Mgr inż. Klementyna Marciniak

Abstract

Identification of regulatory RNAs in methicillin-resistant *Staphyloccocus aureus* (MRSA) using high-throughput sequencing (Term-seq).

Methicillin-resistant Staphylococcus aureus (MRSA) strains pose a significant threat as common causes of bacterial infections in hospitals, often resistant to available antibiotics such as daptomycin, vancomycin, and linezolid. The continuous emergence of new MRSA isolates with no effective treatment options underscores the critical need to explore novel strategies to combat antibiotic-resistant strains, focusing on innovative mechanisms of action. RNA regulatory mechanisms, including attenuation and riboswitches, have emerged as promising targets for alternative therapies against drug-resistant bacteria. These mechanisms rely on structural changes in functional RNA regions, such as the ribosome binding site (RBS) and termination hairpins. Recent research has highlighted extensive alterations in non-coding RNA expression in response to antibiotic-induced stress in bacteria.

This doctoral research delves into the RNA-dependent regulatory mechanisms in MRSA during vancomycin-induced antibiotic stress, with a particular focus on identifying key components of the RNA regulatory network, namely riboswitches. We determined the threshold concentration of vancomycin at which noticeable adaptation processes occur and employed next-generation sequencing using the Term-seq method. This sequencing approach, designed to analyze regulatory mechanisms based on transcription termination events, involves adapter ligation to all natural 3' ends of mRNA, enabling the identification of full-length and truncated transcripts resulting from riboswitch activity. A comparative analysis of the transcriptomes from MRSA control cultures and those treated with 2 mg/l of vancomycin revealed seven potential riboswitch candidates. Secondary structure predictions were conducted for each identified regulator to validate their significance, followed by an analysis of the genes under their control.

During adaptation stress to vancomycin, the activity of potential riboswitches modulating the expression of seven genes was confirmed, including Peptide ABC transporter, ATP – binding protein (SAOUHSC_00167), Multiple sugar-binding transport ATP-binding protein (SAOUHSC_00175), Monovalent cation/H antiporter subunit D (SAOUHSC_00886), Homoserine kinase (SAOUHSC_01322), Anthranilate Synthase Component I (SAOUHSC_01366), Bifunctional autolysin (SAOUHSC_02023) and Dimethyladenosine transferase (SAOUHSC_00464). According to current knowledge, all of these genes are

implicated in the development of a phenotype characterized by reduced sensitivity to vancomycin.

Subsequently, we conducted biochemical verification of these findings using the PTT-quant method, which allows for the analysis of the transcriptional activity of genes potentially regulated by riboswitches and the absolute quantification of shortened and full-length transcripts. Our results confirmed the functionality of riboswitches under vancomycin stress for four out of the seven candidates. The induction coefficients for potential riboswitches located in the 5'UTR of mRNA were as follows: 2.01 for Peptide ABC transporter, ATP – binding protein (SAOUHSC_00167), 1.09 for Monovalent cation/H antiporter subunit D (SAOUHSC_00886), 3.18 for Homoserine kinase (SAOUHSC_01322), and 2.06 for Dimethyladenosine Transferase (SAOUHSC_00464). These findings highlight the significance of pursuing further research to leverage these identified regulatory mechanisms as novel targets in the development of alternative antibiotic therapies.