Journal of Research in Medical and Dental Science
2020, Volume 8, Issue 7, Page No: 77-80

Available Online at: www.jrmds.in
eISSN No. 2347-2367: pISSN No. 2347-2545

Laccases of Basidiomycetes as Prospective Biocatalysts for Transformation of Antibiotics of Penicillin Series

Ulyana A Krut*, Irina V Spichak, Irina I Oleynikova, Galina M Shaidorova, Alexandra I Radchenko, Elena V Kuzubova

Belgorod State University, Russia

ABSTRACT

Today, one of the promising trends in modern biotechnology is the use of enzymes to obtain new compounds that have antibacterial, fungicidal and anticarcinogenic properties. As a promising biocatalyst for the synthesis and modification of various compounds, fungal laccases have recently been considered, which are a cheap and readily available enzyme for obtaining reaction components.

Purpose of the study: to study the possibility of mushroom laccase use for the modification of known antibiotics to obtain their analogues with new properties.

Materials and methods: The activity of laccase was determined spectrophotometrically by the rate of ABTS oxidation. The main object of the study is the laccases of the basidiomycetes of "white rot" and Lentinus strigosus 1566. The work used 2,6-dimethylphenol (Sigma-Aldrich, USA), 2,2-azino-bis (3-ethylbenzothiazoline 6-sulfonic acid) (ABTS) (Fluka, Switzerland) and glycerin (Serva, Germany). Peptone, soy flour, tetracycline hydrochloride, 6-aminopenicellanic acid, phenylglycine, potassium benzylpenicylate, pyrocatechol, hydroquinone ("Khimreaktiv", Russia).

Result: According to the spectra obtained, the reaction of the antibiotic modification with laccase without the addition of hydroquinone is not carried out. Because hydroquinone is a good substrate for laccase, this compound interacts quickly with the enzyme, with the formation of an oxidation product with an absorption maximum in the range from 200 to 300 nm. An example of 6-aminopenicylic acid modification dynamics by the Lentinus strigosus 1566 laccase in the presence of hydroquinone shows the formation of new peaks in the absorption region of 250 nm, which proves the formation of new products of the antibiotic transformation. When they study the spectral characteristics of 6-aminopenicylic acid interaction with the Lentinus strigosus 1566 laccase for 120 min, no new compounds are formed, because there is no spectrum change. However, there is a significant change in the reaction spectrum of the reaction of 6-APA interaction with hydroquinone under the action of Lentinus strigosus 1566 laccase for 120 min.

Conclusion: In the course of the work, the enzymatic preparation of laccase Lentinus strigosus 1566 was tested for the ability to modify the antibiotic. TLC showed the presence of three new compounds with-0.18; 0.15; 0.11; 0.07 (absent in all controls). Changes of absorption spectra in the range of 250 nm in the reaction of hydroquinone and 6-APA interaction under the action of Lentinus strigosus 1566 laccase also indicate the presence of new compounds.

Key words: Laccases of basidiomycetes, Lentinus strigosus, biocatalyst, enzymes, Penicillin's

HOW TO CITE THIS ARTICLE: Ulyana A Krut, Irina V Spichak, Irina I Oleynikova, Galina M Shaidorova, Alexandra I Radchenko, Elena V Kuzubova, Laccases of Basidiomycetes as Prospective Biocatalysts for Transformation of Antibiotics of Penicillin Series, J Res Med Dent Sci, 2020, 8 (7): 77-80.

Corresponding author: Ulyana A Krut

e-mail✉: Krut@bsu.edu.ru

Received: 06/10/2020

Accepted: 23/11/2020

INTRODUCTION

Today, one of the promising trends in modern biotechnology is the use of enzymes to obtain new compounds that have antibacterial, fungicidal and anticarcinogenic properties [1,2].

As a promising biocatalyst for the synthesis and modification of various compounds, fungal laccases have recently been considered, which are a cheap and readily available enzyme for reaction component obtaining [2,3]. Besides, this enzyme has a wide range of attacked substrates, a rich arsenal of catalyzed reactions, and high stability [4].

