown studies of two inducible genes cationic amino acid transporter (*CAT2*) and *ODC* in gastric macrophage, indicated that addition of spermine or knockdown of *CAT2* inhibits L-arginine uptake, lowers iNOS protein levels and NO production, whereas knockdown of *ODC* has the opposite affect. High *ODC* activity of macrophage was reported in chronic gastritis which results in formation of polyamine spermine. Increased polyamine spermine concentration in turn decreases iNOS expression and NO generation in *H. pylori* stimulated macrophage that is essential for survival of pathogen during chronic diseases (Figure 4). The constitutive expression of *rocF* arginase also facilitates the production of ammonia and also favors the production of nitric oxide in stimulated macrophages [35]. Taken together, these findings indicate that in case of chronic gastritis up regulation of *ODC* in gastric macrophages impairs host defense against *H. pylori* by suppressing iNOS derived NO production [35, 36].

The enhanced gastric epithelial cell apoptosis observed during infection with *Helicobacter pylori* has been suggested to be of significance in the etiology of gastritis, peptic ulcers, and neoplasia. To investigate the cell death signaling induced by *H. pylori* infection, activation of caspase-8, -9 and -3 along with the expression of the proapoptotic *Bcl-2*

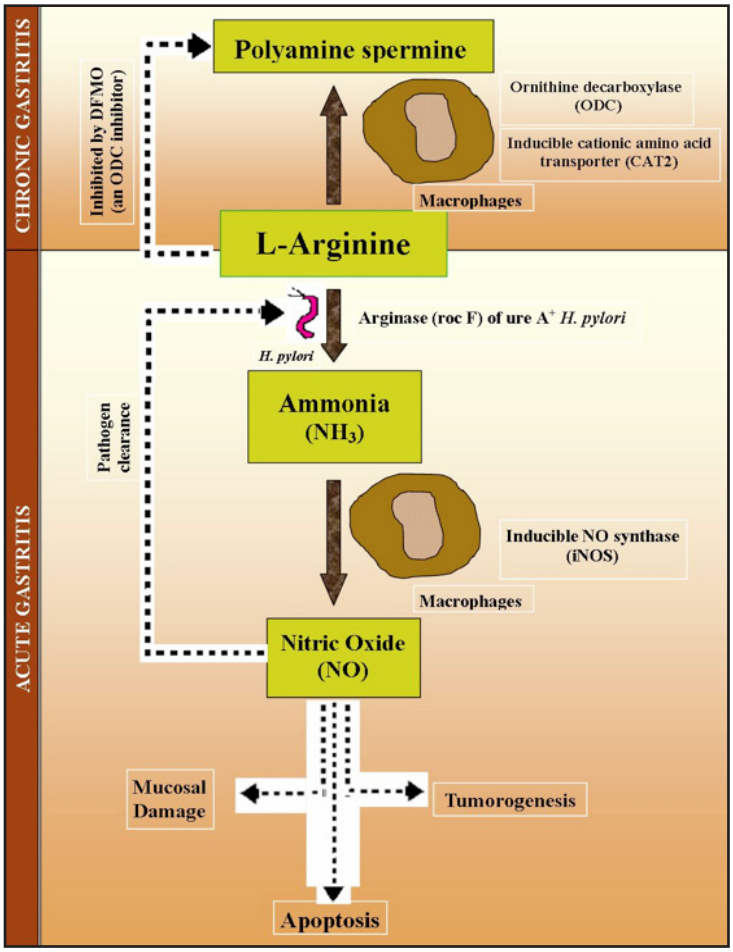

FIGURE 4. Modulation of enzyme activities (arginase, CAT2, iNOS, ODC) and NO production during acute and chronic gastritis.

family proteins *Bad* and *Bid* was studied in human gastric epithelial cells [37]. According to Shibayama et al. [37], *H. pylori* induces apoptosis through a pathway involving the sequential induction of apical caspase-8 activity, the proapoptotic proteins *Bad* and *Bid,* caspase-9 activity, and effector caspase-3 activity. Activation of the pathway is independent of *cagA* or vacuolating toxin. Even a membrane fraction of *H. pylori* is sufficient to activate this pathway.

In chronic cases *H. pylori* infection could lead to peptic/gastric ulcers and adenocarcinoma. Two related mechanisms could promote cancer by *H. pylori* infection. According to first mechanism, there is enhanced production of free radicals (ROIs) near *H. pylori* and a concomitant increased rate of host cell mutation. Second mechanism the "perigenetic pathway" involves enhancement of the transformed host cell phenotype by means of alterations in cell proteins such as adhesion proteins [38]. It has been proposed that *H. pylori* induce inflammation and locally high levels of TNF-α and/or interleukin 6 (IL-6). According to the proposed perigenetic mechanism, inflammation-associated signaling molecules such as TNF-α can alter gastric epithelial cell adhesion and lead to the dispersion and migration of mutated epithelial cells without the need for additional mutations in tumor suppressor genes such as genes coding for cell adhesion proteins [39].

