

Living positive:

Comparative Bioinformatics of the NhaA Antiporter in *H. pylori*

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ABSTRACT

This case study analyzes an unknown DNA fragment from *Helicobacter pylori* to explore its genetic composition and biological relevance. Sequence analyses identified conserved genes involved in acid adaptation, including the Na⁺/H⁺ antiporter (NhaA), which supports pH regulation and survival in acidic environments. Protein and phylogenetic analyses revealed structural differences from *Escherichia coli*, suggesting evolutionary adaptation to gastric conditions. An in silico pUC19 plasmid incorporating the NhaA gene was designed to model expression and functional studies. These results provide a framework for future experimental validation and give an insight for potential applications.

INTRODUCTION

Helicobacter pylori is a spiral-shaped, gram-negative bacterium that colonizes the human stomach and infects nearly 44% of the global adult population (Chen et al., 2024). *H. pylori* infection is a major public health concern, strongly associated with gastritis, peptic ulcer disease, and gastric cancer (Kao et al. 2016). Transmission commonly occurs during childhood through oral-oral or fecal-oral routes, particularly in regions with poor sanitation and overcrowding (Li et al., 2023). In this case study, we examine a *Helicobacter pylori* DNA fragment to identify its encoded proteins and explore the characteristics and evolutionary relationships of the Na⁺/H⁺ antiporter.

