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Synthesis of phenoxazinone-type pharmaceutical compounds by fungal biomass

Synteza fenoksazynów przy użyciu biomasy grzybowej

INTRODUCTION

Phenoxazinone-like compounds have been found in various biological organisms and many of them are used as versatile agents with antifungal, antiviral, antitumor, or antibacterial activities. An example from the nature are phenoxazinone antibiotics produced by many various microorganisms [4, 5], among them *Streptomyces antibioticus* [7] and *Pycnoporus cinnabarinus* [2]. The main enzymes involved in the synthesis of phenoxazinone-type compounds are phenoxazinone synthase, laccase and peroxidases [2, 6]. The chemical method of synthesis of this type of compounds follows through condensation of nitroso-compounds at elevated temperature and in the presence of oxidative chemicals; however, the obtained products are characterized by low water-solubility, which limits their application as biologically active compounds. Rising new interest in the production and distribution of phenoxazinone antibiotics caused a fungal laccase to be discovered as the environmental friendly catalyst for the conversion of *ortho*-aminophenol into phenoxazinone products [1, 3]. This conversion follows through (1) the deprotonation of phenoxy-substituents, (2) the formation of reactive radicals-type intermediates, and (3) the non-enzymatic coupling of these later into new phenoxazinone-type structure (Fig. 1). However, the synthesis and purification of laccase are still too expensive which limits its use for industrial purposes but this enzyme could be replaced by fungal cultures with a well-known ability to secrete laccase. The aim of this study was to examine the ability of fungus *Fomes fomentarius* to transform definite organic precursor into phenoxazinone compound.

MATERIAL AND METHODS

Organism and culture conditions. *Fomes fomentarius* (*FF25*) originated from the Fungal Collection of Biochemistry Department of Maria Curie-Skłodowska University (Lublin, Poland). Two-week-old liquid cultures of *FF25* were homogenized and added to the Erlenmeyer flasks (250 ml) or special air-lift bioreactor (1200 ml) containing special glucose-peptone medium

(GPA) and plastic mesh scourer (PMS) as a carrier for fungal biomass immobilization. Biomass immobilization process lasted the next seven days in the case of Erlenmeyer flasks (140 rpm) and two weeks in the case of air-lift bioreactor. After the carrier was overgrown by the mycelium, the cultivation fluid was decanted and the flasks and the air-lift bioreactor were poured over the biotransformation mixture containing precursor AHBS dissolved in an appropriate medium. At the same time, controls with the precursor but without mycelium were carried out to check autooxidation of precursor.

Transformation of AHBS. The reaction mixture contained precursor 3-amino-4-hydroxybenzenesulfonic acid (AHBS), at three different concentrations, suspended in appropriate medium – sodium tartrate buffer pH 6 (system A), distilled water (system B), 100-times diluted GPA medium (system C), or 10-times diluted GPA medium (system D). The transformation in Erlenmeyer flasks was carried out during 48 hours or 120 hours, and the transformation in the air-lift bioreactor was performed during ten subsequent batches of precursor suspended in 10-times diluted GPA medium, each batch lasted 48 hours.

Analytical assays. The consumption of AHBS over time was monitored using capillary electrophoresis system (MEKC) at 210 nm while new product was detected spectrophotometrically at 436 nm. The activity of extracellular laccase (LAC) was determined following the oxidation of 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) at 414 nm according to Forte et al. [3] to verify the biological activity of all biotransformation systems.

RESULTS

The aim of this study was to check the ability of fungal strain *Fomes fomentarius* (*FF25*) to transform *ortho*-aminophenol (AHBS) into new phenoxazinone-type compound. The transformations of three different concentrations of precursor were prepared in four transformation media differing in the concentration of carbon and nitrogen sources. *FF25* had the ability to transform lower concentration of AHBS (1 mM) even when the strain was incubated in water or in buffer (Fig. 2). The higher concentrations of AHBS (5 mM and 10 mM) were not transformed as effectively as the lower one, regardless of the transformation system applied, but the best results were obtained for the reaction mixture containing GPA medium (system C and D) in the case of both 5 mM and 10 mM AHBS. The contribution of the non-enzymatic transformation of AHBS (without fungal biomass) did not exceed 1% of the absorbance of product (data not shown) obtained as the result of fungal biomass action (Fig. 2). All results were confirmed by the MEKC analysis of the rate of AHBS transformation. 1 mM AHBS was transformed completely after 24 hours (Table 1).

