Introduction
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*Pseudomonas aeruginosa* PA01 is an important human opportunistic pathogen responsible for causing various types of infections including bacteremia, pneumonia and urinary complications in humans. The genomic studies have indicated that this organism is inherently resistant to various antibiotics and has the ability to adapt and thrive in many ecological niches, including humans. In addition, the range of antibiotic therapy for the control of *Pseudomonas* mediated infections is becoming increasingly limited because of the rapid rise in the multi-drug resistance in the clinical isolates thereby resulting in the increased mortality rate. This grim situation has forced the scientists to design new anti-*Pseudomonas* inhibitors. In case of bacterial inhibitors, the selection of drug targets usually involves the enzymes of metabolic pathways essential for the microbes but not required by the humans. The amino acid, meso-diaminopimelic acid (DAP), is the second last intermediate of (S)-lysine biosynthetic pathway and is an integral component of the cell wall of Gram-negative bacteria including *Pseudomonas*. The presence of cell wall is an absolute necessity for a bacterium as it is responsible for myriads of cellular functions. It not only imparts cell shape to bacteria but also protects them from lysing under high internal osmotic pressure caused by high concentrations of organic and inorganic molecules. The cell wall of DAP-negative bacteria is very fragile and the organism lyses immediately. The absolute requirement of DAP by bacteria along with the non-requisite and non-production of this metabolite by humans may make DAP biosynthetic pathway as an excellent drug target for designing specific enzyme inhibitors.

One of the potential therapeutic target of this pathway is dihydrodipicolinate synthase (DHDPS; E.C. 4.2.1.52) which catalyzes the first committed step in the (S)-lysine biosynthetic pathway which involves the condensation of pyruvate and (S)-aspartate-β-semialdehyde [(S)-ASA] to give 4-hydroxytetrahydricolinate (HTPA), which further undergoes non-enzymatic dehydration to give (S)-2,3-dihydrodipicolinate (DHPD), which is the substrate for the next enzyme, dihydrodipicolinate reductase (DHDPR) in the (S)-lysine biosynthetic pathway. This pathway in bacteria leads to *de novo* synthesis of (S)-lysine, essential for protein synthesis, and DAP, which is a
vital constituent of the peptidoglycan layer in the bacterial cell wall. Thus, targeting the DAP pathway through disrupting the first committed step, DHDPS, will yield a novel class of therapeutic agents against *Pseudomonas*.

The amino acid sequence of DHDPS from *Ps. aeruginosa* (PsDHDPS) shows a relatively moderate amino acid identity with DHDPS from other bacterial species having maximum identity of 60 % with *Acinetobacter baumannii* (AbDHDPS) (PDB ID: 3PUL) followed by the identity of 56 % with *E. coli* DHDPS (EcDHDPS), a well studied DHDPS model. From many years, all DHDPS enzymes of known structure and confirmed function were found to be homotetramers, with the TIM-barrel monomers arranged as dimer of tight dimers with the sole exception of dimeric DHDPS enzyme from methicillin-resistant *Staphylococcus aureus* (SaDHDPS). Due to this structural variation it is essential to determine the structure of DHDPS proteins from various species so that a comprehensive understanding of the stereochemical details of the target enzyme could be achieved that will help to design inhibitory molecules against DHDPS.

The main focus of the present study is to understand the structure of PsDHDPS, which will further help in the rational design of antimicrobials against *Pseudomonas*.

The objectives of this study are as follows:

1. Cloning and sequencing of *dapA* gene of *Ps. aeruginosa*.
2. Optimization of growth conditions for production of soluble and functionally active rec. *PsDHDPS*.
3. Purification and biophysical characterization of rec. *PsDHDPS*.
5. Three dimensional structure determination of native rec. *PsDHDPS* and its complex with (S)-lysine.