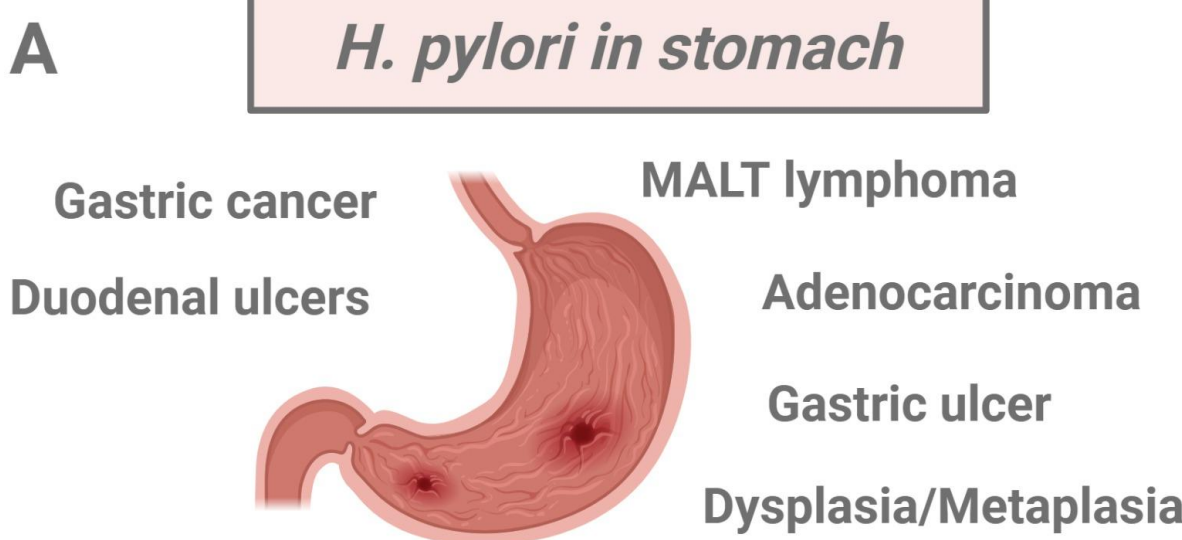
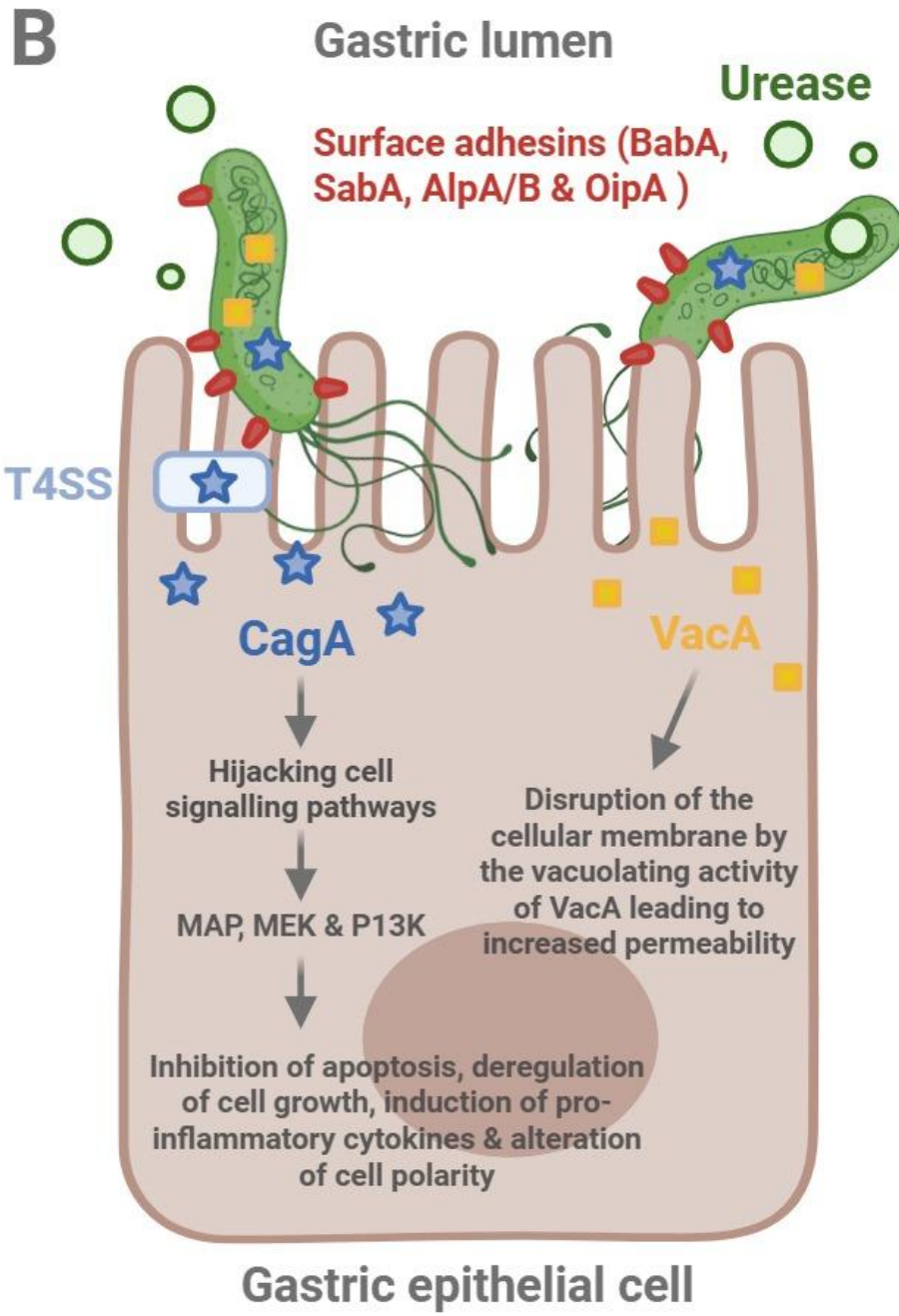


Figure 1 | *Helicobacter pylori* (*H. pylori*) pathogenesis and associated gastric diseases: A. Diagram showing *Helicobacter pylori*-associated gastric disorders, including duodenal and gastric ulcers, adenocarcinoma, MALT lymphoma, and dysplasia/metaplasia arising from chronic infection (Ali & Alhussani, 2024). B. Overview of *Helicobacter pylori* interaction with gastric epithelial cells, highlighting key virulence factors: CagA, VacA, and surface adhesins, affecting host signaling, membrane integrity, and inflammation (Created with Biorender).

