Abstract

The bacterium *Kingella kingae* was first isolated in 1960 by microbiologist Elizabeth O. King and until recently, it was considered a rare cause of human disease. However, over the past 30 years, an increasing number of papers have shown that this bacterium is an important paediatric pathogen, mainly affecting children aged 6 months to 3 years, causing mainly septic arthritis, osteomyelitis, infective endocarditis, and bacteraemia.

K. kingae displays a strong cytotoxic effect against a variety of host cell types, which is caused by the secreted cytolysin RtxA, a member of the RTX (Repeats in ToXin) family. RtxA binds to glycosylated structures of the host cell, subsequently inserts into its cytoplasmic membrane, and forms cation-selective pores, leading to disruption of ion homeostasis and lysis of the attacked cell. RtxA is produced as an inactive protoxin proRtxA. Its activation is mediated by the acyltransferase RtxC, which transfers acyl chains to conserved lysine residues K558 and K689 in the protoxin. It uses the acyl carrier protein ACP as the acyl donor.

Currently, it is unclear how the RtxC, acyl-ACP, and proRtxA proteins interact with each other and which amino acid residues are responsible for these interactions. The aim of this thesis was to identify the residues responsible for these interactions by testing the interactions of RtxC with acyl-ACP and proRtxA, using a bacterial two-hybrid system. Mutant variants of ACP and proRtxA were prepared with substitutions of the residues that, based on *in silico* predictions, could be responsible for the interaction of these proteins with RtxC. Following that, the impact of the mutant variants of ACP and proRtxA on their interaction with RtxC was tested. The residues D36, D39 and D57 were found to be important for the interaction with RtxC in the ACP protein and the residue R697 in the proRtxA protein. In addition, the conserved lysine residue K558 of protoxin was also shown to be partially involved in the interaction with RtxC, whereas the conserved lysine residue K689 had no effect on the interaction with RtxC.

Keywords: *Kingella kingae*, RtxA, acyltransferase, posttranslational modification