Additionally *FF25* was found to catalyze the transformation of 5 mM AHBS in the 1200-ml air-lift bioreactor during ten subsequent batches (60 days) and the maximal absorbance of product was obtained during the 8th batch (Fig. 3).

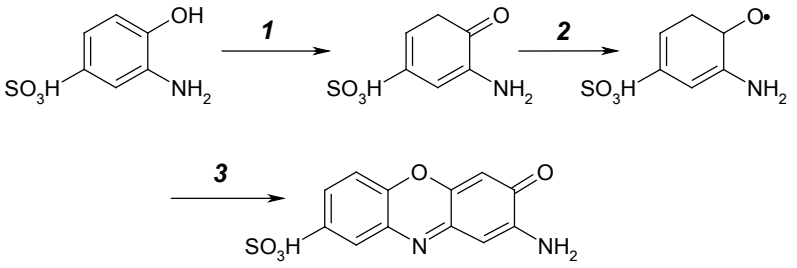

Fig. 1. Mechanism of laccase-mediated transformation of AHBS into phenoxazinone compounds

Applied system	Yield of AHBS transformation (%)				
	1 mM	**5 mM**	**10 mM**		
	24 h	**24 h**	**24 h**	**68 h**	**120 h**
A	**100**	84	48	77	98
B	**100**	**99**	41	61	81
C	**100**	**100**	71	94	98
D	**100**	**100**	**86**	**100**	100

Table 1. The yield of AHBS transformation of three different concentrations using four different transformation systems (A, B, C and D) of *FF25* shaking cultures

