Characterization and crystallization of *Staphylococcus aureus* acetyl-coenzyme A synthetase

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Introduction: The rapid development of antimicrobial resistance is a worldwide problem, endangering the effectiveness of current antibiotics and chemotherapeutic treatments. *Staphylococcus aureus* is a pathogen of greatest concern because of its ineradicable virulence, its ability to adapt to different environmental conditions and its capacity to cause a diverse range of life-threatening infections. New, highly effective antimicrobials are therefore needed. *N*-substituted pantothenamides (PanAms) have been reported to target the bacterial CoA biosynthesis pathway, a pathway crucial for the survival of *S. aureus*. PanAms have been shown to inhibit fatty acid biosynthesis as the essential cofactor is being replaced by CoA antimetabolites formed form the PanAm itself, which then does not have the ability to activate acyl carrier proteins needed for fatty acid biosynthesis. However, Acetyl-CoA formation also relies on CoA and the effect of the CoA antimetabolites have not been investigated acetyl- CoA synthetase (*Sa*Acs), the enzyme responsible for Acetyl-CoA production. We aimed to express, purify and crystalize *Sa*Acs, as well as kinetically characterize *Sa*Acs to evaluate it as a valid target for PanAms.

Methods: Different expression conditions and affinity chromatography were utilized to recombinantly express and purify *Sa*Acs. Next, the activity of the purified *Sa*Acs fractions was investigated using different techniques like HPLC and spectroscopy. Purified *Sa*Acs were screened with commercially available crystallography conditions for crystal formation and diffraction.

Results: *Sa*Acs was successfully expressed and partially purified. Western Blot analysis confirmed a mixture of two *Sa*Acs forms in our purified fractions. HPLC analysis confirmed that both *Sa*Acs forms are active. In addition, the *Sa*Acs mixture showed promising crystallinematerial in optimized crystallography screening solutions.

Discussion and conclusion: Both characterization and crystallization of *Sa*Acs were performed with the purified *Sa*Acs mixture which is not ideal. The ratios between the two *Sa*Acs forms were rationalized against the observed activity and further utilized in the kinetic characterization to obtain more accurate results. Further studies should aim to separate the two *Sa*Acs forms to successfully crystallize *Sa*Acs. If *Sa*Acs is indeed a target for PanAms, co-crystallization of *Sa*Acs and PanAm anti-CoAs can then be exploited to observe the interactions between the two.

References: R. M. Burckhardt, B. A. Buckner, and J. C. Escalante-Semerena, “Staphylococcus aureus modulates the activity of acetyl-Coenzyme A synthetase (Acs) by sirtuin-dependent reversible lysine acetylation,” *Mol. Microbiol.*, vol. 112, no. 2, pp. 588–604, 2019.

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