

WJG 20th Anniversary Special Issues (6): *Helicobacter pylori***Reduced genome size of *Helicobacter pylori* originating from East Asia**

Quan-Jiang Dong, Li-Li Wang, Zi-Bing Tian, Xin-Jun Yu, Sheng-Jiao Jia, Shi-Ying Xuan

Quan-Jiang Dong, Li-Li Wang, Zi-Bing Tian, Xin-Jun Yu, Sheng-Jiao Jia, Shi-Ying Xuan, Central Laboratories and Department of Gastroenterology, Qingdao Municipal Hospital, Qingdao 266000, Shandong Province, China

Zi-Bing Tian, Department of Gastroenterology, the Affiliated Hospital of Qingdao University, Qingdao 266000, Shandong Province, China

Author contributions: Dong QJ and Xuan SY designed the research; Dong QJ and Wang LL drafted the article; Yu XJ and Jia SJ analyzed data; Tian ZB revised the article; and Xuan SY approved the final version.

Correspondence to: Shi-Ying Xuan, MD, PhD, Central Laboratories and Department of Gastroenterology, Qingdao Municipal Hospital, Shinan District, Zhuhai Rd 18, Qingdao 266000, Shandong Province, China. jiangacer@126.com

Telephone: +86-532-88905289 Fax: +86-532-85968434

Received: September 28, 2013 Revised: November 19, 2013

Accepted: January 6, 2014

Published online: May 21, 2014

© 2014 Baishideng Publishing Group Inc. All rights reserved.

Key words: *Helicobacter pylori*; Genome; Mutation; Epidemiology; Recombination

Core tip: Comparative analysis of strains from different *Helicobacter pylori* (*H. pylori*) populations revealed that the genome size of strains from East Asia was reduced. In parallel with this, the number of protein coding genes and the guanine-cytosine content were decreased. The reduced genome of *H. pylori* from East Asia potentially contributes to the high prevalence of *H. pylori* in East Asia.

Dong QJ, Wang LL, Tian ZB, Yu XJ, Jia SJ, Xuan SY. Reduced genome size of *Helicobacter pylori* originating from East Asia. *World J Gastroenterol* 2014; 20(19): 5666-5671 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v20/i19/5666.htm> DOI: <http://dx.doi.org/10.3748/wjg.v20.i19.5666>

Abstract

Helicobacter pylori (*H. pylori*), a major pathogen colonizing the human stomach, shows great genetic variation. Comparative analysis of strains from different *H. pylori* populations revealed that the genome size of strains from East Asia decreased to 1.60 Mbp, which is significantly smaller than that from Europe or Africa. In parallel with the genome reduction, the number of protein coding genes was decreased, and the guanine-cytosine content was lowered to 38.9%. Elimination of non-essential genes by mutations is likely to be a major cause of the genome reduction. Bacteria with a small genome cost less energy. Thus, *H. pylori* strains from East Asia may have proliferation and growth advantages over those from Western countries. This could result in enhanced capacity of bacterial spreading. Therefore, the reduced genome size potentially contributes to the high prevalence of *H. pylori* in East Asia.

INTRODUCTION

Helicobacter pylori (*H. pylori*) is a major human pathogen causing chronic inflammation of gastric mucosa^[1]. Infection by this bacterium is associated with an increased risk for the development of peptic ulcer and gastric cancer^[2,3]. There is a dramatic geographical variation in the prevalence of the infection and *H. pylori* associated diseases. In Western countries, the infection rate is approximately 30%, while it is higher than 60% in Eastern countries^[4]. The incidence of gastric cancer is much lower as well in Western countries^[5].

It is believed that *H. pylori* has established its colonization in human stomachs 100000 years ago^[6]. With the human migration out of the Africa, the bacterium carried by its hosts traced the route of migration reaching Asia through India, and then into South East Asia

and Australia^[7]. It arrived at Europe through Turkey^[7]. The phylogenetic analysis of global strains of *H. pylori* reveals that the bacterium is subdivided into seven populations: hpAfrica1, hpAfrica2, hpEastAsia, hpEurope, hpNEAfrica, hpAsia2 and hpSahul^[6-8]. These populations generally correspond to their geographical origins. Up to date, hundreds of strains have been sequenced or being sequenced^[9-12]. The purpose of this review is to compare the genomic differences of *H. pylori* from different populations, and discuss their implications in the geographical variation of the prevalence of the infection.

