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Original Article

Testing for the ability to modify antibiotics of Panus tigrinus 8/18 Lentinus strigosus 1566 laccase

Teste de capacidade para modificar antibióticos a partir da *lacase de Panus tigrinus* 8/18 e *Lentinus strigosus* 1566

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Abstract

In advanced biotechnology, the utilization of enzymes to achieve new or modified compounds with antibacterial, fungicidal, and anti-cancer specifications is crucial. Mushroom lactases are a hopeful biocatalyst for the synthesis and modification of different compounds. They are an accessible and inexpensive enzyme for the preparation of reaction objects and have recently received attention. Laccase purification was performed from basidiomycete Lentinus strigosus (*LS)* in several stages: Stage 1. On ion-exchange chromatography on TEAE Servacell 23 (400 ml), two distinctly separated laccase activity peaks were observed, eluted from the carrier at 0.21 and 0.27 M NaCl. In order to reduce the loss of enzymes, all fractions with laccase activity were collected, concentrated, and desalted using an ultrafiltration cell (Amicon, United States) with a UM-10 membrane. Stage 2. The resulting preparation with laccase activity was applied to a Q-Sepharose column (60 ml). Two well-separated peaks with laccase activity were obtained during the elution: laccase I (0.12 M NaCl) and laccase II (0.2 M NaCl). Stage 3. In the course of further purification of both enzymes, carried out on anion-exchange carrier Resource Q (6 ml), a broken gradient was used: 0 - 10%, 10 - 20%, and 20 - 100% with 1M NaCl. Stage 4. Both laccase I and laccase II, obtained after Resource Q, were desalted, concentrated to 1 ml each, and applied to a Superdex 75 gel filtration column. As a result, two laccases were obtained in a homogeneous form.

Keywords: laccase, basidiomycetes, enzymes, microorganisms, antibiotics.

Resumo

Na biotecnologia moderna, o uso de enzimas para obter compostos novos ou modificados com propriedades antibacterianas, antifúngicas e anticancerígenas é crucial. Lactases de cogumelos são biocatalisadores promissores para síntese e modificação de diferentes compostos, por serem enzimas baratas e disponíveis para a preparação de componentes de reação, e vem recebendo a devida atenção recentemente. A purificação da lacase foi realizada a partir do basidiomiceto *Lentinus strigosus* em vários estágios: Etapa 1 - na cromatografia de troca iônica em TEAE Servacell 23 (400 ml), foram observados dois picos de atividade da lacase distintamente separados, com eluição do transportador a 0,21 e 0,27 M de NaCl. Para reduzir a perda de enzimas, todas as frações com atividade de lacase foram coletadas, concentradas e dessalinizadas em uma célula de ultrafiltração (Amicon, Estados Unidos) com membrana UM-10; Etapa 2 - a preparação resultante com atividade de lacase foi aplicada a uma coluna Q-Sepharose (60 ml). Durante a eluição, foram obtidos dois picos bem separados com atividade de lacase: lacase I (NaCl 0,12 M) e lacase II (NaCl 0,2 M); Etapa 3 - no decurso da purificação adicional de ambas as enzimas, realizada no Recurso Q de transportador de troca aniônica (6 ml), um gradiente quebrado foi usado: 0-10%, 10-20% e 20-100% com NaCl 1M; Etapa 4 - tanto a lacase I como a lacase II, obtidas após o Recurso Q, foram dessalinizadas e concentradas para 1 ml cada e aplicadas a uma coluna de filtração em gel Superdex 75. Como resultado, duas lacases foram obtidas de forma homogênea.

Palavras-chave: lacases, basidiomicetos, enzimas, microorganismos, antibióticos.

