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Helicobacter pylori, a Gastric Pathogen Causing Persistent Infection

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Abstract *Helicobacter pylori*, a gastric pathogen from the δ, ε subdivision of proteobacteria, is a microaerophilic, Gram-negative, flagellate, spiral bacterium. It shares these properties with the related food-borne pathogen *Campylobacter jejuni*. The genomes of *H. pylori* 26695 and *C. jejuni* NCTC11168 are similar in size and predicted to encode 1,590 and 1,654 proteins, respectively. Despite the close phylogenetic relationship of *H. pylori* and *C. jejuni*, only 55.4% of *H. pylori* genes have orthologs in *C. jejuni*, and strong similarities between them are mainly confined to housekeeping functions. *H. pylori* but not *C. jejumi*, has a unique ammonia-producing system to neutralize gastric acid and pathogenic factors such as VacA and CagA. In addition, it acquired hypervariable properties in the outer membrane proteins and Lewis-antigens of lipopolysaccharide to colonize a wide range of the human host. Thus selective pressures have driven profound evolutionary changes to create specific pathogens appropriate to their niches, from a relatively close common ancestor.

Introduction

Helicobacter pylori is now regarded as one of the most common human pathogens. Infection of this organism causes symptomatic or asymptomatic gastritis that inevitably associates with infiltration of inflammatory cells in lamina propria. Among the infected populations, only a few people get severe diseases such as duodenal and gastric ulceration, gastric adenocarcinoma, and mucosa associated lymphoid tissue lymphoma.[1] Such a variety of the clinical outcome might be due to the difference of pathogenic factors among strains that cause different host responses. The whole genome sequences of *H. pylori* 26695 isolated from a gastritis patient in England and of *H. pylori* J99 isolated from a duodenal ulcer patient in USA have greatly facilitated research into the physiology and virulence genes.[2,3] In addition, the genome sequence of a food-borne pathogen *Campylobacter jejuni* recently published[4] has enabled us to study the physiology and phathogenic factors *in silico* of phylogenetically closely related microorganisms. Here I compare the genes in *H. pylori* and *C. jejuni* to discuss on evolutionary changes that might occur in *H. pylori* to adapt for persistent infection in the unique niche, the human stomach.

1. *H. pylori* and *C. jejuni* are different in their niche and the course of infection

H. pylori and *C. jejuni*, both microaerophilic, Gram-negative, and spiral bacteria, are classified in δ, ε subdivision of proteobacteria. Both microorganisms have polar flagella, but the flagella filaments of *H. pylori* are covered with sheath, while those of *C. jejuni* are naked and modified by sialylation.[5]

H, pylori and *C. jejuni* are pathogens of gastrointestinal tract, but the niche, the course of disease, and the rout of infection are very different. *H, pylori* is a unique microorganism to colonize the human stomach causing chronic gastritis. Initial infection often starts in childhood and persists for decades without antibiotic treatment. On the other hand, *C. jejuni* colonizes the intestine of a variety of animals and birds to cause acute diarrhea. Thus *C. jejuni* shares the pathogenic properties in common with *Salmonella enterica* and pathogenic *Escherichia coli*, the family Enterobacteriaceae from γ subdivision of proteobacteria.

2. The *H. pylori* and *C. jejuni* circular chromosomes are similar in size and the number of encoding proteins

The genome size and the number of encoding proteins of *H. pylori* and *C. jejuni* are very similar (Table 1).[2,4] The bacterial genomes are smaller than those of other Gram-negative, mucosa-colonizing proteobactera such as *Haemophilus influenzae* (1.8 Mbp), *Escherichia coli* K12 (4.6 Mbp), and enterophathogenic *Escherichia coli* O157: H7 (5.5 Mbp). *H, pylori* has two sets of ribosomal RNA (rRNA), while *C. jejuni* has three rRNA operons. This may partly reflect the slower growth rate of *H. pylori* than *C. jejuni* on agar media.