VARIATION OF THE GENOME SIZE AND THE GUANINE-CYTOSINE CONTENT

To explore the genomic differences between *H. pylori* strains, we have determined the genome sequence of *H. pylori* strain D33 isolated from a Chinese patient with gastric cancer. Whole-genome sequencing was performed as described previously^[9,10]. Briefly, the raw reads were trimmed and filtered, yielding a total of 2364383 reads with an average length of 260 nucleotides. This corresponded to about 297-fold genome coverage. A total of 74 contigs were assembled. The annotation of the genome was performed using the Glimmer program. This whole genome shotgun project has been deposited in DDBJ/EMBL/GenBank under the accession number ANIO00000000 (The version described here is the first version, ANIO01000000.) The genome of *H. pylori* D33 had a size of about 1555730 bp, with a guanine-cytosine (GC) content of 38.96%. It possessed 1570 protein coding genes. The D33 genome carries a complete *cag* pathogenicity island. It has been found that *H. pylori* from East Asia had a massive decay of molybdenum-related genes^[13,14]. There are a total of 12 molybdenum-related genes including three genes encoding molybdenum transport proteins, eight genes involved in molybdenum cofactor synthesis, and only one molybdenum-containing enzyme^[15]. Most of them have been fragmented^[14]. Consistent with this, seven molybdenum-related genes of strain D33 were fragmented, while intact *modB*, *moaE*, *moeB*, *mogA* and *mobA* genes were present.

H. pylori D33 had a smaller genome compared to strains from Western countries. To identify the genomic size difference between different *H. pylori* populations, a total of 118 sequenced strains with required data available were assigned into different populations based on results of the phylogenetic analysis. Briefly, sequences of house-keeping genes *atpA*, *efp*, *mutY*, *ppa*, *trpC*, *ureI* and *jpbC* were extracted from strain D33 sequenced in this study, 117 sequenced strains available from GenBank (<http://www.ncbi.nlm.nih.gov>), and 77 strains available from multilocus sequence typing (MLST) database (<http://www.mlst.net>). The sequences of these genes were concatenated and a neighbor-joining tree was constructed (Figure 1). The strains from the MLST database was used as a standard to assign those sequenced strains

into different populations, since the population they belong to is known. These 118 sequenced strains could be assigned into five *H. pylori* populations, including hpEastAsia (30 strains), hpAsia2 (7 strains), hpEurope (39 strains), hpAfrica1 (40 strains) and hpAfrica2 (2 strains). No sequenced strains were assigned into the other two *H. pylori* populations, hpSahul and hpNEAfrica. The average genome size of *H. pylori* was about 1.64 Mbp. However, it is significantly different between the populations. Strains from hpEastAsia had a genome size of 1.60 Mbp on average (Table 1), which is significantly smaller than that from hpEurope ($P < 0.001$) or hpAfrica1 ($P < 0.001$) (Figure 2). The average genome size of strains from hpEastAsia was also smaller than that from hpAsia2 or hpAfrica2, although the difference is not statistically significant probably due to small sample size. These findings suggest that genome reduction occurs in strains of *H. pylori* from East Asia. As shown in Table 1, the genome of *H. pylori* decreased gradually with its spreading. In parallel to the genomic reduction, the number of protein coding genes was decreased ($R = 0.568$) (Figure 3), and the GC content of the genome declined gradually (Table 1).

MECHANISMS OF THE GENOMIC REDUCTION

Bacterial genome size is mainly determined by gains or losses of genes^[16]. Bacteria acquired new genes through duplication of genes or horizontal gene transfer^[17,18]. Genes may be deleted through mutations or recombination events^[19,20]. Genome reduction occurs when gene losses prevail gene gains. The life style of bacteria is a major factor contributing to the genome reduction. Bacterial species have a reduced genome when inhabiting in the host cells or in the extreme conditions^[21,22]. It is, however, unusual that a particular bacterium living in similar environments has reduction of genome. Our comparative analyses of genome found that genome reduction occurs in *H. pylori*. This is unexpected as the bacterium only colonizes in the human stomach. It is likely that variation in host genetic backgrounds dramatically influences physiochemical properties, immune and inflammatory responses of the stomach, leading to genomic alterations of *H. pylori*.