There is a great deal of genetic diversity between various strains of *H. pylori*, so is the outcome of disease. Genotypic diversity of 78 strains of

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H. pylori was dissected at the Institute of Post Graduate Medical Education and Research, Calcutta, India [40]. Study revealed that DNA sequence motifs of *vacAm1* (middle region) alleles are distinct from those of East Asia and the West alleles, whereas the *cagA* sequences of Calcutta and Western strains are closely related. Another virulent factor of *H. pylori*, the *iceA* shows a weak association with disease development. Gene *iceA*2 is associated with most of the Indian strains, whereas the alternative but unrelated gene *iceA1* occupies the same gene locus in approximately 200 strains of *H. pylori* studied from other geographic regions. Around 20% of the Calcutta strains carry an internal deletion in gene *iceA1* which is absent in all the Western strains employed in the study. Thus, the occurrence of internally truncated *iceA1* gene seems to be unique to Calcutta strains. Two mobile DNA elements that were rare in East Asian strains are also common in Calcutta strains. In a separate study, genetic diversity of *H. pylori* strains of Indian subcontinent was also compared with those of European cultures by multilocus sequence typing (MLST) of the 7 housekeeping genes (*atp*A, *efp*, *ure*I, *ppa*, *mut*Y, *trp*C, *yph*C) followed by phylogeographic analysis of the haplotypes [41]. The distribution of *cagPAI* genes within these strains was analyzed using PCR and the geographic type of *cagA* phosphorylation motif EPIYA was determined by gene sequencing. All the Indian *H. pylori* isolates analyzed reveal a European ancestry and belong to *H. pylori* sub-population, *hpEurope*.

Although gastric colonization with *H. pylori* induces histologic gastritis in all infected individuals, but the disease remains asymptomatic as only a minority of patients develop any apparent clinical signs of this colonization. It is estimated that *H. pylori*-positive patients have a 10 to 20% lifetime risk of developing ulcer disease and a 1 to 2% risk of developing distal gastric cancer [42-43]. The risk of development of these disorders in the presence of *H. pylori* infection depends on a genetic make up of bacterial strain, host, and environmental factors that mostly influence the pattern and severity of gastritis [44].

Gastritis can be a brief and sudden illness (acute gastritis) or a long lasting inflammation (chronic gastritis) of gastric tissues that is a result of *H. pylori* infection. Both gastric and duodenal ulcers are strongly related to *H. pylori* infection. Colonization with *H. pylori* virtually always leads to infiltration of both antrum and corpus regions of the gastric mucosa with neutrophilic and mononuclear cells. A close correlation exists between the level of acid secretion and the distribution of gastritis. There is a reduction in acid secretion capacity of the stomach during infection which often results from loss of parietal cells (as in atrophic gastritis) or inhibition of parietal cell function by vagotomy or acid-suppressive drugs, in particular, proton pump inhibitors (PPIs) [42]. Parietal cell function suffers badly due to secretion of local inflammatory molecules such as cytokines, including interleukin-1β (IL-1β). Condition becomes more severe with the augment of nonspecific dyspeptic symptoms, such as fullness, nausea, vomiting and inflammation of both the proximal and distal stomach mucosa and pangastritis. This phase is often associated with hypochlorhydria, which may be followed by spontaneous clearance and resolution of gastritis [45-47]. Approximately 50% of patients with *H. pylori*-associated peptic ulcer disease suffered ulcer recurrence within 1 year [48-49]. Eradication of *H. pylori* dramatically changes the natural course of ulcer and almost completely prevents its recurrence [48-51].

Routes of transmission and detection of peptic ulcers

H. pylori has a narrow host range and is found exclusively in humans and some nonhuman primates [49, 52-54]. *H. pylori* has been detected in saliva, vomitus, gastric refluxate, and feaces of animals. However there is no conclusive evidence regarding transmission of *H. pylori* infection *via.* any of these products [55-60]. Three routes of transmission from the stomach of an infected individual to another have been described in literature. These include (i) the iatrogenic route (*via.* tubes or endoscopes), which is least common, (ii) faecal-oral transmission (the most common) [5-6] and (iii) the oral-oral transmission. There has been no identified association of the infection with sexual transmission [11]. As the *H. pylori* infection remains asymptomatic in early years of infection, thus the progression of disease is generally studied by taking X-ray of the oesophagus, stomach, and duodenum; endoscopy, stool and biopsy testing [61]; blood antibody test, stool antigen test against *H. pylori,* ^{13}C-urea breath test [62].

