Psychrophilic monoamine oxidase from Pseudogymnoascus sp. P3 as a useful tool for amine biotransformations

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The subject of this dissertation were psychrotrophic filamentous fungi, isolated from Antarctic soil, belonging to the collection of microorganisms of the Institute of Molecular and Industrial Biotechnology of the Lodz University of Technology. Strains were identified taxonomically using molecular biology techniques and characterized physiologically, and biochemically.

The cold-loving filamentous fungi were screened for monoamine oxidase activity. The producers of those catalytic proteins were selected based on screening carried out in a medium supplemented with amine inducers. The most efficient source of the target enzyme turned out to be the *Pseudogymnoascus* sp. P3. This strain synthesized an enzyme with an activity of 1.35 U/ml (activity measured towards *n*-butylamine) within 14 days at 20°C. In addition to redox abilities, it has been shown that this strain, can produce various glycosidic hydrolases (e.g., cellulases, xylanases, pectinases), lipolytic enzymes, and proteases, including keratinases, capable of degrading feathers.

Due to the scarcity of literature reports on cold-loving eukaryotic redox enzymes, and industrial importance of those biocatalysts, the subject of doctoral dissertation was the activity of monoamine oxidase (abbreviated as MAO P3) and its application in the biotransformation of amines. In the first stage of the work, optimal conditions for the biosynthesis of the native enzyme were developed, i.e., the culture conditions and the composition of the substrate were selected. After an 8-day shake culture in a medium enriched with the addition of an amine inducer (*n*-butylamine) and vitamin B2, the MAO P3 oxidase activity was 1.867 U/ml. MAO P3 was purified using affinity chromatography, and the highly purified enzyme preparation was biochemically characterized. The adaptation of MAO P3 oxidase to operate at low temperatures is evidenced by the optimum temperature of this enzyme being 30°C, while at 0°C the enzyme retained over 25% of its maximum activity. Monoamine oxidase is a thermolabile protein, it is easily inactivated at temperatures above 30°C, further indicating its psychrophilic character. In addition, MAO P3 has broad substrate specificity for primary and secondary amines. The enzyme has the highest affinity for 6,6-dimethyl-3-azabicyclohexane (K_M 2.4 mM) and the lowest for indoline (K_M 20.4 mM). It is inhibited by clorgyline (IC50 1.8 x 10^{-8} M), which further confirms the correct classification of the enzyme.

As part of the study of the application potential of MAO P3 oxidase, the enzyme was used in cascade reactions with aldo-keto reductases from the thermophilic cyanobacterial strain *Thermostichus lividus*, thus proposing a unique combination of psychrophilic and thermophilic enzymes for the biosynthesis of alcohols, using cyclopentylamine, α-methylbenzylamine, and 2-phenylethylamine. After a cascade reaction lasting 20 hours, it was observed that the first substrate reacted with a molar yield of approx. 22% to cyclopentanol, the second with a yield of 51% to 1-phenylethanol, and the third with a yield of approx. 46% to 2-phenylethanol. This proves a large, unique potential for designing cascade reactions using enzymes with different temperature profiles.

For further characterization of the *Pseudogymnoascus* sp. P3 strain, its genome was sequenced, and its size was determined (approx. 33.9 Mbp). Based on the bioinformatical analysis, over 11,000 protein-coding genes have been identified. Among them, two genes encoding enzymes with motifs characteristic of monoamine oxidases were found, one of which g3222, has a mass similar to that determined by experimental methods.