Genome reduction may be caused by the deletion of intergenic regions^[23]. This leads to an increased density of gene contents and thus a well compacted genome. It is most frequent, however, that genome reduces through removal of non-essential or redundant genes^[24]. Our comparative analysis found that the number of protein coding genes of *H. pylori* was closely associated with the genome size. The number decreased with the reduced size of the genome. This demonstrates that the genome reduction in *H. pylori* is caused essentially by deletion of genes. Compared to strains from Western countries, two groups of genes are frequently removed from those from Eastern countries^[14]. The first group consists of gene encoding outer membrane proteins, including *oipA-2*, *hopN*,

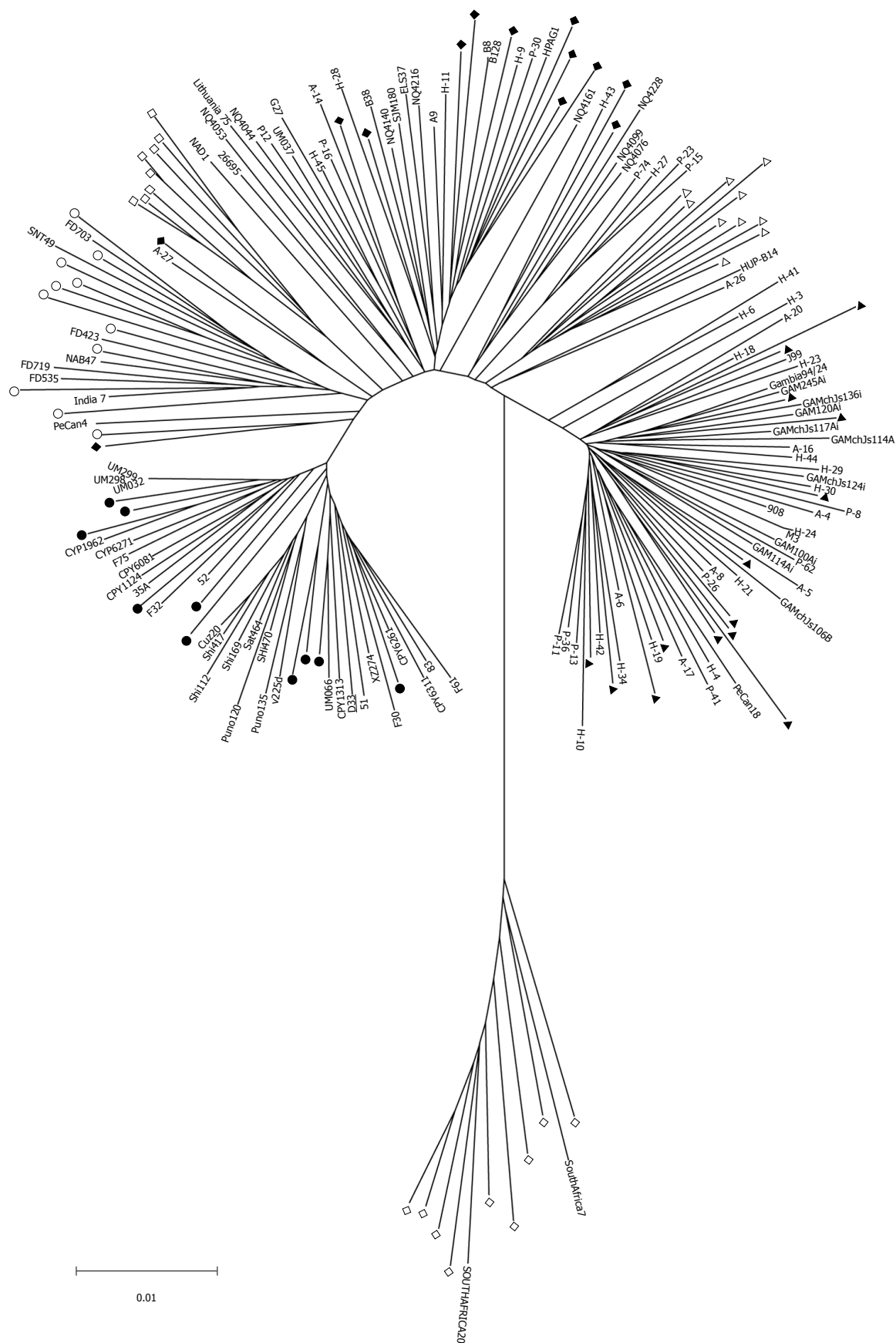


Figure 1 Neighbor-joining tree of 118 sequenced *Helicobacter pylori* strains and 77 strains from multilocus sequence typing database based on the phylogenetic analysis of seven house-keeping genes (*atpA*, *efp*, *mutY*, *ppa*, *trpC*, *urel* and *yphC*). The population to which those 77 strains belong is known. The tree was constructed with Mega 5.0 software. Scale bar indicates substitutions per nucleic acid residue (change/nucleotide site). Classification of population/ subpopulation was as previously described^{6,7}.