A strong correlation exists between a history of ulcer and *H. pylori* infection in the family. Environmental and genetic factors might have influence on susceptibility to the infection. In addition, the high prevalence of *H. pylori* infection in subjects with no family history of ulcer suggests how the living conditions, socioeconomic factors and cultural background of the subjects are important in mounting the prevalence and transmission of *H. pylori* infection. Ahmed et al. [63] assessed the relationship between subjects with a history of gastric or duodenal ulcer and the risk of infection in their offspring in population of South India, which is considered the population being considered at high risk of stomach cancer. It was observed that the transmission of *H. pylori* may be influenced by the presence of ulcer or that *H. pylori* strains causing peptic ulcer may be more infective than other strains as published in earlier studies.

Cure of ulcers

The most proven effective treatment is a 2-week course of treatment called triple therapy. It involves taking two antibiotics to kill the bacteria and either an acid suppressor or a stomach-lining shielder. Two types of acid-suppressing drugs might be used: H2 blockers (e.g. Cimetidine, Ranitidine, Famotidine, Nizatidine) and proton pump inhibitors (eg: Omeprazole, Lansoprazole, Pantoprozole, Rabeprazole, Esomeprazole, Leminoprazole) [64]. Bismuth subsalicylate, a component of Pepto-Bismol, is used to protect the stomach lining from acid. It also kills *H. pylori*. Sucralfate, a basic aluminium sulphate sucrose complex, is another ulcer-preventing agent having anti-pepsin and antiacid properties. It reacts with gastric juice to form a sticky paste which protects the mucosa by coating, and also binds to ulcer-affected sites [65].

Emergence of drug resistance in *H. pylori*

The prevalence of *H. pylori* related chronic gastritis, duodenal and gastric ulcer is quite high in Eastern India [20]. Situation becomes worse due to emergence of drug resistance in *H. pylori*. Various Indian studies highlight the occurrence of drug resistance in *H. pylori* strains isolated from biopsy or stool samples of the patients and as a major obstacle in eradication of this gastro-duodenal pathogen. According to Mukhopadhyay and coworker about 90% of Calcutta strains of *H. pylori* were metronidazole resistant [40]. The drug sensitivity profile of *H. pylori* isolated from different parts of India, namely, Hyderabad, Mumbai and Lucknow was studied at National Institute of Cholera and Enteric Diseases, Kolkata, India [66]. Most of the strains (85%) have shown resistance to metronidazole and 7.5% strains to tetracycline, which is quite high compared to other reports in India. All Kolkata strains are however, highly sensitive to clarithromycin, furazolidone and amoxicillin. Bacterial genotype also has a great influence on the efficiency of

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proton pump inhibitor-based triple-therapy regimen. The efficiency of a multidrug formulation consisting of a proton pump inhibitor and two antibiotics viz. omeprazole, clarithromycin and amoxycillin was analysed in patients of Eastern India [67]. Bacterial *vacA m1* allele was most represented genotype among patients with eradication failures (68%) than in those with successful eradication (39%) ($P<0.05$). No significant association of *vacAs1* (signal sequence allele) or *cag* pathogenicity island status with persistence was detected. Persistent infection and recurrence after eradication therapy is a great problem in *H. pylori* infection [20]. Thus, current antibiotic-based triple therapies are not practical for global control due to the high cost, genotypic variation in *H. pylori* strains, problems with patients' compliance and the emergence of antibiotic-resistant strains [68]. Vaccination against *H. pylori* has therefore been considered as the best approach to control *H. pylori* infection and administration of oral bacterial antigens. In a mice model, the efficacy of the vaccine raised against *H. pylori* urease was tested. This in combination with a mucosal adjuvant protected mice against *Helicobacter* infection that could be further extended to humans to provide protection against this gastric pathogen [69-70].

Probiotics and gut physiology

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 [71]. More recent commercial efforts focus on food supplementation with live probiotic cultures in the form of fermented milk products with either a single strain (*L. acidophilus* La1, commercial name LC1; *B. longum* BB536, commercial name ProCult3, and others) or mixed cultures of various lactobacilli (*L. acidophilus*, *L. plantarum*, *L. rhamnosus*, *L. fermentum*), bifidobacteria (*B. infantis*, *B. bifidum*, *B. longum*), yeasts (*Saccharomyces* sp.) and other microbes (Streptococci). Interestingly, the stimulatory capacity of probiotics have been tested in farm animals where their contribution to improve overall health status, immune system functions, reducing risk of infection and improvement in the yield of poultry and meat products is highly appreciated [72]. 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 [73-74]. A series of review articles have been published in the past year outlining the efficacy of probiotics and prebiotics in human health [75-80].

Summary of health benefits exhibited by probiotics

Probiotic microorganisms in the intestine 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 [71, 81]. 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 produce interferon gamma (IFN-γ) that stimulates immune system of the host by improving phagocytic cell functioning [71,82]. IFN-γ-activated macrophages inhibit growth of pathogenic bacteria like *Mycobacterium* as a result of TfR downregulation [71, 81]. Thus, deprivation from essential growth factors represents an integral part of host defense functions.