The average G+C contents of *H. pylori* and *C jejuni* are 39% and 30.6%, respectively. Four regions of the *H. pylori* genome have a significantly lower G+C content. Two of them contain one or more copies of the insertion sequence (IS605) and are flanked by a 5S ribosomal RNA sequence at one end and a 521-bp repeat (repeat 7) near the other. Other two regions contain either restriction/modification systems or the *cag* pathogenicity island (PAI) (see below). In the *C. jejuni* genome, two regions with significantly lower G+C content correspond to genes of the lipo-oligosaccharide (LOS) and extrapolysaccharide (EP) biosynthesis cluster, respectively. In bacterial genomes, regions that have the G+C content different from the average G+C content of the genome are believed to be the product of lateral transfer. *H. pylori*

Table 1. *H. pylori* and *C. jejuni* genomes

	H. pylori 26695[2]	*C. jejuni* NCTC11168[4]
Size (bp)	1,667,867	1,641,481
(G + C) content (%)	39	30.6
Number of ORF	1,590	1,654
rRNA	23S-5S : 2 16S : 2 5S : 1	16S-23S-5S : 3
Number of tRNA	36	43
IS copies	17 (including partial IS)	1 (partial IS)
Regions of low (G + C) content	4	2
Major genes in the regions	IS605, 5S rRNA *cagPAI* Restriction / modification system	LOS biosynthesis and modification EP biosynthesis and modification

has several copies of insertion sequences, IS 605 and IS606, as well as eight short repeat families in the genome that may be involved in horizontal gene transfer and insertion.[2] Intriguingly, the *C, jejuni* genome has virtually no insertion sequences and very few repeat sequences.[4]

3. *H. pylori* has adapted the unique niche in the stomach

Despite the close phylogenetic relationship of *H. pylori* and *C. jejuni*, strong similarities between them are mainly confined to housekeeping functions ; only 55.4% of *H. pylori* genes have orthologs in *C. jejuni*.[4] In most functions related to survival and pathogenesis, the organisms have remarkably little in common (Table 2). This indicates that selective pressures have driven profound evolutionary changes to create two very different and specific pathogens appropriate to their niches, from a relatively close common ancestor.

1) Acid tolerance of *H. pylori*—an ammonia-producing system

It is now established that *H. pylori* urease, catalyzing hydrolysis of urea to produce two moles of ammonia and one mole of carbon dioxide, plays a major role in colonization.[6] Urease genes consist of two operons, *ureAB* and *ureIEFGH*, encoding apoenzyme AB and accessory proteins for construction of the active site containing Ni^{++}, respectively, A gene *nixA* for Ni^{++} permease is present in the *H. pylori* genome that is required for the synthesis of a large amount of active urease.[7]

Active urease to produce ammonia must be required under acidic conditions, but not at neutral and alkaline conditions. We have found that mRNA transcribed from the *ureIEFGH* operon is stabilized under acidic conditions, while degraded rapidly under neutral and alkaline conditions.[8] In addition, urea, the substrate of urease, is actively incorporated into the bacterial cytoplasm through UreI channel for urea that is opened only under acidic conditions.[9]

Urea in the circulating blood diffuses out to the gut mucous through the tight junction of gastric epithelial cells from submucosal capillaries. This suggests that urea concentration in the gastric mucus gel is high near the epithelial cell surface but low in the luminous side of the gel. The majority of *H. pylori* bacteria are found in the mucous gel that is moving toward duodenum by peristaltic movement, acid secretion, mucous gel production and epithelial cell turnover. *H. pylori* possibly proliferates on the epithelial surface and detaches from the surface to the mucous gel. For persistent infection, the bacteria should move back to the epithelial surface against the flow of the mucous gel. We found the ability of *H. pylori* to move toward urea, termed urea-taxis, i.e., the bacteria can sense the concentration gradient of urea and move to the epithelial surface by rotating sheathed flagella at the end of the spiral body.[10]

In the *H. pylori*-infected mucous gel, urea enters to the bacterial cytoplasm either by diffusion under neutral and alkaline conditions or by channel-gated uptake under acidic conditions and is consumed rapidly by cytoplasmic urease. Urea can be obtained also by arginine hydrolysis. *H. pylori* acquired a permease gene *rocE* to incorporate arginine [2] and rocF for arginase that hydrolyzes arginine to produce urea and L-ornithine[11]. If gastric mucin, a glycoprotein rich in arginine residues, is degraded by a putative glycoprotease (O-sialoglycoprotein endopeptidase) of *H. pylori* that releases arginine[2], the urea-producing system from arginine is another strategy of *H. pylori* to survive in the stomach.

