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Molecular detection of *Helicobacter pylori* infection through *in silico* analysis of the *babA* Gene

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ABSTRACT

Introduction: The *babA* gene of *H. pylori* plays a critical role in pathogenesis and host interaction. This study investigates the phylogenetic relationships of *babA* among various *H. pylori* strains to uncover evolutionary lineages, adaptation mechanisms, and genetic diversity influenced by environmental and geographical factors.

Methods: A phylogenetic tree was constructed using the *babA* gene sequences from diverse *H. pylori* strains. Evolutionary distances were inferred, with specific focus on clustering, genetic divergence, and recombination events. Strain groupings and outliers were evaluated for shared ancestry and ecological adaptations.

Results: The phylogenetic tree revealed multiple clusters reflecting distinct evolutionary lineages. Early-branching strains such as AY744019.1 and KP339412.1 exhibited significant genetic divergence, suggesting unique evolutionary trajectories. Strains like MZ409791.1 and KP339411.1 showed close genetic similarity, implying recent common ancestry or shared ecological environments. Recurrent accessions, such as MZ409795.1, in separate clades indicated potential recombination events that warrants further molecular anlysis. The robust analytical framework underscored evolutionary pressures and highlighted strain-specific adaptations, including longer branches linked to increased virulence or host-specificity.

Discussion: The *babA* gene's genetic diversity underscores its role in the success of *H. pylori* as a pathogen. Variability enhances host immune evasion and adaptability to diverse environments. Future research integrating geographic and genetic data can provide deeper insights into *H. pylori* pathogenesis, guiding more precise diagnostic and therapeutic strategies for managing gastric diseases globally.

Keywords:

H. pylori, *babA*, in silico, infection diagnostic.

INTRODUCTION

Helicobacter pylori (*H. pylori*) is a gram-negative, rod-shaped, motile bacterium that was identified as a vigorous human pathogens. Identification of *H. pylori* as main cause of gastroduodenal disorders can play an inevitable role in efficient management of this type of diseases (1). In past, several biochemical tests include catalase, oxidase, and urease were the important approaches in determination of *H. pylori* as diagnostic method, even in clinical practice. Providing high sensitivity and specificity rate compared to traditional techniques like histology, an accurate PCR test using specific and sensitive primers to detect the *H. pylori* can revolutionized the management of such gastroduodenal disorders. Currently, genetic identification of *H. pylori* is mainly rely on specific genetic regions of *H. pylori* mainly named 16S *rRNA*, *ureA*, *ureB*, *cagA*, and *vacA*, which are mostly associated with the bacterial pathogenicity and survival in acidic gastric environments (2, 3). PCR assays can distinguish between different *H. pylori* strains, allowing genotyping of virulence factors like *cagA* and *vacA*, which are linked to more severe clinical outcomes. Advanced techniques, such as real-time PCR, nested PCR, and multiplex PCR, enable rapid and high-throughput detection, making them ideal for clinical and research settings. Given above evidences, new gene targets

using *in silico* analysis could be a useful approach to find better gene with higher specificity and sensitivity in accurate detection of this persistent infection. The *babA* in *H. pylori* encodes the blood group antigen-binding adhesin (BabA), a critical virulence factor that facilitates bacterial adhesion to the gastric epithelium (4). This adhesion is a key step in *H. pylori* colonization and is strongly associated with severe clinical outcomes, including peptic ulcers and gastric cancer (5-7). Variations and genetic recombination within the *babA* gene, such as *babA/B* chimeric formations, contribute to the pathogen's ability to adapt to diverse host environments and evade immune responses (8-10). Understanding the genetic structure, functionality, and evolutionary patterns of the *babA* gene is crucial for elucidating its role in gastric pathogenesis and developing targeted interventions. *In silico* analysis employs computational tools to investigate genetic sequences, structural features, and functional relationships of genes (11, 12). It is especially valuable for studying microbial pathogens like *H. pylori*, where high genetic variability and recombination rates complicate traditional experimental methods (13, 14). Bioinformatics enables researchers to predict protein structures, identify conserved motifs, and analyze phylogenetic relationships, providing valuable insights into gene evolution and functionality. Utilizing an *in silico* approach for *babA* allows efficient assessment of its genetic diversity, detection of mutations linked to increased virulence, and exploration of its interactions with host factors. This approach is also instrumental in drug discovery, facilitating the identification of molecular targets for therapeutic development. Additionally, *in silico* studies help prioritize experimental efforts by generating hypotheses about the gene's role in pathogenicity and resistance, significantly reducing the costs and time required for comprehensive investigations.