Importance of probiotics is evidenced by their ubiquitous occurrence in natural food products, their GRAS (Generally Recognized as Safe) status, and their ability to exert health benefits beyond basic nutrition. Probiotic organisms display numerous antagonistic activities. These antagonistic effects are mainly exhibited due to production of organic acids, but also of other compounds such as bacteriocins and antifungal compounds [83-87]. Applications of bacteriocin starter cultures and bacteriocin thereof in various food systems were already addressed in a number of review articles [88-90]. Health claims of various probiotic strains include normalization of gastro-intestinal [91-92] and vaginal ecosystem [93, 94], improvement of specific and non-specific immune responses [82], detoxification of carcinogens and suppression of tumors and cancers [95-97], reduction of blood pressure in hypertensive patients [98] and cholesterol [99]. Importance of probiotic lactic acid bacteria in treatment of milk allergies [100] and improvement of mineral absorption capacity of the intestine are also well documented [101].

Mechanism of probiotic action

Lactic acid bacteria have acquired the status of probiotic starter culture in the food industry because of their "GRAS" status and increased consumer awareness of the potential health risks derived not only from food borne pathogens, but also the artificial chemical preservatives used to control them. Their growth lowers pH that inhibits the growth of most of the other microorganisms, the biochemical conversions involved in growth enhance the flavor, improve organoleptic and nutritional properties [102] and many strains produce antagonistic compounds such as organic acids, hydrogen peroxide, diacetyl and bacteriocins [103]. Bacterial species primarily used as probiotic cultures in the International as well as National food industries are *Lactobacillus acidophilus* (La2, La5, Johnsonii, NCFM, DDS-1, SBT-2062), *L. bulgaricus* (Lb12), *L. lactis* (La1, A164, BH5), *L. plantarum* (299v, Lp01), *L. rhamnosus* (GG, GR-1, 271, LB21), *L. reuteri* (SD2112), *L. fermentum* (RC-14), *Bifidobacterium longum* (BB536, SBT-2928), *B. breve* (Yakult), *B. bifidum* (Bb-12), *B. esselnsis* (Danone{Bio Activia}), *B. lactis* (Bb-02), *B. infantis* (Shirota, Immunitass, 744, 01) [104]. Gratia was the first to discover that the antimicrobial property of bacterial cells is exhibited by synthesizing proteinaceous toxins that inhibit the growth of similar or closely related bacterial strain(s) [105]. Later on series of bacteriocin producers have been identified and their importance in food fermentations was tested. Isolated pediocins and their producer strains such as *Pediococcus acidilactici, P. pentosaceous, P. damnosus* are also potential candidate for the development of novel antimicrobial and therapeutic agents [106-109]. Probiotics may exert some of their protective functions through modulation of immune activity and epithelial functions in both the large and small intestine. *In vitro* models suggested that immune and epithelial cells can discriminate between different microbial species through activation of Toll-like receptors [110]. Resta-Lenert and Barrett showed that live *Streptococcus thermophilus* and *Lactobacillus acidophilus* could inhibit the adhesion and invasion of enteroinvasive *E. coli* into human intestinal cell lines [111]. *In vitro* investigations also revealed the ability of *Lactobacillus rhamnosus* GG to prevent cytokine-induced apoptosis in intestinal epithelial cell models through the inhibition of TNF-induced activation of the proapoptotic p38/mitogen-activated protein kinase [112]. Epithelial cells release interleukin-8 in response to pathogenic bacteria but not to probiotic strains [113]. Bacterial DNA of pathogenic strains evoke phosphorylation of the extracellular signal-regulated

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kinase pathway and turn on activator protein-1 [114], and of probiotic strains modulate nuclear factor-κB (NF- κB) pathway in response to TNF-α as indicated in Figure 1 and 2 [115]. Selected probiotics can stimulate host dendritic cells (DCs) regulatory functions by targeting specific pattern-recognition receptors and pathways and confer protection against 2, 4, 6-trinitrobenzenesulfonic acid (TNBS)-induced colitis [116]. The preventive effect of probiotic-pulsed DCs required a high local expression of the immunoregulatory enzyme indolamine 2, 3 dioxgenase, MyD88-, TLR2- and NOD2-dependent signaling and induction of $CD4^+$, $CD25^+$ regulatory cells in an IL-10-independent pathway. A study demonstrated the role of probiotics to counteract stress-induced changes in intestinal barrier function, visceral sensitivity and gut motility in a strain specific manner. Effects are mediated through direct bacterial-host cell interaction and/or via soluble factors. Probiotics may elicit these beneficial responses through various mechanisms viz. competition with pathogens for essential nutrients, induction of epithelial heat-shock proteins, restoration of tight junction protein structure, up-regulation of mucin genes, secretion of defensins, and regulation of the NF-κB signalling pathway. In addition, cannabinoid receptors reduce the perception of intestinal pain [117]. A study has already investigated the adhesion and colonization dynamics of lactobacilli *in vivo* in humans [118]. Exogenously applied lactobacilli are generally able to only temporarily colonize the gastrointestinal tract (GIT). This phenomenon was linked to colonization resistance or the niche exclusion principle, where each niche in the GIT is colonized by well-adapted species [119].