Table 1 Genome size, guanine-cytosine content and number of protein coding genes of *Helicobacter pylori* from different populations

Population	<i>n</i>	Size (Mbp)	GC content	Protein coding genes
hpEastAsia	30	1.6050 ± 0.0327	38.8167% ± 0.1206%	1548.8000 ± 74.3762
hpAsia2	7 ¹	1.6243 ± 0.0282	39.0286% ± 0.0756%	1559.5000 ± 57.2757
hpEurope	39 ¹	1.6467 ± 0.0391	38.9231% ± 0.1287%	1627.8684 ± 74.7411
hpAfrica1	40	1.6558 ± 0.0384	39.1800% ± 0.1137%	1660.0250 ± 58.9108
hpAfrica2	2	1.6500 ± 0.0424	38.5000% ± 0.1414%	1637.5000 ± 92.6310
Total	118	1.6379 ± 0.0417	38.9822% ± 0.1986%	1637.5000 ± 71.3128

¹The number of protein coding gene is only available in 2/7 strains from hpAsia2 and 38/39 strains from hpEurope. GC: Guanine-cytosine.

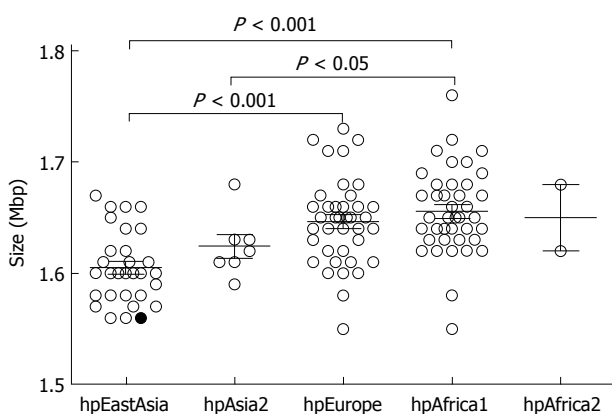


Figure 2 Genome size of strains from different *Helicobacter pylori* populations. Single factor analysis of variance was used for the statistical analysis. The average genome size was significantly different between *Helicobacter pylori* populations.

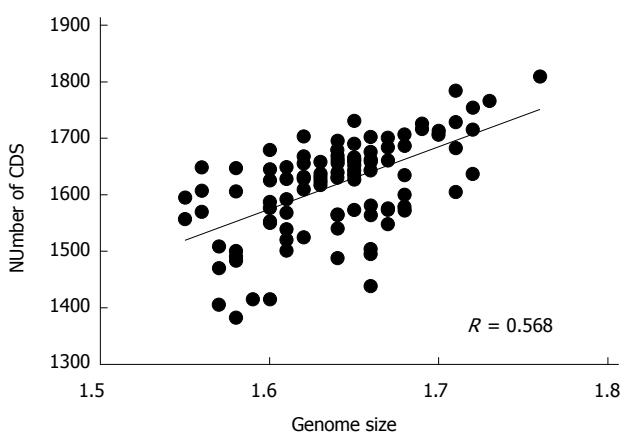


Figure 3 Correlation of the genome size of *Helicobacter pylori* with the number of protein coding genes. CDS: Coding sequence.

babC, *sabB*, *vacA2*, *hombB* and *hopQ*. All of these genes have their homologs and are variable in *H. pylori*^[425,26]. Therefore, their functions are most likely redundant. The other group of genes deleted in Eastern strains are composed of those involved in central metabolism. These genes include molybdenum-related genes as found in our sequenced strain D33 and other Japanese strains^[14], *acoE* encoding acetyl-CoA synthetase^[27], *tas* encoding aldo-ketoreductase involved in carbonyl metabolism and^[28] and *jhp0585* encoding a putative hydroxyl-isobutyrate dehydrogenase which degrades valine^[29]. Functions of

these genes could be complemented by their paralogs in the genome. These findings suggest that *H. pylori* strains originating from Eastern countries could eliminate non-essential genes from their genome, resulting in the reduction of genome.

Bacterial genome size is well correlated with the GC content^[30]. Events of G-C to adenosine-thymine (C-T) mutations are more common than those of C-T to A-G mutations^[31-33]. Thus, GC contents significantly influence the genome size. Our comparative analysis found that the GC content drops with the gradual reduction in the genome size of strains from hpAfrica 1 and 2, to hpEurope and hpAsia2, and finally hpEastAsia. This indicates that mutation is a major factor contributing to the genome reduction in *H. pylori*. Mutations and recombination events frequently occur in this bacterium^[34,35]. It has been estimated that the mutation rate is as high as 1.38×10^5 per site per year. The majority of genes deleted in Eastern strains are possibly caused by mutations. Recombination is also frequent in *H. pylori*^[35]. However, recombination usually has no influence on the GC content. Thus, it is unlikely to be a major reason causing the genome reduction.

