

Short Note

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Short Note

# Detection of Two Phylogenetic Clusters in *Helicobacter pylori* 23S rRNA Phylogeny Suggests Emergence of Two Distinct Phylotype of the Species

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**Abstract:** Phylogenetic tree analysis is commonly used to understand the evolutionary distance between different species or strains of the same species. This work originally aimed to gain an understanding and visual depiction of the evolutionary distance in 23S rRNA gene of different *Helicobacter pylori* strains. Using an in-house MATLAB multiple sequence alignment and phylogenetic tree analysis software, the phylogenetic tree constructed for 23S rRNA gene reveals two major lineages with further subdivisions in each cluster. This suggests two major evolutionary forces driving the evolution of the 23S rRNA gene. In comparison, there is relatively short evolutionary distance in the 16S rRNA gene phylogenetic tree of *H. pylori*, suggesting lack of major evolutionary forces driving mutational changes of the gene. Overall, the data suggests that the large ribosome subunit which houses the 23S rRNA of *H. pylori* is undergoing significant conformational changes that may be a response to the frequent use of various antibiotics that target the ribosome of *H. pylori*.

**Keywords:** *Helicobacter pylori*; large ribosome subunit; small ribosome subunit; 16S rRNA gene; 23S rRNA gene

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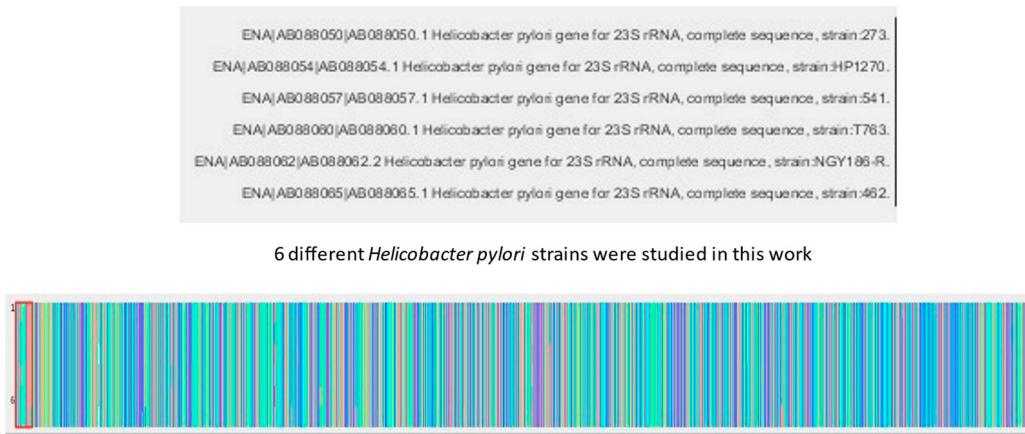
**Subject areas:** biochemistry, structural biology, molecular biology, evolutionary biology, bioinformatics,

In a prokaryotic cell, three ribosomal RNAs (16S rRNA, 23S rRNA, and 5S rRNA) dominate the encoding of phylogenetic information. Specifically, the 16S rRNA is most commonly used to identify various microorganisms due to the rich diversity in phylogeny sequence information encoded in the gene [1] [2] [3]. On the other hand, the 23S rRNA resides in the large subunit of the ribosome, and is less often used in understanding the phylogeny of microbial species [4] [5].

In particular, the 23S rRNA could be used to understand the evolution of the interactions between the 23S rRNA and ribosomal proteins that assemble the large ribosome subunit. Similarly, the 16S rRNA phylogeny tells the evolutionary story of interactions between the ribosomal proteins in the small ribosome subunit and 16S rRNA. This work attempts to use multiple sequence alignment and phylogenetic tree construction to understand the phylogeny of 23S rRNA and 16S rRNA in *Helicobacter pylori* to help tease out its future evolutionary trajectory.

Figure 1 shows the workflow of the study where 23S rRNA gene complete sequence of six *H. pylori* strains were downloaded from European Nucleotide Archive via the de Silva database portal. The sequences were processed via multiple sequence alignment in an in-house MATLAB multiple sequence alignment and phylogenetic tree construction software. Finally, the results of the multiple sequence alignment were used to construct the phylogenetic tree of 23S rRNA of *H. pylori*. The above procedures were repeated for 16S rRNA gene of *H. pylori*.

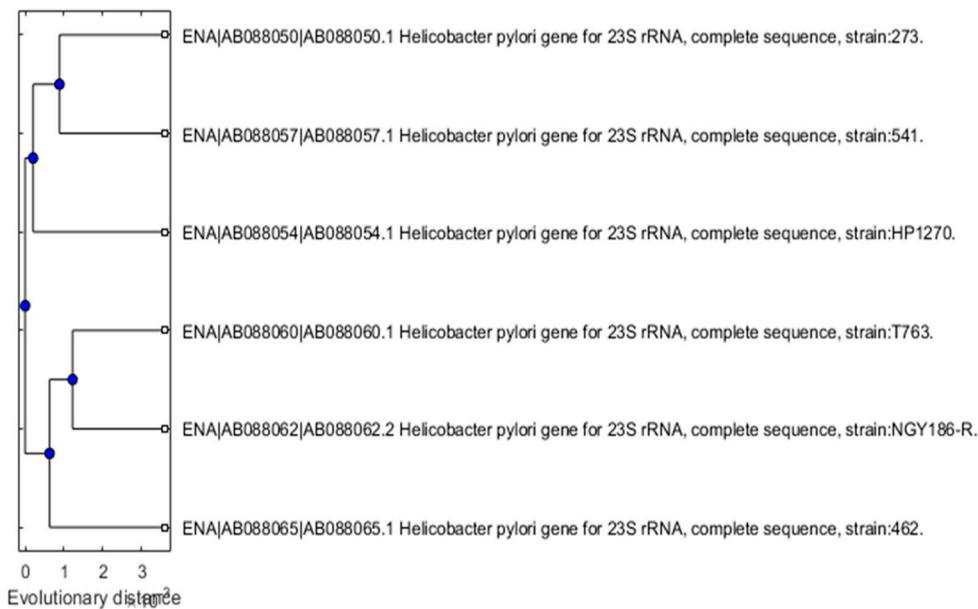
**Multiple sequence alignment and phylogeny of *Helicobacter pylori* strains at the level of 23S rRNA gene**