The *C. jejuni* genome has neither *rocE* nor *rocF*, but has genes for arginine biosynthesis, *argBCDEFGH* similar to *E. coli* and other bacteria.[4] Thus the selective pressure of gastric acid has driven evolutionary changes to create a unique system for acid tolerance in *H. pylori*.

2) Pathogenic factors

Genes functioning in pathogenesis of *H. pylori* and *C. jejuni* have little in common. In *H. pylori*, *vacA* for vacuolating cytotoxin (VacA) and *cagPAI* are well documented, whereas in *C. jejuni*, *ciaB* and *ctd*, a factor involved in intracellular invasion and in a cytolethal distending toxin, respectively, are found.[4]

The N-terminal signal sequence of VacA of *H. pylori* is cut by the Sec system to release

Table 2. Unique genes in *H. pylori* and *C. jejuni* genome

	H. pylori[2]	*C. jejuni*[4]
Genes for arginine metabolism	*rocE*: arginine permease *rocF*: arginase *ureAB, ureIEFGH*: urea hydro lysis *nixA*: Ni transport	*argBCDFGH*: arginine biosynthesis
Pathogenic genes	*cagPAI*: Type IV secretion system *vacA*: vacuolation, apoptosis	*ciaB*: invasion *cdt*: cytolethal distending toxin
Genes for surface-exposed molecules	*hop* family: outer membrane protein family *α 2 fucT, α 3 fucT*: Lewis antigem biosynthesis	*neuABC*: sialic acid biosynthesis *ktdAB, kpsDEMT*: extracellular polysaccharide biosynthesis

protoxin to the periplasm. The C-terminal portion of the VacA protoxin is an autotransporter that forms a β-barrel structure in the outer membrane and acts as a protein channel to secrete the 88-kDa mature VacA toxin of the middle portion.[13] Secreted VacA attaches to the host cell surface, forms an oligomeric structure and then binds to a tyrosine phosphatase β-receptor (RTPTβ).[13] The firm binding may allow VacA to be incorporated to the endosome, and its active fragment is possibly released to the cytoplasm to cause apoptosis through mitochondrial damages.[14]

The *CagPAI* is a 40-kb DNA segment that might be generated by horizontal transfer.[12] It contains genes for the Type IV secretion system that acts to inject proteins or DNAs into the target cells.[15] The *cagA* gene for CagA, cytotoxin associated gene, sits at the terminal end of *cagPAI*. CagA is injected by the secretion system and then phosphorylated in the host cytoplasm causing actin polymerization.[16,17] The CagPAI secretion system may also inject some unkown proteins that are involved in activation of a signal transduction system to produce inflammatory-cytokines such as IL-8,[18] since IL-8 production is dependent on *cagPAI*, but not on *cagA*.

Why does *H. pylori* cause chronic persistent infection, while *C. jejuni causes* acute infection? The stomach lamina propria is absent from inflammatory cells before infection, and there is no bacteria competing *H. pylori* to colonize. Therefore, it will be easy for acid-tolerable *H. pylori* to get colonized. On the contrary, the intestine lamina propria is rich in inflammatory cells such as neutrophiles and monocytes, since colonization of commensal bacteria established inflammatory cell infiltration just after birth as a host defense mechanism. In addition, there is a microflora in the intestine that prevent colonization of pathogens. Thus *C. jejuni* has to invade and destruct the host cells for proliferation.

