Antibody production against *Staphylococcus aureus* CoA biosynthesis enzymes and their application in protein level quantification

Karli Bothma, Dirk Bellstedt and Marianne de Villiers

Department of Biochemistry, Stellenbosch University

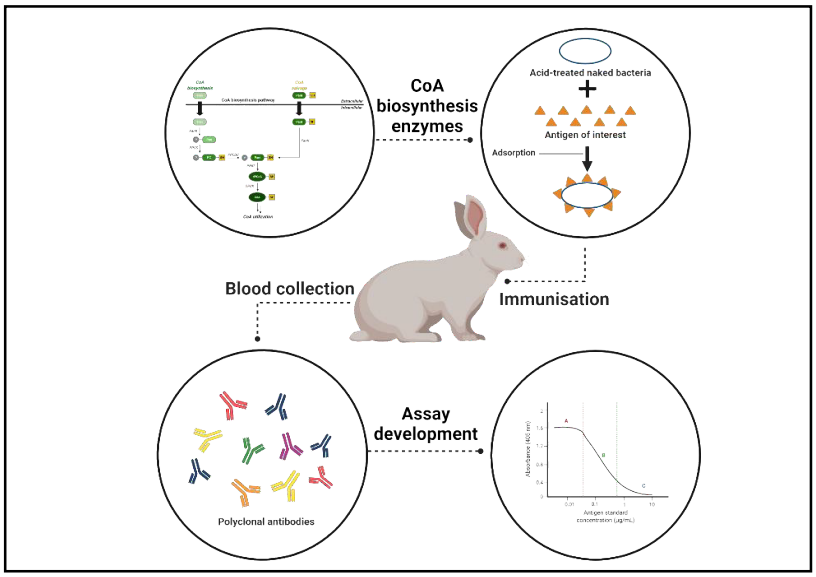

Figure 1: Schematic overview of the workflow from antibody production to assay development.
Rabbits were immunised with the CoA biosynthesis enzymes of *S. aureus* adsorbed to acid-treated naked bacteria. Polyclonal antibodies were obtained against these enzymes and were employed in the development of an ELISA for the detection and quantification of these enzymes.

Introduction: Antimicrobial resistance has become an increased burden worldwide. Therefore, the identification of novel drug targets and antimicrobial drugs are currently of high priority. A drug target that has gained increasedattention is the coenzyme A (CoA) biosynthesis pathway. CoA is an essential cofactor that is necessary for life in allorganisms, making it an attractive target for the development of new antimicrobial drugs. The CoA biosynthesis pathway of *Staphylococcus aureus*, which is the leading cause of hospital-associated infections, was the focus of this study. Although various studies have investigated this pathway in *S. aureus* as a possible drug target, knowledgegap regarding the levels of the CoA biosynthesis enzymes (PanK, CoaBC, PPAT and DPCK) under physiological conditions are currently unknown. We aimed to develop immunological techniques as tools to quantify these enzyme levels at different growth phases of *S. aureus*.

Methods: As depicted in Figure 1, recombinantly expressed *S. aureus* CoA biosynthesis enzymes adsorbed to acid-treated, naked *Salmonella minnesota* R595 was used to immunise Flemish Giant rabbits to produce polyclonal antibodies. These antibodies were characterized utilizing western blot and enzyme-linked immunosorbent assays (ELISA), and ultimately used in the optimisation of an ELISA for the quantification of the CoA biosynthesis enzyme levels of *S. aureus*.

Results: Characterisation of the antibodies revealed that high-titre antibodies were obtained from the immunisation of the rabbits. Indirect competition ELISAs with promising standard curves were obtained for the quantification of each of the enzymes.

Discussion and conclusion: Highly sensitive ELISA methods for the detection and quantification of all four CoA biosynthesis enzymes were developed. These ELISAs may provide a cost-effective and unique method for the quantification of the enzymes of the CoA biosynthesis pathway of *S. aureus* and other pathogens.

References:
Spry, C., Kirk, K., and Saliba, K. J. (2008) Coenzyme A biosynthesis: An antimicrobial drug target. *FEMS Microbiol. Rev.* **32**, 56–106; Bellstedt, D. U., Human, P. A., Rowland, G. F., and Van der Merwe, K. J. (1987) Acid-treated, naked bacteria as immune carriers for protein antigens. *J. Immunol. Methods*. **98**, 249–255

Keywords: *Staphylococcus aureus*, CoA biosynthesis enzymes, Polyclonal antibody production, Protein level quantification, Western blot, ELISA