Characterization and applications of bacterial L-asparaginase from environmental samples

SUMMARY OF

THESIS

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Summary

- Of the 30 bacterial isolates, one isolate CSPS4 was selected based on thermal screening which showed maximum activity at higher temperatures over the other isolates. Bacterial isolate CSPS4 exhibited glutaminase as well as asparaginase activity. It was used for further investigation.

- Morphological, biochemical, and molecular (16S rRNA) characterization identified the bacterial isolate CSPS4 as a *Pseudomonas aeruginosa* strain.

- Production of L-asparaginase is enhanced using the 'one variable at a time approach (OVAT). In the Placket Burman (PB) analysis pH, sucrose, and temperature are found as significant factors to influence L-asparaginase production. Overall, a two-fold increase was observed after statistical optimization of asparaginase.

- Wild-type asparaginase was recovered by fractional precipitation using acetone to achieve the purified asparaginase from the crude sample (extracellular).

- On characterization, wilt-type purified asparaginase showed a molecular weight of ~35 KDa on SDS-PAGE. Further biochemical characterization, l-asparaginase is identified as a thermo-acidophilic enzyme exhibiting optimum pH and temperature of 6.0 and 60 °C, respectively. These properties render this enzyme novel from other available L-asparaginases of *Pseudomonas* spp. L-asparaginase activity remained unaffected by different modulators.

- Sequence analysis of Asn_PA protein depicted three highly conserved motifs (VVILATGGTIAG, DGIVITHGTDTLEETAYFL, and, LRKQGVQIIRSSHVNAGGF) exhibiting two catalytically important residues i.e., T^{45} and T^{125}.

- Homology modeling-based structure model for Asn_PA was generated using MODELLER 10.4 with 4PGA as the top-matched template. The predicted structure was validated and energy was minimized. Molecular docking was carried out cantered at the active site for asparagine and glutamine as its

substrate ligands. The enzyme-substrate interaction analysis showed binding affinities of -4.8 and -4.1 kcal/mol for asparagine and glutamine respectively.

- Molecular dynamics (MD) simulation studies showed better stability of Asn_PA at temperatures of 60°C, over 40, 50, and 80°C which makes this enzyme a novel L-asparaginase from mesophilic *P. aeruginosa* strain. The trajectory analysis showed that RMSD, Rg, and, SASA values correlate well with each other in the different tested temperatures during the MD analysis. Thus, the present findings encourage extensive characterization of the Asn_PA using laboratory experiments to understand the structural behavior of the active site loop in an open or closed state with and without the substrate molecules.

- An attempt was made to clone and express the l-asparaginase encoding gene of *Pseudomonas aeruginosa* CSPS4 into the pET28a (+) vector and expressed into *E. coli* BL21(*DE3*) for characterization of the protein. The recombinant rAsn_PA enzyme was purified by affinity chromatography using Ni-NTA^{2+} resins.

- Molecular weight analysis using SDS-PAGE unveiled rAsn_PA as a monomeric protein of molecular weight ~ 35 kDa. On characterization, the recombinant rAsn_PA showed optimum pH and temperature of 6.0 and 60 °C, respectively, along with significant stability at 50–70 °C, along with 50% residual activity at 80 °C after 3 h of incubation. Similarly, the rAsn_PA exhibited asparaginase activity over a broad pH range between 4 and 8.

- The rAsn_PA was not significantly inhibited in the presence of detergents. The rAsn_PA was grouped into the asparaginase-glutaminase family II due to the glutaminase activity.

- The purified asparaginase of isolate CSPS4 showed antitumor activity by exhibiting a cytotoxic effect on three different cell lines, where IC50 of purified rAsn_PA was 2.3 IU, 3.7 IU, and 20.5 IU for HL-60, MOLM-13, and K-562 cell lines, respectively.

- Thus, recombinant rAsn_PA of *P. aeruginosa* CSPS4 may also be explored as an antitumor agent after reducing or minimizing the glutaminase activity. Thermo-acidophilic properties of rAsn_PA make it a novel enzyme that needs to be further investigated.

- This L-asparaginase with novel properties has been successfully tested for acrylamide reduction in commercial fried potato chips which establishes its applicability in food industries.

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