Genes and proteins supporting probiotic function

Caco-2 or HT-29 human-derived adenocarcinoma cells are important models to study adherence of probiotics to Human epithelial cell [120]. In a genome-wide microarray-based genotyping, Pretzer et al. [121] identified mannose-specific adhesin in *L. plantarum* WCFS1. This glycoprotein is encoded by *lp_1229* gene (renamed as *msa*). With the availability of *in silico* approaches for genome wide analysis it became possible to identify genes encoding proteins that facilitate adherence of bacteria to intestinal cells as well as for novel proteins mediating probiotic adherence to GIT. Such techniques are less labour-intensive, fast and highly precise. It saves a lot of time for initial screening, though validation of the results demand wet lab experiments. An *in silico* study showed that the genes encoding a fibronectin-binding protein (*FbpA*), a mucin-binding protein (*Mub*), and a surface layer protein (*SlpA*) all contribute to the ability of *L. acidophilus* NCFM to adhere to Caco-2 cells, confirming that adhesion is determined by multiple factors. Sortase and sortase dependent proteins (SDPs) such as *srtA* and the *lspA*, are also important adhesion factors of *L. salivarius* UCC118 [122]. Surprisingly, two peculiar cytoplasmic proteins of *L. johnsonii* NCC533; an elongation factor Tu (EF-Tu) and a heat shock protein *GroEL* have an important role in the binding of NCC533 to Caco-2 and HT-29 intestinal epithelial cells and mucins [123, 124]. Both the proteins of NCC533 have been located at the cell surface, although no secretion or cell wall-binding motifs are present to explain this observation. S-layer proteins (like *SlpA* and *CdpA*) of lactobacilli have been commonly implicated in the adherence of lactobacilli to Caco-2 cells [125-126].

Prevention of *H. pylori* infection

The adhesion of *H. pylori* to epithelial cells is important in determining the outcome in *H. pylori*–associated diseases [127]. In the gastric mucosa, *H. pylori* possibly interact with epithelial cells through secretory components or as a result of adherence [12]. There is substantial evidence that probiotics modulate *H. pylori* colonization of the gastric mucosa through production of lactic acid, bacteriocins or antibiotics. Different reports support this hypothesis and have proven the efficiency of probiotic microorganism in treatment of *H. pylori* infection (as given in Table 2). *In vitro* studies showed that *Lactobacillus johnsonii* La1, *L. salivarius*, *L. acidophilus,* and *Weissella confusa* inhibit the attachment of *H. pylori* to intestinal HT-29 cells [61, 146] or to MKN 45 gastric cell lines [128,135]. Probiotics interfere in adhesion of *H. pylori* to epithelial cells and their capacity to attenuate *H. pylori*-induced gastritis in man is addressed in a reveiw by Felley and Michetti [147]. Previous reports have suggested a role of probiotics in treatment and prevention of *H. pylori* infection through probiotic-induced inhibition of *H. pylori* growth and adherence to epithelial cells and co-activation of host immune system [129, 147, 148]. Both *in vitro* and *in vivo* mouse model of Ushiyama *et al.* demonstrates inhibitory effect of *Lactobacillus gasseri* on *H. pylori* colonization [149]. In a double-masked, randomized, controlled clinical trial, 326 school children from a low socioeconomic area of Santiago, Chile, with *H. pylori* infection were treated with both live and heat-killed strains of *Lactobacillus johnsonii, Lactobacillus paracasei* and/or carrier once daily for 4 weeks. A ^{13}C-urea breath test demonstrated a significant decrease in *H. pylori* colonization in children receiving live *L. johnsonii* but not the other groups [150]. Both of these studies support the complementary effect of probiotics in the management of *H. pylori* infection. The sharing of glycolipid specificity is a pre-requisite for the *Lactobacillus* strains to have a therapeutic effect on *Helicobacter pylori* eradication [134]. The *MUC5AC* glycoprotein has been identified as the primary receptor for *H. pylori* in the human stomach [151].

TABLE 2. Probiotic cultures with a potential to treat *Helicobacter pylori* infections.