MATERIALS AND METHODS

Sequence Puzzling

The *babA* gene sequences of *H. pylori* were retrieved from the National Center for Biotechnology Information (NCBI) database (www.ncbi.nlm.nih.gov). Phylogenetic analysis was conducted to examine the evolutionary relationships and homology among the retrieved *babA* gene sequences. The sequences between 700-1500 bp approximately were selected. A total of 71 partial *babA* sequences from *H. pylori* strains were aligned to the reference sequence using the SeaView software. FASTA files obtained from NCBI were further analyzed as part of the study. To investigate the phylogenetic relationships, a maximum likelihood analysis was performed using SeaView's sequence puzzle method. This feature enabled the construction of probability-based phylogenetic trees, effectively capturing the evolutionary relationships among the *babA* sequences.

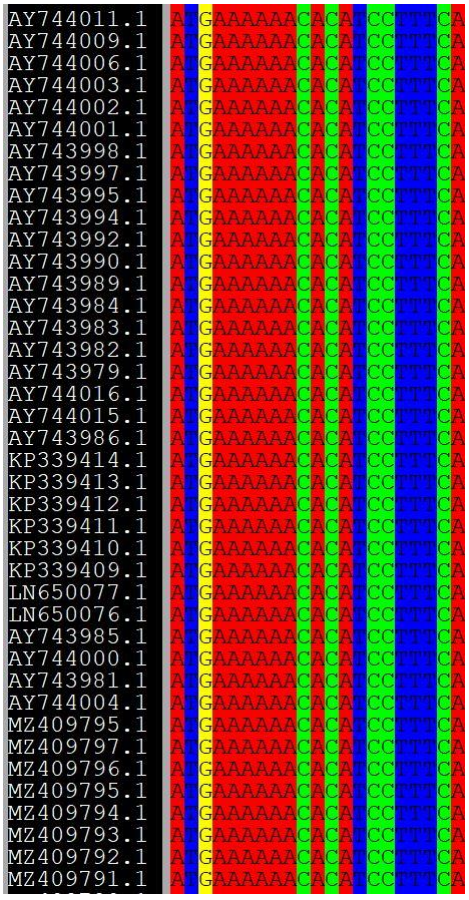

Figure 1: Aligned version of available 71 *babA* sequences

RESULTS

The phylogenetic tree for the *babA* gene in *H. pylori* elucidates the evolutionary relationships among various strains based on genetic similarity (see Figure 1, 2). The phylogenetic tree reveals multiple clusters, indicating distinct evolutionary lineages within the *babA* gene. Our sequence puzzling reveals that there is a clearly conserved region in this sequence that may be the target for designation of a specific location for primers and a new developed PCR. Certain strains, such as AY744019.1 and KP339412.1, occupy early-branching positions in the tree, suggesting

significant genetic divergence. These outlier strains may represent unique evolutionary routes. Strains like MZ409791.1 and KP339411.1 (Figure 2) group closely together, implying a recent common ancestry or high genetic homogeneity. This proximity may indicate shared ecological niches or hosts. The repeated presence of specific accessions (e.g., MZ409795.1) in separate branches suggests potential recombination events or sequencing redundancies within the *babA* gene. The tree demonstrates several divergent lineages, with early-branching strains such as AY744019.1 and KP339412.1 pointing to unique evolutionary pathways potentially shaped by geography or host specificity. Clusters with minimal divergence, such as MZ409791.1 and KP339411.1, highlight strains with recent shared ancestry or overlapping ecological environments. The appearance of accessions like MZ409795.1 in multiple clades suggests recombination events that likely enhance the adaptability and survival of *H. pylori* in diverse hosts.

DISCUSSION

The application of *in silico* analysis to decipher closely related sequences represents a novel approach for exploring bacterial genomes. This method enables the identification of conserved regions or highly mutated sequences, providing critical insights into genomic variation (15, 16). The phylogenetic evaluation of the *babA* gene in *Helicobacter pylori* offers valuable perspectives on the evolutionary relationships among various strains, shedding light on significant genetic diversifications and potential drivers of pathogenicity (17). Our phylogenetic tree shows clusters that mirror evolutionary lineages in the *babA* gene. These clusters suggest that geographical and environmental factors may influence the genetic diversity observed in *H. pylori*. Early-branching lineages, such as AY744019.1 and KP339412.1, exhibit significant genetic divergence, indicating distinct evolutionary pathways likely shaped by ecological niches or host interactions. Our study revealed that strains such as MZ409791.1 and KP339411.1 exhibit minimal genetic divergence, indicating a recent common ancestry or shared environmental conditions—a novel finding that has not been previously reported. Additionally, the repeated occurrence of accessions such as MZ409795.1 in separate clades suggests possible recombination events. These events likely contribute to the bacterium's adaptability and its ability to persist in diverse host environments. Strain-specific adaptations, indicated by longer branch lengths in certain atypical strains, imply higher mutation rates or selective pressures that may enhance virulence or host specificity. Genetic variation in the *babA* gene underscores its critical role in enabling *H. pylori* to establish persistent colonization and contribute to the development of gastric diseases. These variations enhance the bacterium's ability to evade the host immune response, increasing its survival and adaptability as a pathogen in the harsh environment of the human acidic stomach (18, 19). This pioneering in silico study underscores the immense potential of computational approaches in deciphering the complexity of the *babA* gene. Despite being the first of its kind, the findings highlight that the *babA* gene fragment is an excellent candidate for further research, including clinical sample investigations. These analyses strongly advocate for utilizing the relatively conserved sequences of the *babA* gene to develop and implement a rapid and reliable PCR-based diagnostic method for *H. pylori* infection. By seamlessly connecting genetic data with clinical and geographical trends, this study lays a solid foundation for groundbreaking strategies to diagnose and combat *H. pylori*. Ultimately, such advancements hold the promise of significantly reducing the global burden of gastroduodenal disorders associated with this pathogen.

