DOI: 10.46991/JISEES.2025.SI1.114

Analysis of D-Amino Acid Oxidases Using Computer Technologies

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ABSTRACT

Oxidase of D-amino acid (DAAO, EC 1.4.3.3) is FAD containing enzyme catalyzing oxidation of D-amino acids to the corresponding α-keto acids and ammonia ion. DAAO is widely applied in biotechnological processes, namely in production of 7-aminocephalosporic acid (7-ACA) from cephalosporin C (CPC). 7-ACA is the precursor of many semisynthetic cephalosporin antibiotics of various generations, such as cefotaxime, ceftriaxone, etc. It is promising time DAAO as biomarker of diverse pathologies (neurodegenerative (schizophrenia), malignant tumors (gastric cancer, breast milk gland cancer, hepatocellular carcinoma, pancreatic cancer)) since these diseases are accomplied by significant changes in both the concentration of certain D-amino acids and activity thereof. Aim of this study is to conduct analysis of D-amino acid oxidases using computer technology. Software BLASTp and databases UniProt NCB were applied to engage for analysis 11 microbial genera producers of D-amino acid oxidase – were selected for analysis. Among them, 6 genera of yeast: *Candida*, *Lipomyces*, *Ogataea*, *Rhodotorula*, *Schizosaccharomyces*, *Trigonopsis* and 5 genera of mycelial fungi were sorted at *Aspergillus*, *Fusarium*, *Penicillium*, *Rasamsonia*, *Talaromyces*. Homologous of DAAO were found in the studied microbial species. High level of homology was identified with fungi, whereas with bacteria homology level did not exceed 30 %. To identity open reading frames for DAAO translation of nucleotide sequences was conducted in six possible reading frames (3 in the 5'→3' direction and 3 in the reverse 3'→5' direction) to identify DAAO. *In silico* analysis with SnapGene instruments confirmed the presence of a single open reading frame (ORF), starting from start codon ATG and ending with stop codon TAG, which with high possibility encodes polypeptide of 356 amino acids long. Analysis of the amino acid composition has enabled to reveal high content of leucine (Leu 9.3%), valine (Val 8.1%) and glycine (Gly 9.0%), evidencing the presence of α-helices or β-sheets typical for structural proteins or enzymes. Theoretically calculated molecular weight of protein encoded by this polypeptide is ~39.2 kDa. Theoretical isoelectric focusing point (pI) 6.52 is the consequence of prevalence of mildly acidic residues (Asp, Glu) over basic moieties (Arg, Lys). Low level of cysteine (Cys 1.7 %) is typical with the intracellular localization of the enzyme. The deduced data on the structure of DAAO will enable to develop strategy for its heterologous expression in industrially valuable organisms.

Keywords: D-amino acid oxidase, enzyme, amino acid sequence, biotechnology

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