Multiple sequence alignment of 23S rRNA gene of different *H. pylori* strain shows high level of conservation in sequence of the gene

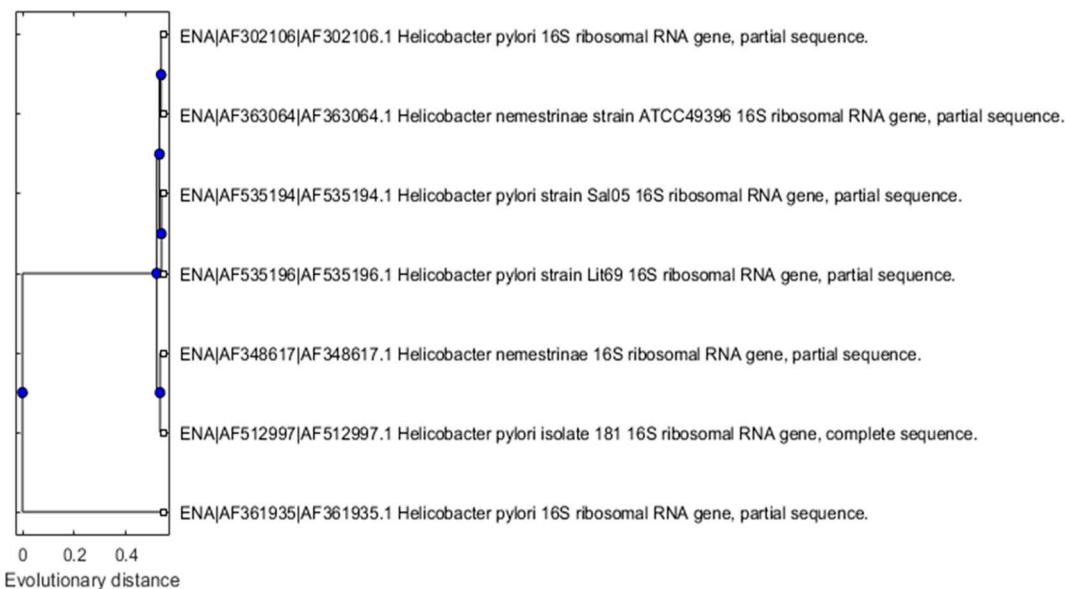
**Figure 1.** Identities of the 6 *Helicobacter pylori* strains studied in this work. Multiple sequence alignment of their 23S rRNA gene sequence show high level of conservation in sequence of the gene.

Phylogenetic tree analysis of 23S rRNA gene of different strains of *H. pylori* in Figure 2 reveals that the sequence has diverged into two main lineages with further subdivisions. This suggests that two major selection forces are acting on the evolutionary trajectories of the 23S rRNA gene, which portends a further where, at the level of 23S rRNA gene, two different functional phenotypes in ribosomes would emerge in *H. pylori* strains. Hence, one category of *H. pylori* may grow faster compared to the other due to higher functional status of their 23S rRNA gene.



**Figure 2.** Phylogenetic tree based on the 23S rRNA gene of *Helicobacter pylori*.

On the other hand, phylogenetic tree analysis in Figure 3 showed that except for one strain, all other strains of *H. pylori* show high level of similarity in their 16S rRNA gene, which indicates that this gene does not encode sufficient sequence divergence to explain the phylogeny of the different *H. pylori* strains. In addition, the evolutionary distance of the outlier strain is also not too far away from other *H. pylori* strains.



**Figure 3.** Phylogenetic tree based on the 16S rRNA gene of *Helicobacter pylori*.

Combining the data and implications of the two phylogenetic tree constructions for 23S rRNA and 16S rRNA gene of *H. pylori* reveals that the large ribosome subunit of *H. pylori* is evolving fairly quickly compared to the small ribosome subunit. One possible selection force mediating this quick evolutionary trajectory of the large ribosome subunit of *H. pylori* is the use of antibiotics targeting the ribosome of *H. pylori* that engenders the adaptation of the 23S rRNA gene sequence that affords its new binding site for different ribosomal proteins in new orientations. These changes could result in changes to the shape and conformation of the large ribosome subunit that negates the binding of antibiotics used. On the other hand, lack of evolution of the 16S rRNA gene of *H. pylori* meant that the shape and conformation of the small ribosome subunit of *H. pylori* remains stable.

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