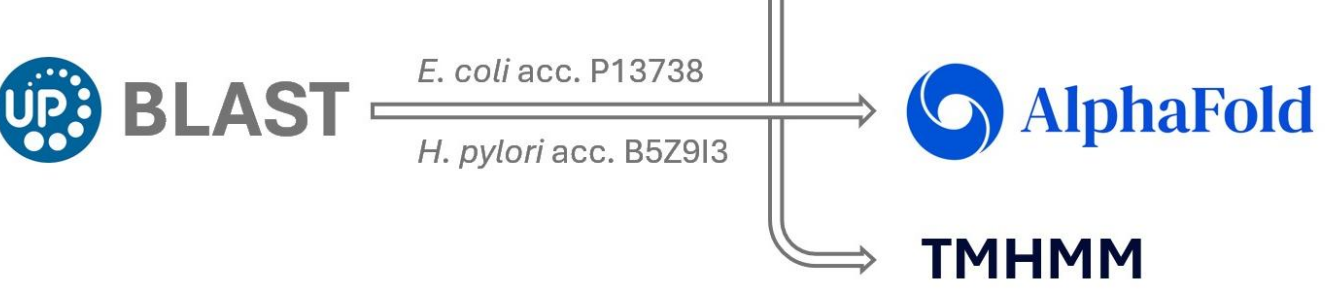


METHODS

A - Gene Annotation



B - Structure Comparison



C - Phylogenetic Tree



D - Plasmid Design

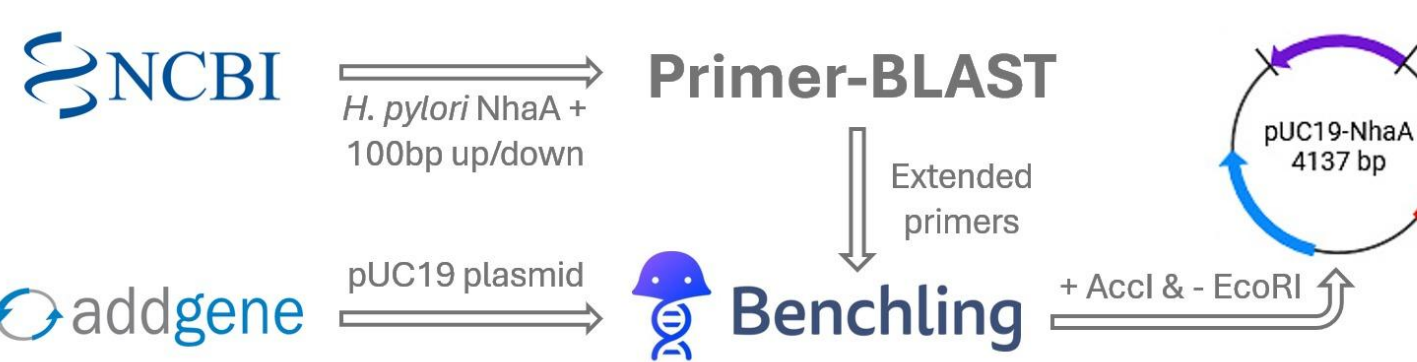


Figure 2 | Overview of the bioinformatics case study workflow: A. Gene annotation was performed with KBase and BLASTp to identify coding sequences and their function. B. Protein structures were predicted and compared through BLAST, SWISS-MODEL, AlphaFold, and TMHMM. C. Phylogenetic relationships among NhaA homologs were inferred with Clustal Omega and visualized in iTOL. D. Finally, *H. pylori* NhaA was cloned into the pUC19 vector using *AccI* and *EcoRI* for plasmid construction.

RESULTS

Table 1 | Genomic features identified within the *Helicobacter pylori* DNA fragment using Kbase annotation tools.