Strain	References
L. salivarius WB1004	128
L. acidophilus (johnsonii) La1	61
L. acidophilus	129
L. acidophilus CRL 639	130
L. gasseri OLL2716	131
L. GG	132
Bacillus subtilis	133
L. reuteri	134
Weissella confusa	135
Lactobacilli and Bifidobacteria	136, 137
L. casei strain Shirota	138
L. casei strain Shirota and L. acidophilus	139
L. casei subsp. DG	140
L. brevis	141
L. rhamnosus R0011 and L. acidophilus R0052	142
Bacillus clausii	143
L. reuteri and L. paracasei	144
L. salivarius	145

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Probiotics have been suggested to increase efficacy of *H. pylori* eradication therapy by preventing antibiotic-associated side effects and thus increasing compliance. Cremonini *et al.* [152] randomized 85 patients with *H. pylori* undergoing eradication with triple therapy to one of four groups: *Lactobacillus casei* subspecies *rhamnosus, Saccharomyces boulardii, L. acidophilus* plus *Bifidobacterium lactis*, or placebo. In all probiotic-supplemented groups, there was a significantly lower incidence of antibiotic-associated diarrhea and taste disturbance relative to placebo. Nevertheless, there was no difference in *H. pylori* eradication or compliance rates between the various groups.

The effects of multi-species probiotic combination on *H. pylori* infection in terms of adhesion, epithelial cell damage, apoptosis and inflammatory responses in Caco-2 cells were evaluated by Myllyluoma et al. [153]. All probiotics used in the study inhibited *H. pylori* adhesion. *L. rhamnosus* GG, *L. rhamnosus* Lc705, *P. freudenreichii* subsp. *shermanii* Js, and the combination inhibited *H. pylori*-induced cell membrane leakage. *L. rhamnosus* GG, *L. rhamnosus* Lc705, and the combination initially improved epithelial barrier function but increased the *H. pylori*-induced barrier deterioration after incubation for 24 to 42 h. *L. rhamnosus* GG, *L. rhamnosus* Lc705, and *P. freudenreichii* subsp. *shermanii* Js inhibited *H. pylori*-induced IL-8 release, whereas *L. rhamnosus* GG, *L. rhamnosus* Lc705, and *B. breve* Bb99 suppressed PGE2 release. None of these anti-inflammatory effects persisted when the probiotics were used in combination. The combination thus increased the levels of IL-8, PGE2, and LTB4 released from *H. pylori*-infected epithelial cells. The proinflammatory actions of the individual components dominated the anti-inflammatory effects when the probiotic bacteria were used in combination. Therapeutic response could be optimized if probiotic strains are characterized before they are used in combination.
In a clinical trial on *H. pylori* patients, the effect of fermented milk-based probiotic preparations on *H. pylori* eradication was evaluated at Sitaram Bhartia Institute of Science and Research, New Delhi [154]. The search identified 10 eligible randomized controlled trials. Data were available for 963 patients, of whom 498 were in the treatment group and 465 in the control group. The pooled odds ratio for eradication by intention-to-treat analysis in the treatment versus control group was 1.91 (P<0.0001); test for heterogeneity (Cochran's Q=5.44; P=0.488). The pooled risk difference was 0.10 (95% CI 0.05-0.15; P<0.0001) by the fixed effects model (Cochran's Q=13.41; P=0.144). The pooled odds ratio for the number of patients with any adverse effect was 0.51 (95% CI 0.10-2.57; P=0.41); random effects model; heterogeneity by Cochran's Q=68.5; P<0.0001). Fermented milk-based probiotic preparations improve *H. pylori* eradication rates by approximately 5-15%, whereas the effect on adverse effects is heterogeneous.

The drug sensitivity profiles of *H. pylori* isolated from different parts of the World have indicated that the pathogen has acquired resistance to the antibiotics due to point mutations and decreased binding of the antibiotics to the ribosomes [40, 66, 155]. Thus, current antibiotic-based triple therapies are not practical for global eradication due to the genotypic variation in *H. pylori* strains and the emergence of antibiotic-resistant strains [68]. Very few studies have actually focused on role of bacteriocins produced by probiotic bacteria in treating *H. pylori* infection (Table 3). Lacticin A164 of *Lactococcus lactis* subsp. *lactis A164* and lacticin BH5 of *L. lactis BH5* are two bacteriocins of lactococcal origin with anti-*Helicobacter* activity that tell pathogen in a dose-dependent manner [156]. Two more anti-*Helicobacter pylori* bacteriocins namely bulgaricin BB18 produced by *L. bulgaricus* BB18 and enterocin MH3 produced by *E. faecium* MH3 have recently been identified [157]. These are potential antimicrobial agents and in conjunction with their producers, may have use in applications to contribute a positive effect on the balance of intestinal microflora.

TABLE 3. Anti-Helicobacter pylori bacteriocins.