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CONFLICT OF INTEREST

None of the authors declare potential conflict of interest.

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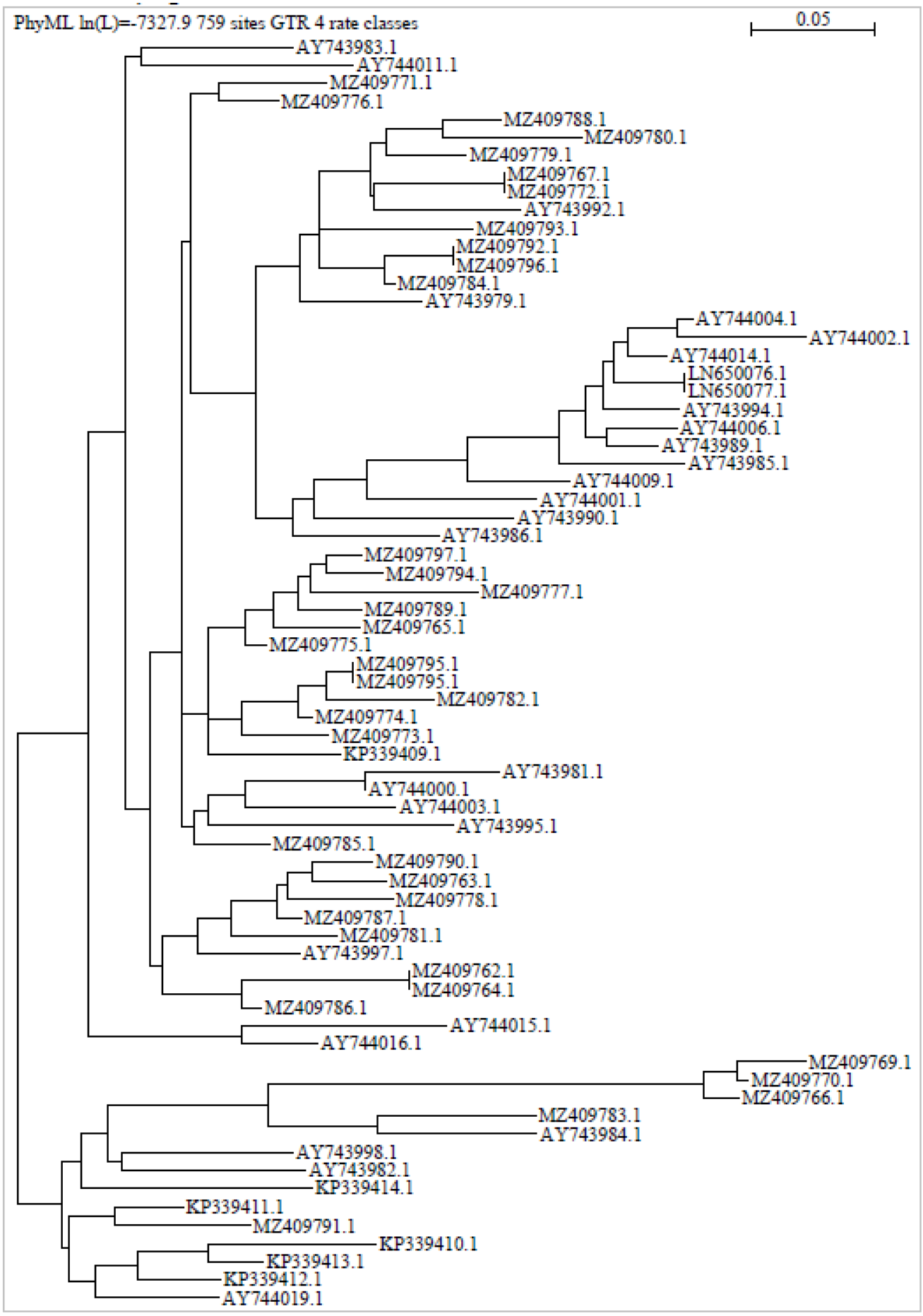

Figure 2: divergence of various *babA* sequences in phylogenetic tree

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