Summary & Conclusions
Ps. aeruginosa was grown in NB medium at 37°C. Log phase cells were harvested and their genomic DNA was isolated using CTAB method.

2. A set of forward and reverse primers having restriction sites for the enzyme NdeI and XhoI were designed on the basis of dapA gene sequence using Gene Runner software.

3. The PCR reaction was optimized. A hot start (94°C/3 min) was followed by 30 cycles of amplification each with denaturation at 94°C for 30 sec, annealing at 58°C for 45 sec and extension at 72°C for 1 min 30 sec. An end filling step of 72°C/10 min was given in the end. The PCR reaction yielded a single band of approximately 879 bp on 1% agarose gel corresponding to the expected size of dapA gene.

4. The PCR product was purified from agarose gel and digested by restriction enzymes NdeI and XhoI. The digested product was ligated to an expression vector pET-28c(+) which has been digested with the same enzymes as the amplified product. The ligation mixture was transformed into E. coli DH5α cells followed by retransformation in E. coli BL21(DE3) made competent by CaCl₂ method.

5. The transformed E. coli cells were plated on LB-agar plates supplemented with antibiotic Kan₅₀.

6. Positive clones were screened for the presence of insert by the plasmid isolation and restriction digestion.

7. E. coli cells having rec. plasmid pNART1 [pET-28c(+)/dapA] were grown in LB-broth with Kan₅₀ till the OD₆₀₀ reached 0.6. At this point, the culture was induced with 1 mM IPTG and kept at 37°C for 3 hrs. SDS-PAGE analysis of induced cells lysed in sample loading buffer showed a thick band of expressed protein corresponding to PsDHDPS (approximately 31.0 kDa).

8. One clone showing high level of expression of the rec. protein was subjected to forward and reverse DNA sequencing. The result of one
clone shared 99.3 % identity with dapA gene as reported in the complete genome sequence of *Ps. aeruginosa*. The sequence was submitted to GenBank and appears with accession number GU166748.

9. In order to attain maximum protein in soluble fraction the induction conditions were optimized. It was found that maximum soluble expression of rec. *PsDHDPS* was obtained at IPTG conc. of 0.4 mM and incubated at 20°C for 17 hrs.

10. Due to the His tag attached to the rec. *PsDHDPS*, the single step protein purification was done by Ni-NTA column. An imidazole gradient was used to elute the desired protein. The purified *PsDHDPS* was eluted at imidazole conc. of 200 mM. We were able to purify approximately 20 mg of rec. *PsDHDPS* from 2 litres of culture.

11. The secondary structure elements of *PsDHDPS* were studied by Far-UV CD spectroscopy and their relative contents in rec. *PsDHDPS* were α-helices = 48 %, β-sheets = 12 %, β-turns = 11 % and the rest 29 %.

12. The tryptophan fluorescence spectrum of rec. *PsDHDPS* revealed that the all of its three tryptophan residues were exposed in the native protein.

13. Rec. *PsDHDPS* was stable up to 65°C and further increase in the temperature leads to changes in the secondary structure.

14. The enzymatic activity of rec. *PsDHDPS* was found to be maximum at pH 8.0 and temperature 37°C. Any further increase of pH and temperature has resulted in the drop of the enzymatic activity of rec. *PsDHDPS*.

15. The $K_m$ values of the rec. *PsDHDPS* for the substrates, pyruvate and (S)-ASA were found to be 0.90±0.13 mM and 0.17±0.02 mM respectively.

16. The three-dimensional structure determination showed that DHDPS forms a homodimer which is stabilized by several hydrogen bonds and van der Waals forces at the interface. The active site formed with
residues Thr44, Tyr107 and Tyr133 is found to be stereochemically suitable for catalytic function. It may be noted that Tyr107 of the catalytic triad belongs to the partner molecule in the dimer.

17. The structure of the complex of PsDHDPS with (S)-lysine determined at 2.65 Å resolution revealed the positions of three lysine molecules bound to the protein

In the present study, we describe the structural, biochemical and biophysical characteristics of rec. PsDHDPS, an important drug target for the control of growth of *Ps. aeruginosa* PA01. X-ray crystallographic studies revealed that rec. PsDHDPS existed as a tight homo-dimer, whereas majority of other bacterial DHDPS structures exist as tetramers except SaDHDPS which also exists as dimer. The dimeric interface of PsDHDPS is more stable than that of EcDHDPS, NmDHDPS, StDHDPS. This was due to the presence of two crucial hydrogen bonds involving Ser247 O' of one monomer and Lys109 O of the second monomer in PsDHDPS, whereas Ser247 is replaced by Pro247 in other tetrameric structures. Despite of these structural differences, PsDHDPS possess nearly similar enzymatic characteristics with other tetrameric homologues (EcDHDPS, NmDHDPS). This could be due to the conserved amino acid residues involved in substrate binding (Thr45, Arg138, Lys161, Asp187, Asp188) and catalysis (Thr44, Tyr107 and Tyr133). Our studies also revealed that the PsDHDPS has three (S)-lysine binding sites as in EcDHDPS. The coordinates and structure factors for rec. PsDHDPS and (S)-lysine bound complex have been deposited with the Protein Data Bank (PDB codes 3PS7 and 3PUO). Structure determination of PsDHDPS with other complexes are still in progress which will further help in designing of inhibitory molecules against PsDHDPS in future.