Bacteriocin	Strain	Reference
Lacticin A164	*Lactococcus lactis subsp. Lactis A164*	156
Lacticin BH5	*Lactococcus lactis subsp. lactis BH5*	156
Bulgaricin BB18	*Lactobacillus delbrueckii ssp. bulgaricus BB18*	157
Enterocin MH3	*Enterococcus faecium MH3*	157

Exploring genome and transcriptome of *H. pylori* to identify potential drugs targets

Microarray technology is a powerful tool to get an overview of cell responses to environmental changes at the transcriptional level. A single environment change or exposure to pathogen can induce a large number of changes in the transcriptome content of a particular tissue. Differential gene expression needs a further validation at the transcriptome level (through RT-PCR) or at proteome level (through ELISA, western blotting, 2D gel electrophoresis, MALDI-TOF etc.). Statistical analysis of the microarray expression data generated in transcriptomics study needs a skilled personnel and is time consuming too. Sometimes normalization of data needs to be done in order to get final result. That is why the application of this technology to the study of heterogeneous microbial populations presents a huge methodological challenge. Generally, the biochips used with mixed cultures are not pangenomic and serve rarely for transcript detection. Most of them are devoted to the detection of microbial species in complex ecosystems through ribosomal DNA sequences or to the detection of a reduced number of DNA sequences without quantifying their expression levels [158-161]. Some articles mention the use of DNA biochips for mRNA quantification, but these chips are mostly restricted to a limited number of mRNAs [162-164]. The use of pangenomic biochips to study mixed cultures has been mentioned only in few research articles [165, 166].

The transcriptional profile of gastric epithelial cell lines cocultured with *H. pylori* and the global gene expression of whole gastric mucosa has been described in a study by Resnick and coworkers [167]. mRNA from 10 patients with peptic ulcer disease, before and after *H. pylori* eradication were reverse transcribed and applied on to Affymetrix cDNA microarray chips. Differentially expressed genes were identified and subset was validated by real-time polymerase chain reaction (RT-PCR). A total of 13,817 transcripts decreased and 9680 increased after *H. pylori* eradication. Applying cut-off criteria (p<0.02, fold-change threshold 2.5) reduced the sample to 98 differentially expressed genes. Genes detected included those previously implicated in *H. pylori* pathophysiology such as interleukin-8, chemokine ligand 3, β-defensin and somatostatin, as well as novel genes such as gastrokine-2 (*GDDR*) (*TFIZ1*), chemokine receptor-7 and -8, and gastrokine.

A serious obstacle in transcriptome studies is massive cross-hybridization between the foreign cDNA and the genome specific DNA chip. A very simple method was proposed to considerably decrease this nonspecific hybridization, consisting of adding the microbial partner's DNA [166]. Co-culture technique was followed to study gene expression changes in *L. lactis* to the presence of *Saccharomyces cerevisiae*. Although no differences between growth kinetics were observed for the pure and the mixed cultures of *L. lactis*, the mRNA levels of 158

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genes were significantly modified. More particularly, a strong reorientation of pyrimidine metabolism was observed when *L. lactis* was grown in mixed cultures. These changes in transcript abundance were demonstrated to be regulated by the ethanol produced by the yeast and were confirmed by an independent method (quantitative reverse transcription-PCR). It is important to highlight here that co-culture technique opted by Maligoy et al. [166] can be extended further to study probiotic-*H. pylori* interaction *in vitro* and can provide useful information on the actual mechanism by which probiotic bacteria prevents colonization, and/or influence clearance of the *H. pylori* infection from human gastroduodenal region. Sharma and coworker presented a genome-wide map (1.67Mb) of *H. pylori* transcriptional start sites and operons [168]. Polycistrons are more complex and uncoupled in *H. pylori* as hundreds of transcriptional start sites were discovered upstream to annotated genes lying within the operons. Further, control of gene expression in *H. pylori* is exhibited through anti-sense transcription. 60 small RNAs including the e-subdivision counterpart of the regulatory 6S RNA and associated RNA products, and potential regulators of *cis*- and *trans*-encoded target messenger RNAs were identified. Availability of primary transcriptome of *H. pylori* helps in identifying novel therapeutic targets and the information obtained could be applied to rational remodeling or "tailoring" of human-associated probiotic microorganism in order to enhance their anti- *H. pylori* activity and associated functions. Clearly, the best approach would be targeting transcriptional regulators of virulence and associated factors in *H. pylori* in order to provide an effective cure against this major gastric pathogen.