REDUCED GENOME SIZE AND PREVALENCE OF *H. PYLORI* IN EAST ASIA

The prevalence of *H. pylori* shows considerable geographical variation^[36]. In Eastern countries, the infection is much higher compared to Western countries. Family transmission is a major route for the bacterium to spread^[37]. Spread of *H. pylori* is influenced by environmental conditions including socio-economic status, inhabiting conditions and hygiene levels^[38,39]. These factors may lead to an increased exposure to the bacterium. Spread of the bacterium is also influenced by the individual susceptibility determined by host genetic backgrounds. The single nucleotide polymorphism of the Toll-like receptor gene is closely associated with increased susceptibility to the infection^[40]. Bacterial factors related to the spread are, however, unclear.

Bacteria spend the majority of their energy for synthesis of proteins^[41]. Genome reduction with reduced number of protein coding genes, thus, requires less cost of energy^[42]. Furthermore, genome reduction would

decrease the energy cost for the maintenance of DNA structure and replication^[43]. These would promote bacterial growth and proliferation^[44]. The lowered energy cost for bacteria means less nutrient needs. Therefore, bacteria with a reduced genome have an increased capacity of adapting to unfavorable environments^[16]. Our comparative analysis found that strains from the hpEastAsia population have a smaller genome than those from hpEurope. Thus, *H. pylori* originating from East Asia could have an enhanced capacity of bacterial proliferation and growth, facilitating the spreading of the bacterium. It is conceivable that the reduced genome size contributes to the higher prevalence of *H. pylori* in East Asia.

CONCLUSION

H. pylori shows an unusual variation of the genome size. The reduced genome size of strains from East Asia potentially contributes to the high prevalence of the bacterium in the region. It is worth of further studies to investigate the influence of the genome reduction on the proliferation, growth and spread of *H. pylori*. This will benefit for the understanding of mechanism of the spreading and the prevention of the infection.