Function	Start	Strand	Length
Transcription-repair-coupling factor	3,540	-	3000
Bactofilin family protein	3,858	-	315
Peptidoglycan DD-metalloendopeptidase Csd1	4,781	-	909
M23 family metalloproteinase	5,743	-	927
Dihydrofolate synthase/folypolyglutamate synthase	6,918	-	1176
LPS assembly lipoprotein LptE	7,420	-	513
Leucine-tRNA ligase	9,837	-	2421
Protein translocase subunit SecF	11,166	-	972
Protein translocase subunit SecD	12,752	-	1578
Sec translocon accessory complex subunit YajC	13,045	-	294
Na ⁺ /H ⁺ antiporter NhaA	14,409	-	1317
RecBCD enzyme subunit RecB	17,228	-	2796
30S ribosomal protein S2	17,455	-	843
Elongation factor Ts	18,297	+	1068

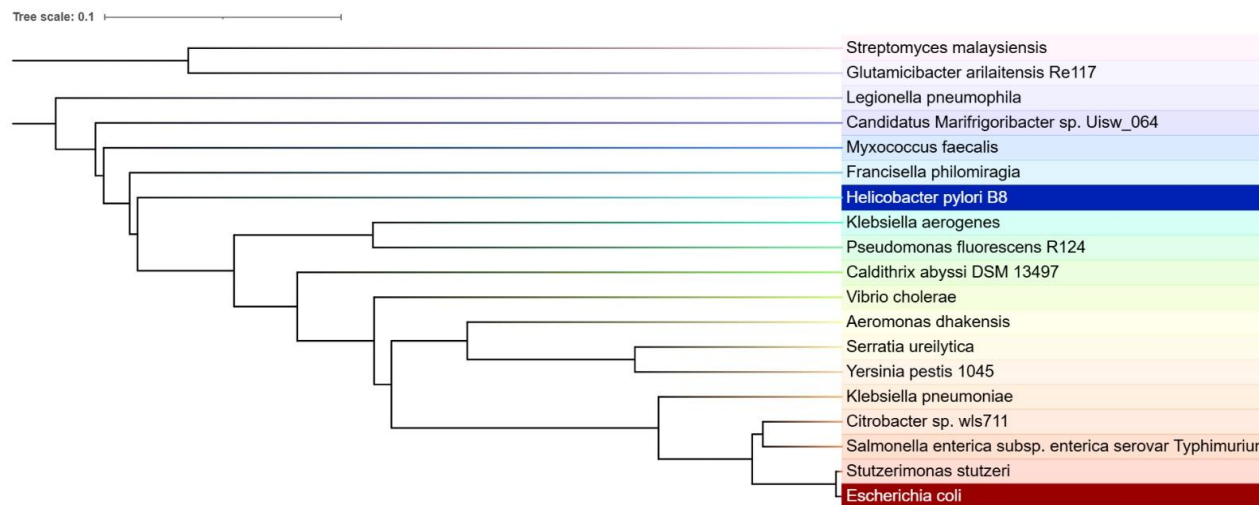


Figure 3 | Phylogenetic tree of NhaA proteins in different species. *H. pylori*, *E. coli* and 17 other random NCBI search hits were used to create the tree. The branch lengths represent evolutionary distance, indicated by a scale on top of the tree. Different colours separate the different species. The proteins of interest, from *H. pylori* and *E. coli*, are given a bolder colour.

