Physiological, molecular and biochemical characterization of cold-adapted microscopic fungi from the Institute of Molecular and Industrial Biotechnology

MSc. Eng. Katarzyna Małgorzata Wiśniewska

Supervisor PhD. DSc. Eng. Aneta Białkowska, Associate Professor

Abstract

The subject of this dissertation were cold-adapted strains of yeast and yeast-like fungi belonging to the collection of microorganisms of the Institute of Molecular and Industrial Biotechnology, Lodz University of Technology. Their characterization, initialized by the author during her Master's Thesis and continued as part of her doctoral project, included analysis of physiological, biochemical and molecular features. The obtained results were used to prepare metrics for 20 strains isolated from Antarctic soil. The metric informs about the taxonomic identification of a strain, its morphology, physiology and selected biochemical features.

One strain, *Aureobasidium bupleuri* G3 IBMiP, with unique enzymatic abilities was selected based on the characteristics of cold-adapted yeast and yeast-like fungi. As part of its deep biochemical characteristics, an efficient way to obtain a highly purified enzyme preparation was designed, as well as indications for its potential industrial application were made.

Genome size and karyotypes of psychrophilic and psychrotrophic yeasts *Goffeauzyma gilvescens*, *Naganishia globosa*, *Goffeauzyma gastrica* and *Naganishia albida* were characterized using flow cytometry (FCM) and pulsed field gel electrophoresis (PFGE) for the first time. Most of the studied strains were haploids, two strains with a diploid number of chromosomes were identified (*Babjeviella inositovora* and *Rhodotorula mucilaginosa*) as well as two aneuploids (*Candida sake* and *Naganishia adeliensis*), for which the ratio of FCM genome size to the size of an individual copy determined using PFGE was 1.5 and 1.59, respectively.

The screening of 44 cold-adapted strains for the biosynthesis of enzymes adapted to low temperatures showed that the majority of the studied microorganisms displayed the esterolytic (33 strains), amylolytic (26 strains) and phytic acid hydrolysis (19 strains) activity. Moreover, 14 of the tested strains showed proteolytic and β-galactosidase activity, 6 — pectinolytic, and 1 — xylanolytic activity. What was interesting from the biotechnological point of view, activity of cold-adapted laccase was found for 4 strains. Submerged cultivation of one of them, identified as *A. bupleuri* G3 IBMiP, demonstrated a particularly high activity of this enzyme. Further research was aimed at a closer biochemical characterization of this strain, with particular emphasis on its ability for laccase biosynthesis.

Optimal conditions for the biosynthesis of *A. bupleuri* G3 IBMiP laccases were developed, including the identification of compounds increasing their accumulation, i.e. copper ions, Tween 80 and ascorbic acid. Activity of enzyme preparation of laccases in reaction with 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) acid at optimal pH 3.5 was 215 U/L after 15 days of cultivation in a medium with waste material — brewery spent grain, and approx. 130 U/L after 25 days in a defined medium. The optimal temperature for the preparation activity was in the range of 30-40 °C, at 10 °C it retained over 60% of its maximum activity. Enzyme preparation of laccases was characterized by high thermal lability — the half-life at 40 °C is only 60 minutes, which is a potential advantage enabling for an easy thermal inactivation of the enzyme.

For a further biochemical characterization of the *A. bupleuri* G3 IBMiP, its genome was sequenced and its size was determined (approx. 23.4 Mbp). Based on bioinformatics analysis, over 10,000 protein coding genes have been identified. Among them, four multicopper oxidase genes with motifs characteristic for laccases were found, one of which is responsible for the biosynthesis of KbLcc1 laccase.

Identification of the sequence of the gene encoding the KbLcc1 laccase enabled the expression of this protein in the heterologous mesophilic host *Pichia pastoris*. The highest activity of the recombinant protein (approx. 130 U/L) was achieved after 7 days of submerged cultivation in the expression medium supplemented with 2.0 mM Cu^{2+}. Recombinant KbLcc1 laccase was purified and precisely characterized: the enzyme with a molecular weight of approx. 69 kDa is a protein kinetically adapted to low temperatures, displaying high specificity for syringaldazine (K_M 0.021 mM, k_{cat} 37.85 s^{-1}) and sinapic acid (K_M 0.014 mM, k_{cat} 1.68 s^{-1}). The enzyme is stabilized by nickel, calcium and magnesium ions, and strongly inhibited by acetone and iron ions (Fe^{2+} and Fe^{3+}). The presence of hexane (40%, v/v) causes an almost 60% increase in enzyme activity.

Concerning the industrial application potential of *A. bupleuri* laccases, it was shown that both the native preparation and the recombinant enzyme catalysed the decolorization of synthetic dyes from the triphenylmethane group (basic fuchsin, crystal violet and Coomasie Brilliant Blue R-250) and triazine dyes (methylene blue). The highest degree of decolorization was achieved for crystal violet (over 40%). Additionally, recombinant KbLcc1 laccase has the ability to convert ferulic acid to vanillin. After 5 days of incubation of the enzyme in the presence of ABTS as a mediator, approx. 22 mg/L of vanillin was obtained with a molar yield of approx. 14%.