REFERENCES

- Blaser MJ. Helicobacter pylori and gastric diseases. *BMJ* 1998; **316**: 1507-1510 [PMID: 9582144 DOI: 10.1136/bmj.316.7143.1507]
- Blaser MJ, Atherton JC. Helicobacter pylori persistence: biology and disease. *J Clin Invest* 2004; **113**: 321-333 [PMID: 14755326 DOI: 10.1172/JCI20925]
- Peek RM, Crabtree JE. Helicobacter infection and gastric neoplasia. *J Pathol* 2006; **208**: 233-248 [PMID: 16362989 DOI: 10.1002/path.1868]
- Lehours P, Yilmaz O. Epidemiology of Helicobacter pylori infection. *Helicobacter* 2007; **12** Suppl 1: 1-3 [PMID: 17727452 DOI: 10.1111/j.1523-5378.2007.00541.x]
- Hu Y, Fang JY, Xiao SD. Can the incidence of gastric cancer be reduced in the new century? *J Dig Dis* 2013; **14**: 11-15 [PMID: 23134264 DOI: 10.1111/j.1751-2980.2012.00647.x]
- Moodley Y, Linz B, Yamaoka Y, Windsor HM, Breurec S, Wu JY, Maady A, Bernhöft S, Thiberge JM, Phuanukoonnon S, Jobb G, Siba P, Graham DY, Marshall BJ, Achtman M. The peopling of the Pacific from a bacterial perspective. *Science* 2009; **323**: 527-530 [PMID: 19164753 DOI: 10.1126/science.1166083]
- Falush D, Wirth T, Linz B, Pritchard JK, Stephens M, Kidd M, Blaser MJ, Graham DY, Vacher S, Perez-Perez GI, Yamaoka Y, Mégraud F, Otto K, Reichard U, Katzowitsch E, Wang X, Achtman M, Suerbaum S. Traces of human migrations in Helicobacter pylori populations. *Science* 2003; **299**: 1582-1585 [PMID: 12624269 DOI: 10.1126/science.1080857]
- Linz B, Balloux F, Moodley Y, Manica A, Liu H, Roumagnac P, Falush D, Stamer C, Prugnolle F, van der Merwe SW, Yamaoka Y, Graham DY, Perez-Trallero E, Wadstrom T, Suerbaum S, Achtman M. An African origin for the intimate association between humans and Helicobacter pylori. *Nature* 2007; **445**: 915-918 [PMID: 17287725 DOI: 10.1038/nature05562]
- Sheh A, Piazuelo MB, Wilson KT, Correa P, Fox JG. Draft Genome Sequences of Helicobacter pylori Strains Isolated from Regions of Low and High Gastric Cancer Risk in Colombia. *Genome Announc* 2013; **1**: pii: e00736-13 [PMID: 24051318 DOI: 10.1128/genomeA.00736-13]
- Armitano RI, Zerbetto De Palma G, Matteo MJ, Revale S, Romero S, Traglia GM, Catalano M. Draft Genome Sequences of Helicobacter pylori Strains HPARG63 and HPARG8G, Cultured from Patients with Chronic Gastritis and Gastric Ulcer Disease. *Genome Announc* 2013; **1**: pii: e00700-13 [PMID: 24009126 DOI: 10.1128/genomeA.00700-13]
- Behrens W, Bönig T, Suerbaum S, Josenhans C. Genome sequence of Helicobacter pylori hpEurope strain N6. *J Bacteriol* 2012; **194**: 3725-3726 [PMID: 22740658 DOI: 10.1128/JB.00386-12]
- Guo Y, Wang H, Li Y, Song Y, Chen C, Liao Y, Ren L, Guo C, Tong W, Shen W, Chen M, Mao X, Guo G, Zou Q. Genome of Helicobacter pylori strain XZ274, an isolate from a tibetan patient with gastric cancer in China. *J Bacteriol* 2012; **194**: 4146-4147 [PMID: 22815458 DOI: 10.1128/JB.00804-12]