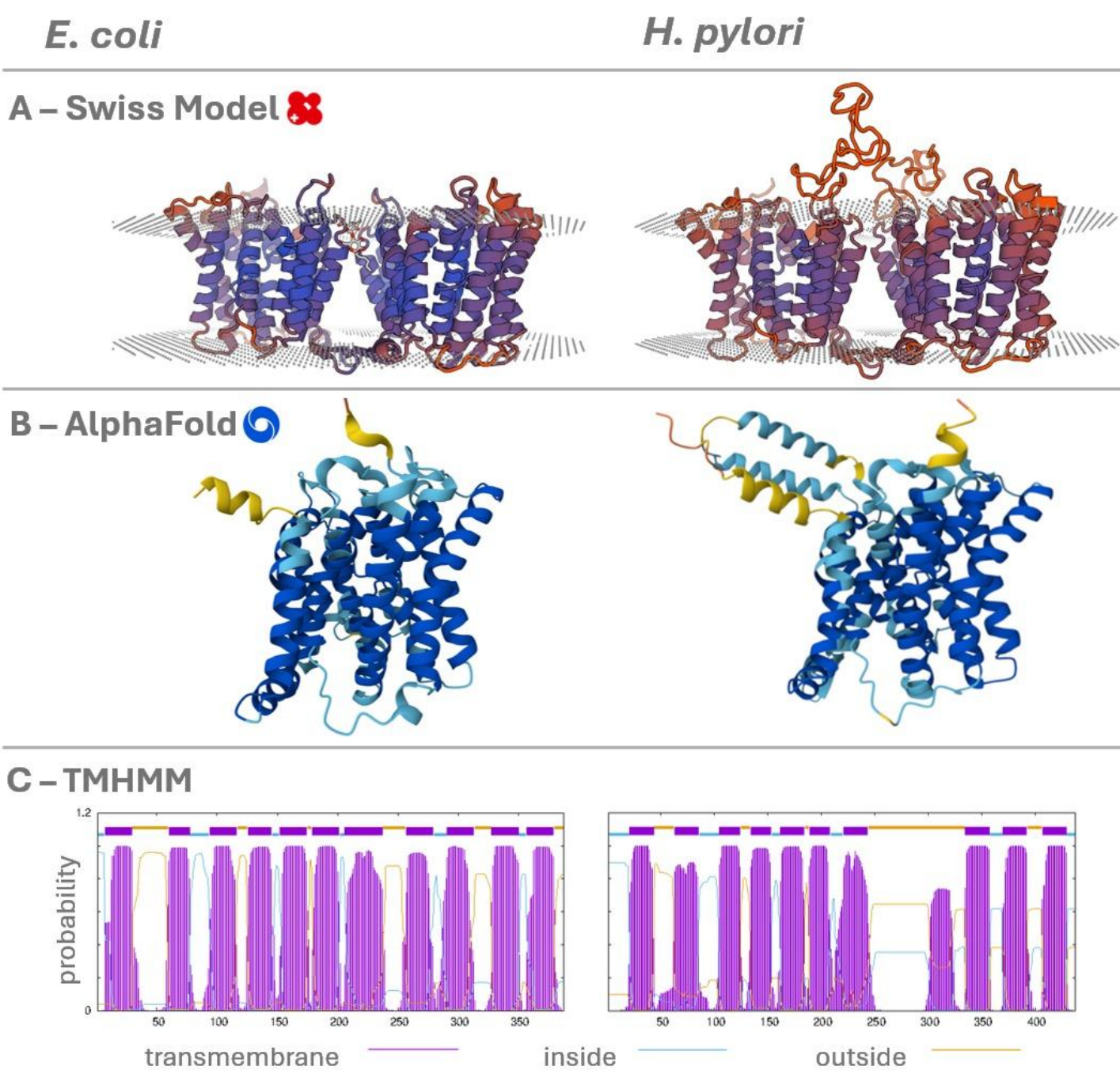


Figure 4 | Structural comparison of NhaA in *E. coli* and *H. pylori*. A. Swiss-Model predicted 3D structures of dimerized NhaA with membrane visualization (grey) B. AlphaFold-predicted NhaA structures colored by model confidence (pLDDT): dark blue (very high) to red (very low). C. TMHMM topology (fuchsia: transmembrane; blue: inside; orange: outside).

