

Crystal Structure of the Conserved Hypothetical Protein Xcc441 from *Xanthomonas campestris* Reveals a Novel Pentameric Assembly of Four-Helix Bundles

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Genome sequencing projects have produced abundant amino acid sequences of proteins, but a full understanding of the biological roles of these proteins will require knowledge of their structure and function. Because the function of a protein is tightly coupled to its three-dimensional structure, therefore structure determination can be used as a tool to infer molecular function of a protein. An integrated structural and functional genomic project of *Xanthomonas campestris* pv. *campestris* strain.17, a gram-negative bacterium that is phytopathogenic to cruciferous plants and causes black rot, was initiated in 2002 to provide experimental or computational three-dimensional structures for all tractable proteins on a genome-wide scale. One of the key goals of this project is to perform structural characterization for proteins with unknown function in hoping to find novel protein folds.

In this study, the ORF Xcc441 of *X. campestris* codes for a conserved hypothetical protein Xcc441, with a predicted molecular mass of 14.6 kDa, was selected for structural study by X-ray crystallography. A protein-protein BLAST search with this sequence revealed 22 homologous sequences with E values below 6×10^{-10} and sequence identity ranging from 35% to 100%. Most of these homologous sequences are annotated as hypothetical proteins. Interestingly, a Pfam database search suggests that Xcc 441 protein may belong to the ribosomal 23S rRNA protein family with unknown function. No structural information is currently available for this protein family. To determine the crystal structure of Xcc 441, we have purified and crystallized this protein

in orthorhombic space group $P2_12_12$, and selenomethionine derivatized protein crystals that diffracted to a resolution of 2.5 Å have been prepared. Preliminary analysis indicates that Xcc 441 assemble as a homopentamer and forms a novel funnel-like structure of 20 helices (Fig. 1). The functional implication of this structure is currently being analyzed.



Figure 1. Ribbons representation of funnel-like Xcc441 homopentamer. Each subunit is differentially colored.

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