- Dong QJ, Zhan SH, Wang LL, Xin YN, Jiang M, Xuan SY. Relatedness of Helicobacter pylori populations to gastric carcinogenesis. *World J Gastroenterol* 2012; **18**: 6571-6576 [PMID: 23236231 DOI: 10.3748/wjg.v18.i45.6571]
- Kawai M, Furuta Y, Yahara K, Tsuru T, Oshima K, Handa N, Takahashi N, Yoshida M, Azuma T, Hattori M, Uchiyama I, Kobayashi I. Evolution in an oncogenic bacterial species with extreme genome plasticity: Helicobacter pylori East Asian genomes. *BMC Microbiol* 2011; **11**: 104 [PMID: 21575176 DOI: 10.1186/1471-2180-11-104]
- Tomb JF, White O, Kerlavage AR, Clayton RA, Sutton GG, Fleischmann RD, Ketchum KA, Klenk HP, Gill S, Dougherty BA, Nelson K, Quackenbush J, Zhou L, Kirkness EF, Peterson S, Loftus B, Richardson D, Dodson R, Khalak HG, Glodek A, McKenney K, Fitzgerald LM, Lee N, Adams MD, Hickey EK, Berg DE, Gocayne JD, Utterback TR, Peterson JD, Kelley JM, Cotton MD, Weidman JM, Fujii C, Bowman C, Watthey L, Wallin E, Hayes WS, Borodovsky M, Karp PD, Smith HO, Fraser CM, Venter JC. The complete genome sequence of the gastric pathogen Helicobacter pylori. *Nature* 1997; **388**: 539-547 [PMID: 9252185 DOI: 10.1038/41483]
- Moran NA. Microbial minimalism: genome reduction in bacterial pathogens. *Cell* 2002; **108**: 583-586 [PMID: 11893328 DOI: 10.1016/S0092-8674(02)00665-7]
- Gressmann H, Linz B, Ghai R, Pleissner KP, Schlapbach R, Yamaoka Y, Kraft C, Suerbaum S, Meyer TF, Achtman M. Gain and loss of multiple genes during the evolution of Helicobacter pylori. *PLoS Genet* 2005; **1**: e43 [PMID: 16217547]
- Furuta Y, Kawai M, Yahara K, Takahashi N, Handa N, Tsuru T, Oshima K, Yoshida M, Azuma T, Hattori M, Uchiyama I, Kobayashi I. Birth and death of genes linked to chromosomal inversion. *Proc Natl Acad Sci USA* 2011; **108**: 1501-1506 [PMID: 21212362 DOI: 10.1073/pnas.1012579108]
- Björkholm B, Sjölund M, Falk PG, Berg OG, Engstrand L, Andersson DI. Mutation frequency and biological cost of antibiotic resistance in Helicobacter pylori. *Proc Natl Acad Sci USA* 2001; **98**: 14607-14612 [PMID: 11717398 DOI: 10.1073/pnas.241517298]
- Aras RA, Kang J, Tschumi AI, Harasaki Y, Blaser MJ. Extensive repetitive DNA facilitates prokaryotic genome plasticity. *Proc Natl Acad Sci USA* 2003; **100**: 13579-13584 [PMID: 14593200 DOI: 10.1073/pnas.1735481100]
- Kikuchi Y, Hosokawa T, Nikoh N, Meng XY, Kamagata Y, Fukatsu T. Host-symbiont co-speciation and reductive genome evolution in gut symbiotic bacteria of acanthosomatid stinkbugs. *BMC Biol* 2009; **7**: 2 [PMID: 19146674 DOI: 10.1186/1741-7007-7-2]
- Stepkowski T, Legocki AB. Reduction of bacterial genome size and expansion resulting from obligate intracellular lifestyle and adaptation to soil habitat. *Acta Biochim Pol* 2001; **48**: 367-381 [PMID: 11732608]
- Dufresne A, Garczarek L, Partensky F. Accelerated evolution associated with genome reduction in a free-living prokaryote. *Genome Biol* 2005; **6**: R14 [PMID: 15693943 DOI: 10.1186/gb-2005-6-r14]