1. Introduction

Usage of enzymes to produce new compounds or modify antibacterial compounds is important (Zengin et al., 2018; Saleem et al., 2020; Zhao et al., 2020; Tukenova et al., 2021). Fungal laccases are an inexpensive and readily accessible enzyme for earning reaction components. They have recently been considered as an important biocatalyst for the synthesis and reformation of diverse compounds. In addition, this enzyme has an extensive

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range of attacked substrates, a wealthy arsenal of catalyzed reactions, and great sustainability (Ferraroni et al., 2007). In modern biotechnology, researchers are interested in using laccases with the possibility of biodegradation under mild conditions. Mild conditions are conditions that have three conditions, which are: (i) low temperatures, (ii) under relative zero pressure, and (iii) without the usage of toxic solvents. However, we see the feasibility of a one-stage reaction to earn the needed compounds using laccases as biocatalysts to be particularly relevant (Myasoedova et al., 2008; Kolomytseva et al., 2019).

Laccases are able to polymerize the primary molecules through C-C, C-O, C-S, C-N crosslinks and form di-, tri-, and polymer products. Also, laccases can oxidize various molecules containing phenolic rings with the formation of oxidized products (Williamson, 1994; Myasoedova et al., 2015).

Laccase (EC 1.10.3.2, p-diphenol: oxygen oxidoreductase), a copper-containing blue oxidase found in plants, fungi, and bacteria, catalyzes one-electron oxidation of a wide range of substrates, mainly phenols and aromatic amines, reducing oxygen to water (Thurston, 1994; Burke and Cairney, 2002; Bains et al., 2003; Alcalde, 2007; Madhavi and Lele, 2009; Babu et al., 2012).

Considering the broad substrate specificity, ease of use, and stability, fungal laccases serve as enzymatic catalysts in the transformation of antibiotics (Rave et al., 2019; Guardado et al., 2019; Navada and Kulal, 2019; Aldayel et al., 2021). The objective of the research was to study the possibility of using fungal laccases to modify famous antibiotics to earn their analogs with new features.

2. Methodology

The main objects of the article were laccases of basidiomycetes of "white rot" *Panus tigrinus* 8/18 and Lentinus strigosus (*LS)* 1566. The fungi were grown by submerged cultivation. Laccase activity was determined spectrophotometrically by the rate of ABTS oxidation (2,2-Azino-bis (3-ethylbenzothiazoline 6-sulfonic acid), ε_{436} = 29300 $M^{-1}cm^{-1}$ at 436 nm on a UV-160 spectrophotometer (Shimadzu, Japan).

The enzymes were purified from the culture liquid of fungi collected at the peak of laccase activity using ion-exchange chromatography on TEAE Servacell 23 (400 ml). We determined the mass of the enzyme subunit using SDS-PAGE electrophoresis (Andlar et al., 2018; Yesilada et al., 2018). Antibiotic modification products were identified using thin layer chromatography and spectrophotometry (Li et al., 2018).

3. Results and Discussion

The study of the dynamics of laccase activity showed that laccase reached its maximum activity in the fungus *P. tigrinus* 8/18 on the 5th day and amounted to 50 ME/ml, while the fungus *LS* 1566 had a higher level of biosynthesis of this enzyme (250 ME/ml) and the peak of activity shifted on the 3rd day of cultivation (Figure 1).

As shown in Figure 1, the laccase activity of *LS* F-1566 is significantly higher than that of *P. tigrinus* 8/18. Therefore, for further work, we used *LS* F-1566 culture liquid. At the stage of ion-exchange chromatography on TEAE Servacell 23 (400 ml), two peaks of laccase activity were observed, eluted from the support at 0.21 and 0.27 M NaCl (see Figure 2).

The data on the elution peaks of laccase activity shows that there are two isoforms of laccase: laccase I and laccase II. The study with SDS electrophoresis showed that both laccases are monomers with molecular weights of 62 and 60 kDa (laccase I and laccase II, respectively) (Figure 3). In further studies, the dominant isoform – -laccase I – was selected.

