

Discovery of Novel Dual Phosphatase Kinase Regulating Pyocyanin Secretion in *Pseudomonas aeruginosa*

Dey, Riju (School: Shorewood High School)

The bacterium *Pseudomonas aeruginosa* causes many human and plant diseases, infecting over 50,000 people annually. ~75% of *Pseudomonas* proteins are characterized; however, pathogenic mechanisms of *Pseudomonas* infection remain unclear. To understand these mechanisms, we focused on an uncharacterized protein containing a eukaryotic-like protein kinase and phosphatase domain, referred to as the dual-phosphatase kinase 1 (Dpk1). Deletion of the *dpk1* gene from the genome did not affect its normal growth, suggesting that Dpk1 provides nonessential functions. In silico modeling of the Dpk1 protein shows that it contains a bonafide eukaryotic-like protein kinase at the C-terminus and a phosphatase domain at the N-terminus. We found that overexpression of the wild-type kinase domain, but not its kinase inactive mutant, was toxic in the budding yeast. The in vivo data was further confirmed by an in vitro kinase assay. We also found that the Dpk1 phosphatase domain could dephosphorylate ATP, suggesting that both the kinase and phosphatase domains were functional. Interestingly, we observed a decreased secretion of the toxin pyocyanin when the *dpk1* gene was deleted from the genome, whereas pyocyanin secretion was restored when complemented with the Dpk1 gene. Collectively, our data suggest that Dpk1 has functional protein kinase and phosphatase domains and a significant role in the secretion of the toxin pyocyanin.

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