Abstract

Phenazine biosynthesis is a characteristic of several bacterial genera including *Pseudomonas, Erwinia, Burkholderia, Brevibacterium and Streptomyces*. It involves the production of a group of water-soluble nitrogen-containing aromatic pigments called phenazines. Phenazines biosynthesis, though a topic of research for various studies was still not completely understood. The objective of this study was to elucidate the structure of the enzymes PhzA, B and G of the phenazine biosynthesis pathway of *Pseudomonas fluorescens 2-79* and to gain an insight into their functions as well as the intermediates formed during biosynthesis of Phenazine -1-carboxylic acid (PCA).

Structural investigation of PhzA and BcepA highlighted interesting and thus far unknown aspects of these enzymes. Structurally, both these enzymes were found to belong to the ketosteroid isomerase (KSI) family of proteins, displaying an α+β fold characteristic to this family. PhzA and BcepA are the most recently recognised members of this family, with BcepA being the first member displaying an 'arm-exchange' mode of dimerisation. The enzyme PhzG, which shows sequence similarity to pyridoxamine 5'-phosphate-oxidase from *E.coli,* was found to be a FMN-dependent oxidase on the elucidation of its structure. Moreover, the structure of PhzG complexed with PCA was also solved, which helped to further clarify its role as an oxidase in the phenazine biosynthesis pathway.

Functional studies were carried out during the course of this study by using a bacterial two-hybrid system, APCI mass spectroscopy, and the oxygen electrode. A new methodology using APCI-MS was established in this work, for detection of the activity of various enzymes and the intermediates formed during the biosynthesis of PCA. Using this method, two new, key intermediates, a proposed Schiff's base with molecular weight 274Da as well as a partially aromatized PCA with Mw 228Da were discovered. Moreover, this work also led to the resolution of the sequential order of enzymes acting in this pathway, which is PhzE, D, F, B, G and A.