Laccases are ligninolytic extracellular enzymes that are widespread in nature. Due to their high stability and substrate specificity, laccases are applied a lot in biotechnological processes, such as modification of biomaterials containing lignin, discoloration of fibers, fabrics and paper, paint decomposition, industrial effluent treatment, detoxification of xenobiotics, as well as the production of biopolymers, antioxidants, anticancer drugs, antibiotics, steroids and other valuable compounds. They are used to create biosensors for the determination of various phenolic compounds, oxygen, azides, morphine, codeine, catecholamines, and plant flavonoids [5]. Laccases are also used in the food industry to remove phenolic compounds from beverages and baked goods, and for cosmetic purposes to whiten hair and skin.

The interest of researchers in the use of laccases in modern biotechnology is supported by the possibility of biocatalysis under mild conditions (at low temperatures, using atmospheric pressure and without the use of toxic solvents). But the possibility of carrying out a one-stage reaction to obtain the required compounds using laccases as biocatalysts is especially relevant [6,7].

Due to the presence of a unique reaction mechanism with the formation of highly active free radicals as intermediates, laccases are able to polymerize the starting molecules by the means of C-C, C-O, C-S, C-N crosslinks and form di-, tri- and polymer products. In addition to polymerization reactions, laccases are capable of oxidizing various molecules containing phenolic rings to form oxidized products [8-10].

STUDY PURPOSE

Study of the possibility of fungal laccase use for the modification of known antibiotics to obtain their analogs with new properties.

MATERIALS AND METHODS

The activity of laccase was determined spectrophotometrically by the rate of ABTS oxidation. The main object of the study is laccases of basidiomycetes of "white rot" and Lentinus strigosus 1566. We used the following substances in the work: 2,6-dimethylphenol (Sigma-Aldrich, USA), 2,2-azino-bis (3-ethylbenzothiazoline 6-sulfonic acid) (ABTS) (Fluka, Switzerland) and glycerin (Serva, Germany). Peptone, soy flour, tetracycline hydrochloride, 6-aminopenicellanic acid, phenylglycine, potassium benzylpenicylate, pyrocatechol, hydroquinone ("Khimreaktiv", Russia).

Experiment methods

The main object of the study is laccases of "white rot" basidiomycetes and Lentinus strigosus 1566. It was obtained from the collection of basidiomycetes of the Botanical Institute named after Komarov RAS (BIN), St. Petersburg. The culture was isolated from the basidiospores of the fruiting body growing on spruce (South Urals). The cultures of L. strigosus 1566 were maintained on stocks with wort agar with periodic reseeding and stored at 4°C.

Growth characteristics: Inoculum of L. strigosus 1566 fungi were grown for 7 days on soybean-glycerol medium, in 750 ml conical flasks with 100 ml of medium, on a shaker at 200 rpm. The medium had the following composition (g/l): NH_4NO_3-0.2; KH_2PO_4 0.2; K_2HPO_4 0.02; $MgSO_4$ x $7H_2O$-0.01; peptone-0.5; soy flour-0.5; glycerin-2 ml. Before inoculation, the inoculum was ground with porcelain beads on a shaker for 10 min at 200 rpm and added homogenized mycelium at the rate of 5 ml per 100 ml of medium.

The cultivation of mushrooms was carried out using a nutrient medium of the following composition, (g/l): glucose - 20.0; yeast extract - 5.0; peptone -5.0; $MgSO_4$ x $7H_2O$- 1.0, on a shaker at 200 rpm. On the 4th day of cultivation, the corresponding inductor (2,6-dimethylphenol for L. strigosus 1566 (1 mm)) and $CuSO_4$ (2 mm) were added. Immobilization of the mycelium was carried out on polycaproamide fiber, which was introduced in the amount of 1 g per flask during the inoculation period.

The activity of laccase was determined spectrophotometrically by the rate of ABTS oxidation, ε436=29300 M^{-1} cm^{-1} at 436 nm via UV-160 spectrophotometer (Shimadzu, Japan). The reaction mixture contained 1 mM of ABTS, 20 mm of Na-acetate buffer (pH 5.0), and an enzyme preparation. The average rate (for 1 minute) of conversion of 1 mcm of substrate in 1 ml of 20 mm Na-acetate buffer (pH 5.0) was taken as the unit of laccase activity.

When they study the spectral characteristics of

the 6-APC modification, control reactions of the starting compounds with laccase were set.