The resulting enzymatic provision of *LS 1566* laccase was experimented for its capability to correct the precursors of antibiotics of various classes (penicillin, cephalosporin, tetracycline, imidazole, and erythramycin).

As can be seen in Figure 4, our experiments showed a modification of the antibiotic of the penicillin series. Formation of new compounds with R_f - 0.18; 0.15; 0.11; 0.07, absent in all control variants, was observed in the reaction mixture using 6-APA, hydroquinone, and laccase preparation.

The study of the spectral features of the 6-APA correction found that the reactions of antibiotic correction with laccase do not proceed without adding hydroquinone (Morsi et al., 2020). When this enzyme is added to a mixture

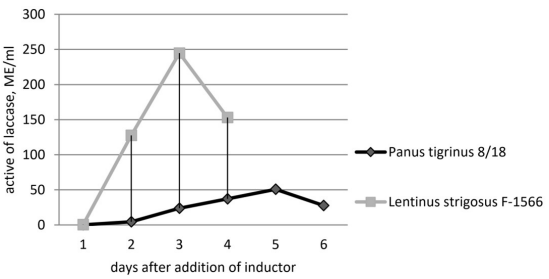

Figure 1. Dynamics of laccase activity of fungi *P. tigrinus* 8/18 and *LS* F-1566.

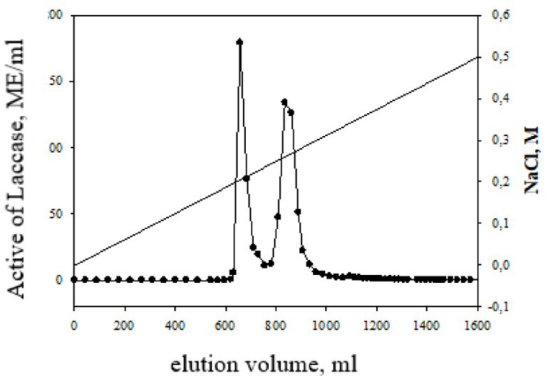

Figure 2. Elution of laccase activity from the column «TEAE servacell 23».

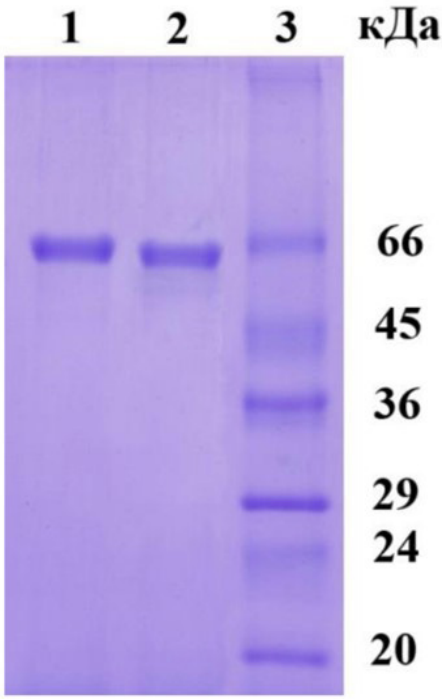

Figure 3. SDS electrophoresis (12% PAGE gel) of pure preparations of laccase I (1) and laccase II (2), isolated from the fungus LS 1566.3 - marker proteins.