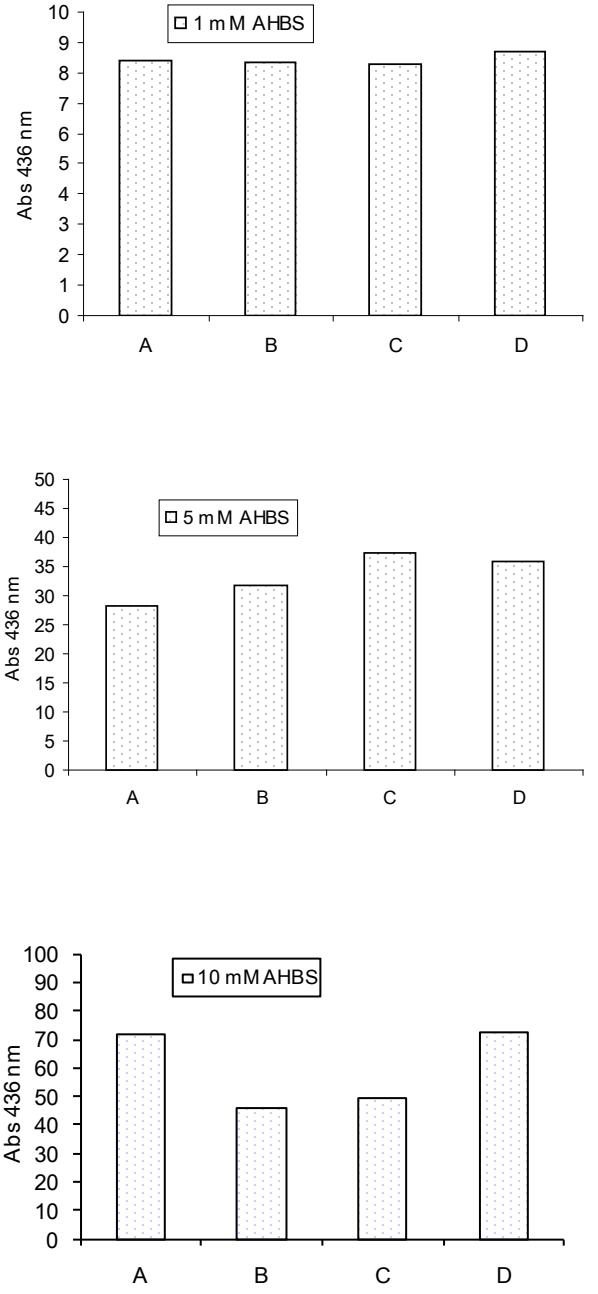

Fig. 2. The effect of applied transformation system (A, B, C, D) on maximal absorbance of product obtained in the case of AHBS transformation by *FF25* shaking cultures after 24 hours for 1 and 5 mM AHBS and after 120 hours for 10 mM AHBS

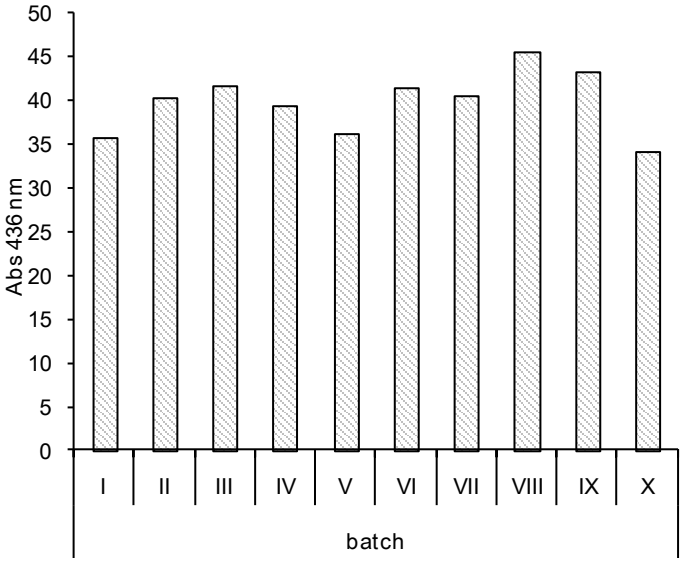

Fig. 3. Absorbance of phenoxazinone product obtained during ten subsequent batches of AHBS transformations (5 mM) by *FF25* strain cultivated in air-lift

CONCLUSIONS

Immobilized fungal biomass was found to be an effective catalyst for transformation of 3-amino-4-hydroxybenzenesulfonic acid into phenoxazinone-type compound, which previously was described only for purified laccase [3]. The use of the whole-cell system simplifies the transformation process, reduces the time, and does not require harmful and toxic oxidizing agents.

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SUMMARY

The phenoxazinone-type compound was achieved by transformation of 3-amino-4-hydroxybenzenesulfonic acid (AHBS) using fungus *Fomes fomentarius* (*FF25*). The oxidation of three different concentrations of AHBS was prepared in four transformation media differing in the concentration of carbon and nitrogen sources. The lower concentration of AHBS was transformed by immobilized fungal biomass even when the strain was incubated in water or in buffer. The product of this biotransformation belongs to phenoxazinone-type structures which are present in many pharmaceuticals.

Key words: phenoxazinone, antibiotics, biotransformation, *Fomes fomentarius*, laccase

STRESZCZENIE

Wykazano zdolność biomasy grzybowej *Fomes fomentarius* (*FF25*) do efektywnej transformacji kwasu 3-amino-4-hydroksybenzenosulfonowego (AHBS) w pochodną fenoksazynową o znanych właściwościach farmakologicznych. Najlepszym układem zastosowanym do transformacji prekursora AHBS, warunkującym jednocześnie długą aktywność biologiczną biomasy grzybowej, była rozcieńczona pożywka glukozowo-ziemniaczana. Dowiedziono, że szczep *FF25* jest zdolny do wydajnej transformacji 5 mM AHBS w ciągu 60 dni hodowli, w dziesięciu kolejno po sobie następujących transformacjach.

Słowa kluczowe: fenoksazyny, antybiotyki, biotransformacja, *Fomes fomentarius*, laktaza