Biochemical and structural characterization of ClpK from *Klebsiella pneumoniae*

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Klebsiella pneumoniae is an antibiotic resistant pathogen which belong into to group of pathogens referred to as ESKAPE pathogens. This group consists of *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacter*. These bacterial species are responsible for more than 40% of infections in intensive care units therefore causing economic burden especially in developing countries. Recently, a caseinolytic (Clp) protein, ClpK was identified from a clinical *K. pneumoniae* isolate which was responsible an outbreak in Denmark. Clp proteins are found in both eukaryotes and prokaryotes. These proteins play a major rolein cell protein homeostasis and help the cell survive harsh environmental conditions. This newly identified ClpK was associated with the thermal stress survival capability of *K. pneumoniae*. We used bioinformatic analysis to understand the distribution of Clp proteins across the *Klebsiella* species. Out of the investigated strains, only 34% contained the *clpk* gene. *In silico* analysis and molecular dynamicsimulations showed that the protein was mainly α-helical and highly dynamic. The gene encoding for ClpK was cloned into a pColdI vector. This was followed by successful expression and purification of ClpK homogeneity using affinity and anion exchange chromatography. Biophysical characterization of ClpK showed that this protein predominantly α-helical and this is in agreement within silico analysis of the protein structure. The purified protein is biologically active and hydrolyses ATP in a concentration dependent manner. Additionally, ClpK was found to be stable at temperatures up to 70^{o}C.

Keywords:
Klebsiella pneumoniae, Caseinolytic proteins, ClpK, Protein expression and purification, Moleculardynamic simulations, Spectroscopic analysis, ATPase assay.

Reference:
Bojer, M.S., et al., Heat resistance mediated by a new plasmid encoded Clp ATPase, ClpK, as a possible novel mechanism for nosocomial persistence of Klebsiella pneumoniae. PloS one, 2010.5(11): p. e15467.
Bojer, M.S., et al., ClpP-dependent and-independent activities encoded by the polycistronic clpK- encoding locus contribute to heat shock survival in Klebsiella pneumoniae. Research in microbiology, 2013. 164(3): p. 205-210.