- 10.1186/gb-2005-6-2-r14]
- 24 **Mendonça AG**, Alves RJ, Pereira-Leal JB. Loss of genetic redundancy in reductive genome evolution. *PLoS Comput Biol* 2011; **7**: e1001082 [PMID: 21379323 DOI: 10.1371/journal.pcbi.1001082]
 - 25 **Alm RA**, Bina J, Andrews BM, Doig P, Hancock RE, Trust TJ. Comparative genomics of *Helicobacter pylori*: analysis of the outer membrane protein families. *Infect Immun* 2000; **68**: 4155-4168 [PMID: 10858232 DOI: 10.1128/IAI.68.7.4155-4168.2000]
 - 26 **Oleastro M**, Cordeiro R, Ménard A, Yamaoka Y, Queiroz D, Mégraud F, Monteiro L. Allelic diversity and phylogeny of *hombB*, a novel co-virulence marker of *Helicobacter pylori*. *BMC Microbiol* 2009; **9**: 248 [PMID: 19954539 DOI: 10.1186/1471-2180-9-248]
 - 27 **Doig P**, de Jonge BL, Alm RA, Brown ED, Uria-Nickelsen M, Noonan B, Mills SD, Tummino P, Carmel G, Guild BC, Moir DT, Vovis GF, Trust TJ. *Helicobacter pylori* physiology predicted from genomic comparison of two strains. *Microbiol Mol Biol Rev* 1999; **63**: 675-707 [PMID: 10477312]
 - 28 **Kratzer R**, Wilson DK, Nidetzky B. Catalytic mechanism and substrate selectivity of aldo-keto reductases: insights from structure-function studies of *Candida tenuis* xylose reductase. *IUBMB Life* 2006; **58**: 499-507 [PMID: 17002977 DOI: 10.1080/15216540600818143]
 - 29 **Alm RA**, Ling LS, Moir DT, King BL, Brown ED, Doig PC, Smith DR, Noonan B, Guild BC, deJonge BL, Carmel G, Tummino PJ, Caruso A, Uria-Nickelsen M, Mills DM, Ives C, Gibson R, Merberg D, Mills SD, Jiang Q, Taylor DE, Vovis GF, Trust TJ. Genomic-sequence comparison of two unrelated isolates of the human gastric pathogen *Helicobacter pylori*. *Nature* 1999; **397**: 176-180 [PMID: 9923682 DOI: 10.1038/16495]
 - 30 **Musto H**, Naya H, Zavala A, Romero H, Alvarez-Valín F, Bernardi G. Genomic GC level, optimal growth temperature, and genome size in prokaryotes. *Biochem Biophys Res Commun* 2006; **347**: 1-3 [PMID: 16815305 DOI: 10.1016/j.bbrc.2006.06.054]
 - 31 **Hildebrand F**, Meyer A, Eyre-Walker A. Evidence of selection upon genomic GC-content in bacteria. *PLoS Genet* 2010; **6**: e1001107 [PMID: 20838593 DOI: 10.1371/journal.pgen.1001107]
 - 32 **Lind PA**, Andersson DI. Whole-genome mutational biases in bacteria. *Proc Natl Acad Sci USA* 2008; **105**: 17878-17883 [PMID: 19001264 DOI: 10.1073/pnas.0804445105]
 - 33 **Rocha EP**, Feil EJ. Mutational patterns cannot explain genome composition: Are there any neutral sites in the genomes of bacteria? *PLoS Genet* 2010; **6**: e1001104 [PMID: 20838590 DOI: 10.1371/journal.pgen.1001104]
 - 34 **Didelot X**, Nell S, Yang I, Woltemate S, van der Merwe S, Suerbaum S. Genomic evolution and transmission of *Helicobacter pylori* in two South African families. *Proc Natl Acad Sci USA* 2013; **110**: 13880-13885 [PMID: 23898187 DOI: 10.1073/pnas.1304681110]
 - 35 **Suerbaum S**, Smith JM, Bapumia K, Morelli G, Smith NH, Kunstmann E, Dyrek I, Achtman M. Free recombination within *Helicobacter pylori*. *Proc Natl Acad Sci USA* 1998; **95**: 12619-12624 [PMID: 9770535 DOI: 10.1073/pnas.95.21.12619]
 - 36 **Goh KL**, Chan WK, Shiota S, Yamaoka Y. Epidemiology of *Helicobacter pylori* infection and public health implications. *Helicobacter* 2011; **16** Suppl 1: 1-9 [PMID: 21896079 DOI: 10.1111/j.1523-5378.2011.00874.x]
 - 37 **Kivi M**, Tindberg Y. *Helicobacter pylori* occurrence and transmission: a family affair? *Scand J Infect Dis* 2006; **38**: 407-417 [PMID: 16798686 DOI: 10.1080/00365540600585131]
 - 38 **Tindberg Y**, Bengtsson C, Granath F, Blennow M, Nyrén O, Granström M. *Helicobacter pylori* infection in Swedish school children: lack of evidence of child-to-child transmission outside the family. *Gastroenterology* 2001; **121**: 310-316 [PMID: 11487540 DOI: 10.1053/gast.2001.26282]
 - 39 **Brown LM**. *Helicobacter pylori*: epidemiology and routes of transmission. *Epidemiol Rev* 2000; **22**: 283-297 [PMID: 11218379 DOI: 10.1093/oxfordjournals.epirev.a018040]
 - 40 **Mayerle J**, den Hoed CM, Schurmann C, Stolk L, Homuth G, Peters MJ, Capelle LG, Zimmermann K, Rivadeneira F, Gruska S, Völzke H, de Vries AC, Völker U, Teumer A, van Meurs JB, Steinmetz I, Nauck M, Ernst F, Weiss FU, Hofman A, Zenker M, Kroemer HK, Prokisch H, Uitterlinden AG, Lerch MM, Kuipers EJ. Identification of genetic loci associated with *Helicobacter pylori* serologic status. *JAMA* 2013; **309**: 1912-1920 [PMID: 23652523 DOI: 10.1001/jama.2013.4350]
 - 41 **Lane N**, Martin W. The energetics of genome complexity. *Nature* 2010; **467**: 929-934 [PMID: 20962839 DOI: 10.1038/nature09486]
 - 42 **Ranea JA**. Genome evolution: micro(be)-economics. *Heredity* (Edinb) 2006; **96**: 337-338 [PMID: 16249801 DOI: 10.1038/sj.hdy.6800766]
 - 43 **Ranea JA**, Grant A, Thornton JM, Orengo CA. Microeconomic principles explain an optimal genome size in bacteria. *Trends Genet* 2005; **21**: 21-25 [PMID: 15680509 DOI: 10.1016/j.tig.2004.11.014]
 - 44 **Giovannoni SJ**, Tripp HJ, Givan S, Podar M, Vergin KL, Baptista D, Bibbs L, Eads J, Richardson TH, Noordewier M, Rappé MS, Short JM, Carrington JC, Mathur EJ. Genome streamlining in a cosmopolitan oceanic bacterium. *Science* 2005; **309**: 1242-1245 [PMID: 16109880 DOI: 10.1126/science.1114057]

P- Reviewers: Franceschi F, Nakajima N

S- Editor: Gou SX **L- Editor:** Wang TQ **E- Editor:** Liu XM





Published by **Baishideng Publishing Group Inc**

8226 Regency Drive, Pleasanton, CA 94588, USA

Telephone: +1-925-223-8242

Fax: +1-925-223-8243

E-mail: bpgooffice@wjgnet.com

Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>

<http://www.wjgnet.com>



ISSN 1007-9327



9 771007 932045