Peptide vaccines available against *H. pylori*

The human microbiome could be manipulated by such "smart" strategies to prevent and treat acute *H. pylori* infection and a variety of other disorders. Information obtained from metagenomics and the human microbiome will tremendously expand our knowledge of the genetic composition of microbial species associated with human gut. This information can be directly applied to engineer human-associated probiotic microorganism and enhances their associated functions. In an attempt to use *Lactococcus lactis* for oral delivery of vaccine against *H. pylori*, fragment E of *ureB* (*ureBE*) was cloned from a clinical isolate of *H. pylori* [169]. A prokaryotic expression vector, pAMJ399, with the *ureB* fragment E and the *Staphylococcus aureus* protein A anchor fragment (*spaX*), was constructed. The fusion protein was expressed under the control of the P170 promoter in *Lactococcus lactis*. Western blot assay of lactococcal cell wall extracts with a polyclonal chicken antiserum confirmed the immunity of the expressed recombinant protein that was located on the cell surface. These results provide the first report of a surface display system in lactic acid bacteria for the delivery of oral vaccines against *H. pylori*.

Genome-wide association study (GWAS) or whole genome association study (WGAS)

GWAS is an examination of genetic variation across a given genome, designed to identify genetic associations with observable traits. Millions of single-nucleotide polymorphisms, and thousands types of copy number variations are found in large or small segments of the human genome. These genetics variations may either directly induce phenotypic changes or tag nearby mutations that influence individual variation and susceptibility to disease, thus, are considered as pointers to the region of the genome where the disease-causing problem is likely to reside. Since the entire genome is analyzed for investigating genetic of a particular disease, GWAS allow the genetics of a disease to be investigated in a non-hypothesis-driven manner [170]. However, because of population stratifications, studies must take account of the geographical and racial background of participants. To date, GWAS studies have identified risk and protective factors for AIDS [171], asthma [172], breast cancer [173], cardiac arrest [174], gastrointestinal diseases viz. celiac disease [175], colorectal cancer [176,177], crohn's disease [178-180], esophageal cancer [181], pancreatic cancer [182,183], type I diabetes [184], type II diabetes [185,186], heart failure [187], hypertension [188], macular degeneration [189], multiple scleresis [190], neuro-degenerative alzheimer's disease [191], obesity [192], rheumatoid arthritis [193], schizophrenia [194-196], urinary bladder cancer [197] and other human disorders. A number of GWAS have been conducted to discern the genetic susceptibility as well as the host pathogen interaction underlying the etiology of gastroduodenal diseases. In their two stage study conducted amongst Japanese 1,384 ulcerative colitis patients and 3,057 controls [198], Asano and coworkers identified strong association of disease with the major histocompatibility complex (MHC) region and three new susceptibility loci: the immunoglobulin receptor gene FCGR2A : rs 1801274, a locus on chromosome 13q12: rs17085007 and the glycoprotein gene SLC26A3: rs2108225. The FCGR2A: rs1801274 is a nsSNP that is critical to receptor's binding affinity for IgG and has been associated with other autoimmune diseases. Franke *et al* identifed two new associations at chromosomes 7q22: rs7809799 and at chromosome 22q13 in ILI7REL: rs5771069 amongst Europeans [199]. However such studies are incomplete in absence of clear cut pathogen characterization which alone can lead to determination of host pathogen susceptibility genes and the pathways involved in such interaction.

Conclusion and future perspectives

H. pylori is a common pathogen of gastro-duodenal region associated with chronic gastritis, peptic and gastric ulcers, gastric adeno-carcinoma and more rarely, lymphoma of the mucosa-associated lymphoid tissue. Pathogen shows a high prevalence in developing and poor countries. Emergence of drug resistance in *H. pylori* is a major obstacle in eradication of this gastric pathogen. Why susceptibility to this gastric pathogen varies with genetic diversity of the population? Answering this question will require a substantial commitment to future research. This will need a substantial effort to identify important genotypic variations that aid in protecting individual against *H. pylori*. Extensive GWAS analyses will help to get insight into mechanism of pathogen attachment, etiology of disease and altered immune responses for pathogen clearance before it establishes itself into the gastric mucosal system. Besides academics, the impact of such studies has been widespread as novel therapeutic agents and better preventive measures could be suggested to control pathogen colonization or to eradicate pathogen in the host gastrointestinal tract and to prevent outbreak of the *H. pylori* infection. The populations indicating higher susceptibility to *H. pylori* might have a differential expression of genes such as adhesion factors and alternative induction mechanism of disease could only be identified following a well planned GWAS. The potential of probiotic lactic acid bacteria in aggravating resistance to *H. pylori*, to kill pathogen through production of anti-*H. pylori* bacteriocins and/or other antagonistic factors is revealed here. The application of probiotic organisms in food preservation as well as prevention of human gastro-intestinal diseases is emerging these days. Mechanism by which probiotics confer resistance to *H. pylori* is still to be deciphered and requires a separate GWAS on human populations taking oral doses of probiotic organism. Moreover, GWAS helps to investigate genetics of a disease and identify novel therapeutic targets. Information thus obtained could be directly applied to tailor probiotic organisms or vaccines against *H. pylori*. The future perspectives of the study help to display probiotic lactic acid bacteria and/ parts thereof that may provide an effective prevention or cure against this major gastric pathogen.

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