Conference track «2.1 Bioengineering and Physicochemical Biology»

Search for DNA gyrase activity sites in the genome of the thermophilic bacterium *Thermus thermophilus*

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The aim of this work is to create a genome-wide map of DNA gyrase activity sites in the thermophilic bacterium *Thermus thermophilus* and to assess the effect of the prokaryotic Argonaute protein (TtAgo) on its distribution upon exposure to the antibiotic ciprofloxacin.

DNA gyrase is the only type II topoisomerase in *T. thermophilus*, making it a key enzyme for maintaining DNA topology; however, its genome-wide activity has not been previously studied [2]. It has been shown that the TtAgo protein increases the resistance of *T. thermophilus* to ciprofloxacin (Cfx), presumably by participating in the processing of double-strand breaks induced by gyrase inhibition and in the completion of replication [1]. The molecular mechanism of this interaction is unknown, and understanding it requires a comparison of gyrase activity in the presence and absence of TtAgo.

To address this problem, we are constructing recombinant strains of *T. thermophilus* HB27 using plasmids carrying recombination cassettes and spacers of the endogenous I-C CRISPR-Cas system to reduce the population of cells that have not undergone recombination. Using this method, we obtained a strain with a chromosomal copy of the gyrase gene (*gyrA*) carrying a C-terminal SPA tag, which will allow immunoprecipitation of the enzyme. To study the role of TtAgo, a plasmid carrying a spacer targeting the *ago* gene was created, as well as recombination cassettes aimed at obtaining a knockout strain (Δago). The minimum inhibitory concentration (MIC) of ciprofloxacin (Cfx) for the parent strain was also determined, which sets the conditions for subsequent gyrase inhibition. The final stage will be the application of the Topo-Seq method to the obtained strains. This technique, based on the immunoprecipitation of covalent gyrase-DNA complexes stabilized by Cfx, will allow nucleotide-resolution determination of gyrase cleavage sites across the entire genome and comparison of their distribution in wild-type and Δago strains.

The results are expected to provide the first visualization of gyrase activity distribution in the *T. thermophilus* genome and to establish how TtAgo modulates this activity, revealing a novel antibiotic resistance mechanism associated with the prokaryotic immune system.

Источники и литература

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2) Sutormin D., Galivondzhyan A., Musharova O. et al. Interaction between transcribing RNA polymerase and topoisomerase I prevents R-loop formation in E. coli. *Nature Communications*. 2022. Vol. 13. P. 4524.