To test the ability of laccase to modify antibiotics, the reaction was carried out on a shaker at 200 rpm and 29°C. The reaction mixtures (5 ml) contained 20 mM of Na-acetate buffer, 2 mM of hydroquinone, 2 mM of antibiotics, and an enzyme preparation. If necessary, 0.8 mM of hyaluronic acid, 0.8 mM of 4-hydroxy-TEMPO, 0.8 mM of 3-hydroxy-1,2,3-benzo-triazin-4(3H)-one were added as mediators. The reaction mixtures were incubated at 29°C, on a shaker at 200 rpm, for 30 min, 1 hour, and 2 hours. Then the reaction mixtures were acidified with 0.1 M of HCl up to pH 2.0 and the reaction products were extracted.

The reaction mixtures were extracted three times with 15 ml of ethyl acetate at pH = 5, the extracts were combined and dried with anhydrous [Na]_2 [SO]_4. The solvent was distilled off on a rotary evaporator. The dry residues were dissolved in acetone and analyzed by thin layer chromatography (TLC).

The qualitative analysis of the products was carried out on Silica gel 60 F254 plates (MERK, Germany). The mobility of the compounds was determined by the absorption in UV light. At the start, 15 mcl of the obtained extracts were applied. Chromatography was performed in the solvent system of acetone-chloroform-acetic acid (10:9:1). The extracts were dispersed to the distance of 5.5 cm.

Compounds were identified using the Rf value of the compound, which is equal to the ratio of the distance traveled by the substance in a given solvent system to the distance traveled by the solvent front.

RESULTS AND DISCUSSION

According to the spectra obtained, the reaction of the antibiotic modification with laccase without the addition of hydroquinone is not carried out. The spectrum has a well-expressed maximum at 250 nm, which lasts for 120 min. did not change (Figure 1A). Considering that hydroquinone is a good substrate for laccase, this compound interacts quickly with the enzyme, with the formation of an oxidation product with an absorption maximum 200 - 300 nm. (Figure 1B). No significant spectrum changes were observed

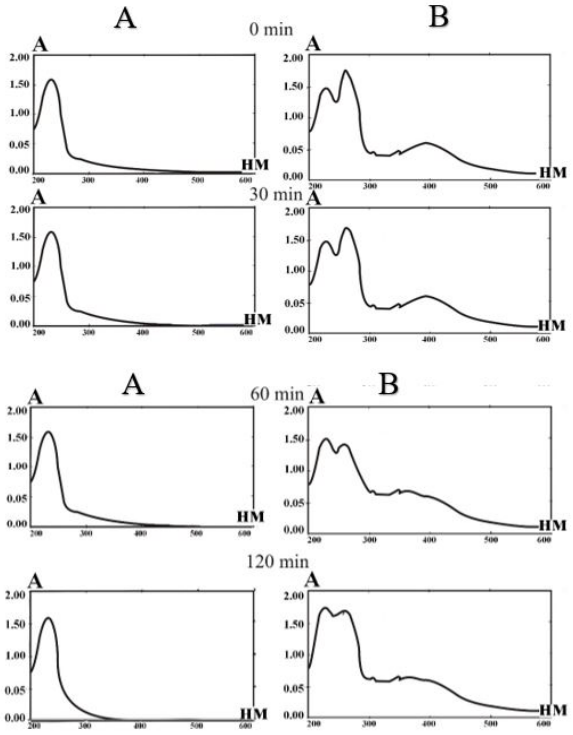

Figure 1: Changes in the absorption spectrum for 120 min: A-The reaction of 6-APA and laccase; B-The reaction of hydroquinone and laccase.

over time. This indicates that there was an accumulation of formed products.

Figure 2 shows the dynamics of 6-APA modification by the laccase Lentinus strigosus 1566 in the presence of hydroquinone. The formation of new peaks occurs in the absorption region of 250 nm, which proves the formation of new products of the antibiotic transformation. When studying the spectral characteristics of 6-APA interaction with the Lentinus strigosus 1566 laccase for 120 min, no new compounds are formed, because there is no spectrum change. However, there is a significant change in the reaction spectrum of 6-APA interaction with hydroquinone under the action of Lentinus strigosus 1566 laccase for 120 min (Figure 2). Perhaps this indicates the formation of heteromolecular antibiotic compounds.

