Functional Characterization of the Pseudomonas aeruginosa ExoY Virulence Factor

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Purpose of the Experiment P. aeruginosa is responsible for the second most common hospital infection. Its ExoY toxin is a promiscuous cyclase similar to other toxins like the B. anthracis Edema Factor and the B. pertussis CyaA. Functional domains of ExoY are poorly understood. I used forward genetic screening to identify functional residues of ExoY, considering the fact that the enzyme is active when expressed in the yeast S. pombe and human cells, while inactive in E. coli. Procedures Used Using PCR I created a population of mutant alleles of exoY which were introduced into an expression vector by gap repair transformation. Screening was done using light microscopy and iodine staining to detect hypomorphic ExoY enzymes. The candidates were sequenced and the single amino acid changes were mapped to a 3D model. The relationship of calmodulin and ExoY was studied by attempting to rescue mutant ExoY activity with mutant alleles of the S. pombe calmodulin gene. Observation/Data/Results 45 candidates displayed low ExoY activity and were characterized. Most lacked an intact exoY gene. From 4 candidates expressing a full-length exoY gene, 2 novel single missense mutants were identified; one (R68G), at the active site of the enzyme, the other (Q134R) required for multimerization. I was unable to conclude about the relationship of ExoY and calmodulin, but the identification of 2 distinct co-factor-binding domains on ExoY may explain that calmodulin is necessary, but not sufficient, for ExoY activation. Conclusions/Applications: The study can be the basis for development of effective, high affinity ExoY-targeted drugs for treatment of P. aeruginosa infections. Future studies will reveal unknown co-factors and promote our knowledge regarding the strtructure and function.

Awards Won:
Fourth Award of $500