Streszczenie w języku angielskim

The increasing antibiotic resistance of *Helicobacter pylori* (*H. pylori*) significantly reduces the effectiveness of eradication therapies and highlights the urgent need to identify novel molecular targets for potential therapeutic substances. This study aimed to characterize selected components of the *H. pylori* RNA degradosome - polynucleotide phosphorylase (PNPase), the RNA helicase RhpA, and the protein PapS with particular emphasis on evaluating PNPase as a potential therapeutic target. Additionally, the function of PapS in RNA metabolism and its interaction with RhpA were investigated.

Recombinant PNPase, RhpA, and PapS were successfully purified. Enzymatic assays for PNPase activity, including RNA polymerization and degradation reactions, were optimized to enable downstream inhibitor screening. The spatial structure of PNPase was resolved, followed by structural-functional analyses and interaction studies of the RhpA-PapS complex. PNPase was shown to form a homotrimeric assembly and to retain enzymatic activity under conditions compatible with high-throughput screening applications. PapS did not exhibit polyadenylation activity toward single-stranded RNA; however, it catalyzed the sequential addition of the 3′-terminal CCA sequence to tRNA, consistent with its role as a CCA-adding enzyme. A transient and moderate interaction between PapS and RhpA was detected.

Transcriptomic analysis revealed limited changes in gene expression in the *H. pylori* G27 Δ*pnp* and Δ*papS* strains, whereas deletion of *rhpA* resulted in extensive transcriptomic remodeling. Although *pnp* deletion did not abolish bacterial viability, it markedly impaired adaptive capacity. The Δ*pnp* strain displayed reduced tolerance to temperature stress (slower growth at 39°C and decreased survival at 4°C), increased sensitivity to UV-C radiation and oxidative stress, reduced motility and urease as well as catalase activity, while enhanced ability to biofilm complex formation, which needs further study to elucidate the potential mechanism.

A four-stage screening strategy for PNPase inhibitors was implemented, integrating *in vitro* enzymatic assays, qualitative selection steps, and evaluation using bacterial cells. From a library of 8,562 compounds, 23 candidates were selected for further validation. Among them, niclosamide exhibited the most pronounced PNP-dependent activity profile.

Collectively, these findings indicate that although *H. pylori* PNPase is not essential for viability, it represents a central regulatory node in RNA metabolism that governs stress response and adaptive phenotypes. Therefore, PNPase constitutes a rational and promising target for the development of novel antibacterial strategies.

Keywords: *Helicobacter pylori*, RNA degradosome, PNPase, RhpA, PapS

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