SUMMARY

In the course of the work, the enzymatic preparation of laccase Lentinus strigosus 1566 was tested for the ability to modify the antibiotic. TLC method showed the presence of three new compounds with - 0.18; 0.15; 0.11; 0.07 (absent in all controls). Changes in absorption

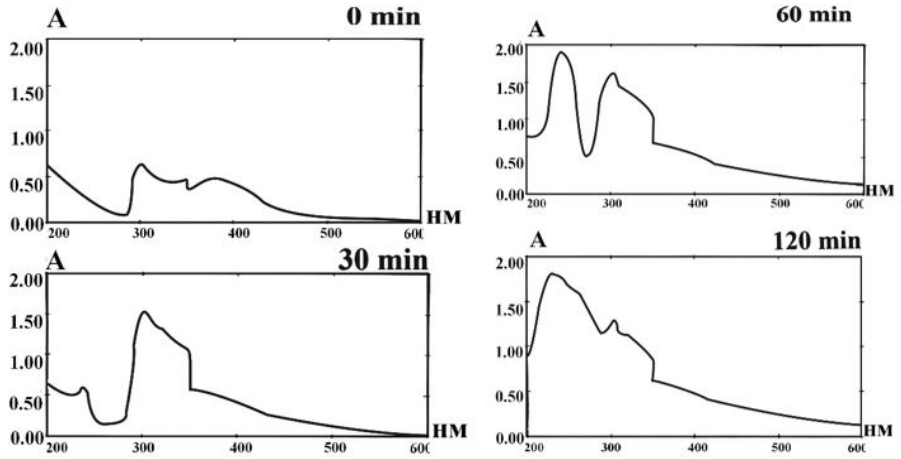

Figure 2: Dynamics of 6-APC modification during its reaction with hydroquinone under the action of Lentinus strigosus 1566 laccase for 120 min.

spectra within 250 nm during the reaction of hydroquinone and 6-APA interaction under the action of Lentinus strigosus 1566 laccase also indicate the presence of new compounds. The research has been done within the framework of State Assignment of the Russian Federation Ministry of Science and Higher Education № FZWG-2020-0021. The authors express their gratitude to Professor, Doctor of Biological Sciences, Honored Scientist of the Russian Federation L.A. Golovleva, as well as the Regional Microbiological Center of Belgorod State University.

REFERENCES

1. Egorov NS. Fundamentals of the doctrine on antibiotics. Moscow State University, Russia 2004; 524.
2. Scryabin GK, Golovleva LA. Use of microorganisms in organic synthesis. Moscow: Nauka, Russia 1981; 332.
3. Baldrian P. Increase of laccase activity during interspecific interactions of white-rot fungi. FEMS Microbiol Ecol 2004; 50:245–253.
4. Chernykh AM, Myasoedova NM, Kolomytseva MP, et al. New laccases of Steccherinum ochraceum 1833 with high biotechnological potential. INMI, Moscow, Russia 2007; 128-129.
5. Smirnov SA, Koroleva OV, Gavrilova VP, et al. 2001. Laccases from basidiomycetes: physicochemical characteristics and substrate specificity in relation to methoxyphenolic compounds. Biochemistry 2001; 66:952-958.
6. Williamson PR. Biochemical and molecular characterization of the diphenol oxidase of Cryptococcus neoformans–Identification as a laccase. J Bacteriol 1994; 176:656–664.
7. Micolash A, Schauer F. Fungal laccases as tools for the synthesis of new hybrid molecules and biomaterials. Microbiol Biotechnol 2009; 82:605-624.
8. Lee D, Jang EH, Lee M, et al. 2019. Unraveling melanin biosynthesis and signalling networks in *cryptococcus neoformans*. Mbio 2019; 10:13-19.
9. Alcalde M. Laccases: Biological functions, molecular structure, and industrial applications. MacCabe AP (eds.). Industrial Enzymes Springer 2007; 144:461–476.
10. Wang X, Yao B, Su. Linking enzymatic oxidative degradation of lignin to organics detoxification. Int J Mol Sci 2018; 19:3373.