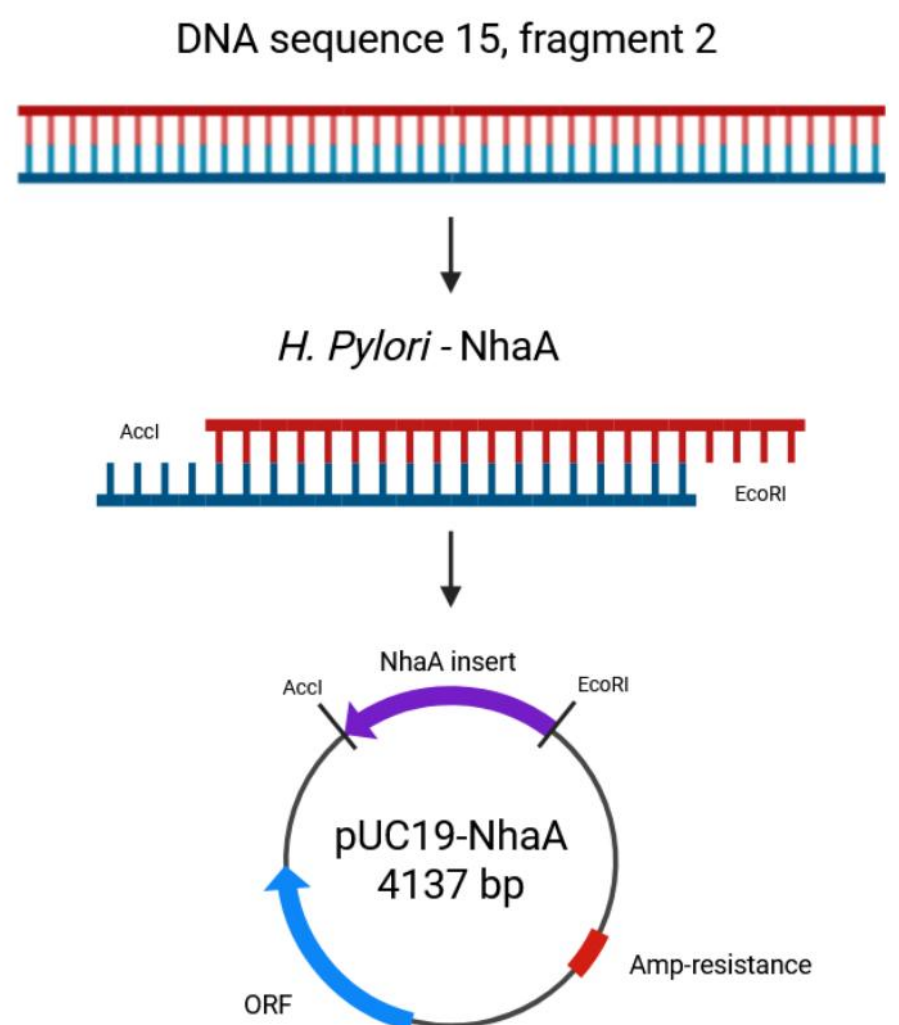


Figure 5 | pUC19 plasmid with *Helicobacter Pylori* pH-dependent sodium/proton antiporter insert. Circular map of the 4137 bp recombinant pUC19 plasmid showing key elements, including the origin of replication (ori), ampicillin resistance gene (ampR), and multiple cloning site (MCS). The pH-dependent sodium/proton antiporter gene from *Helicobacter pylori* was inserted at the lac-operon site using the *AccI* and *EcoRI* restriction enzymes (Created with BioRender).

CONCLUSIONS

The genomic fragment was identified as *Helicobacter pylori* through BLAST and gene annotation, revealing genes such as the Na⁺/H⁺ antiporter (HpNhaA), Sec translocon components, and DNA repair enzymes. Structural comparison showed that HpNhaA contains an inserted extracellular domain (aa 254–294) absent in *E. coli*, likely linked to its function across a broader pH range (6.5–8.5) in the acidic gastric niche (Calinescu et al. 2014). Phylogenetic analysis confirmed its evolutionary divergence from neutral-pH bacteria, reflecting long-term host adaptation (Atherton et al. 2009). A plasmid design was proposed to express *H. pylori* NhaA in *E. coli* for functional validation of these acid-tolerance features (Marcus et al. 2018).

Potential applications:

- Probiotics – so they may pass the gastric digestion
- Agriculture – so they may adapt to the lowering pH levels in our agricultural soils
- Biotech – so they may survive harsher conditions in bioreactors

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