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D-AMINO ACID OXIDASES FROM OGATAEA PARAPOLYMORPHA DL-1

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D-amino acid oxidase (EC 1.4.3.3., DAAO) is FAD-containing flavoprotein catalyzing stereospecific oxidative deamination of various D-amino acids to corresponding alpha-keto-acids and ammonia. DAAO plays important role in living cells and the enzyme is widely used as biocatalysts in several biotech and biosensor applications.

We have identified 4 new orthologous genes encoding potential DAAOs in the genome of thermotolerant methylotrophic yeast *O.parapolymorpha* DL-1. Transcriptome analysis showed marked differences in their expression in glucose- and methanol- grown cells. Gene disruptions, *in situ* enzymatic screens and heterologous expression in *E.coli* strains were used to determine substrate specificity and *in vivo* functions of the encoded proteins. We found that HPODL_02914 and HPODL_02165 genes encode DAAO paralogs with increased expression on glucose. Both genes are capable to support the growth of *O.parapolymorpha* in the medium with D-alanine as sole nitrogen source. The HPDL_02400 gene encodes D-aspartate oxidase. The major DAAO overexpressed on methanol is encoded by HPODL_02082 gene. Comparative analysis of the substrate specificity of the encoded protein with published data on substrate specificity of DAAO from *Candida boidinii* showed notable differences in their relative activity towards D-Met, D-Ser and D-Phe.

Studies are in progress towards identification of subcellular localization, physiological role, enzymatic and physico-chemical properties of encoded DAAO/DDO.

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