4. *H. pylori* and *C. jejuni* have different surface-exposed molecules encoded by hypervariable genes

1) Surface exposed antigens of *H. pylori* and *C. jejuni*

The surface exposed antigens of *H. pylori* consist of outer membrane proteins and lipopolysaccharides (LPS) with Lewis antigens (Table 3), whereas those of *C. jejuni* consist of sialylated LOS, flagellin, and EP that are possibly involved in the Guillain-Barre syndrome of an autoimmune disease (Table 2).[19]

Interestingly, hypervariable sequences, short homopolymeric runs such as Cn, Gn, Tn, An, (CT)n, and (AG)n, are commonly present in the genes encoding surface an-

Table 3. Surface-exposed molecules of *H. pylori* and their deduced functions

Outer membrane protein Hop
HopA, HopD, HopE : porin
HopB (AlpB), HopS (BabA), HopT (BabB), HopZ (AlpA) : adhesin
HopH (OpiA) : chemokine inducer
Other 12 Hop proteins : functions unknown
Lewis antigens and related blood group substances
Lewis X, Lewis Y : adhesin
Lewis A, Lewis B, H type 1, I antigen, Type A antigen : functions unknown

tigens of *H. pylori* and *C. jejuni*.[2,4] Such structures in the genome may cause strand-slipped-misparing during DNA replication, resulting in frame-shift mutation. The rapid variation in the surface properties may have relevance to broadening the host range in colonization.

2) Outer membrane proteins of *H. pylori*

The *H. pylori* 26695 genome has a large paralogous gene family of the porin/adhesin-like outer membrane proteins with 32 members.[2] The family was further divided into two subfamilies of 20 Hop (*Helicobacter* outer protein) and 12 Hor (Hop-related) proteins (Table 3).[20] These proteins have an N-terminal conserved sequence A ↓ EX[D,N]G(↓ represents signal cutting site) and a C-terminal Hop consensus sequence with 7 to 8 amphipathic β-sheet structures. Intriguingly, the Hop/Hor family proteins are variable in size, but the C-terminal Hop consensus sequence is well conserved, suggesting its functional role. In fact, the HopE porin was suggested to form a β-barrel structure with 8 amphipathic β-sheets.[21] A loop extruding from such a structure may recognize a specific ligand on the host cell surface, and serves as an adhesin (BabA), or a signal transduction factor (OpiA) to induce IL-8.[22] The large gene family of outer membrane proteins and the presence of hypervariable region in the genes allow the bacteria to change the ability to adhere and recognize the host cells, and thus may change and/or enlarge the repertoire of target cells.

3) Lewis antigens

The LPS of *H. pylori* is less toxic than those of enteric bacteria, and its O antigen contains various Lewis-related blood group antigens(Table 3).[23] The genome of *H. pylori* has two genes for fucosyl-transferases, α3 *fucT* and α2 *fucT*, and the former contains C tracts.[2] Once the fucosylated LPS was thought to mimic the human Lewis X and Lewis Y blood group antigens, but it is now believed that Lewis X of LPS serves as an adhesin that stimulates infiltration of inflammatory cells through the action of Cag-PAI.[24] Thus the mutation to change the Lewis antigen of LPS may also contribute to change and/or enlarge the repertoire of target cells,

Conclusion

H. pylori is unusual among pathogenic bacteria in its ability to colonize in an environment of high acidity. By comparing the genome of *H. pylori* with that of a food-borne pathogen *C. jejuni*, we can find the strategies of *H. pylori* to colonize the stomach. A general strategy for acid tolerance is to produce ammonia that may be necessary and sufficient for the bacteria to colonize. The host immune system might not be effective to eradicate bacteria liveing in the thick mucous gel.

Two surface exposed molecules, outer membrane proteins and Lewis antigens of LPS, appear to be host-specific and may be involved in adherence, resulting to promote the action

of CagAPI and VacA. The adhesive molecules are hypervariable so that the bacteria can adapt to the host cell ligands during the course of infection. These properties may explain why more than 90% of Japanese population have been infected with cagPAI (+)-vacA (+) strains of *H. pylori*, While a few people develop severe diseases such as peptic ulcers and gastric cancer.

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