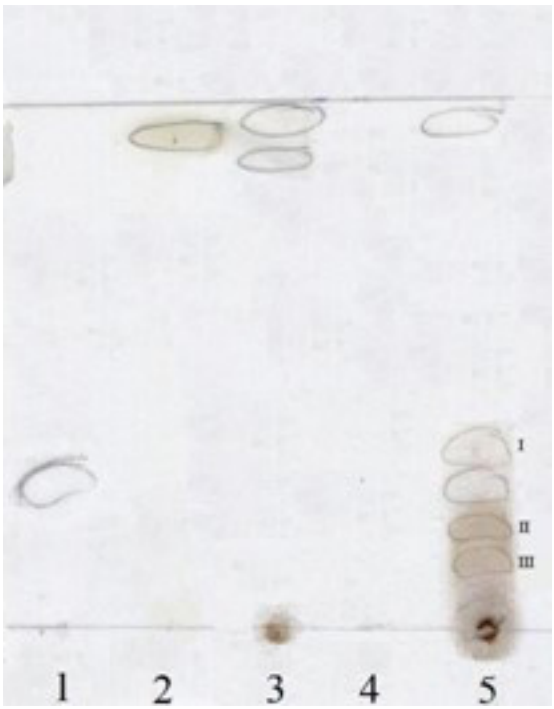

Figure 4. Thin-layer chromatogram of the tested compounds (1-2) and compounds obtained in experiments with the addition of laccase (3-5). 1 - 6-APA; 2 - hydroquinone; 3- hydroquinone + laccase; 4 - 6-APA + laccase; 5 - 6-APA + hydroquinone laccase.

of hydroquinone and 6-APA, new peaks are formed in the absorption region of 250 nm. Given that hydroquinone is a good bed for laccase, in the reaction, it can act as an activator of the formation of new transformation products of the antibiotic 6-APA (see Figure 5).

Mikolasch and Schauer (2009), in their studies, showed the possibility of modifying antibiotics of a number of penicillins using the laccase *Trametes Versicolor*. The products formed were homo- and heteromolecular compounds of the original antibiotic. In our case, investigation the spectral specifications of the interaction of 6-APA with the *LS* 1566 laccase for 2 hours, no new compounds are created, as no spectrum changes occur. Nevertheless, we observe a remarkable variation in the spectrum of the reaction of the interaction of 6-APA with

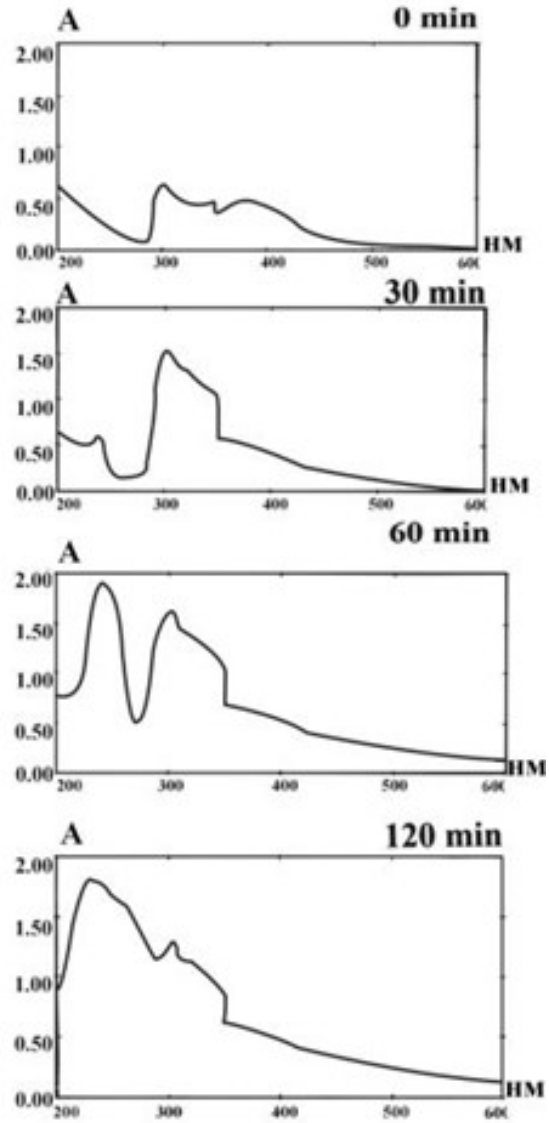

Figure 5. Dynamics of 6-APA correction upon its reaction with hydroquinone under the action of *LS 1566* laccase for 2 hr.

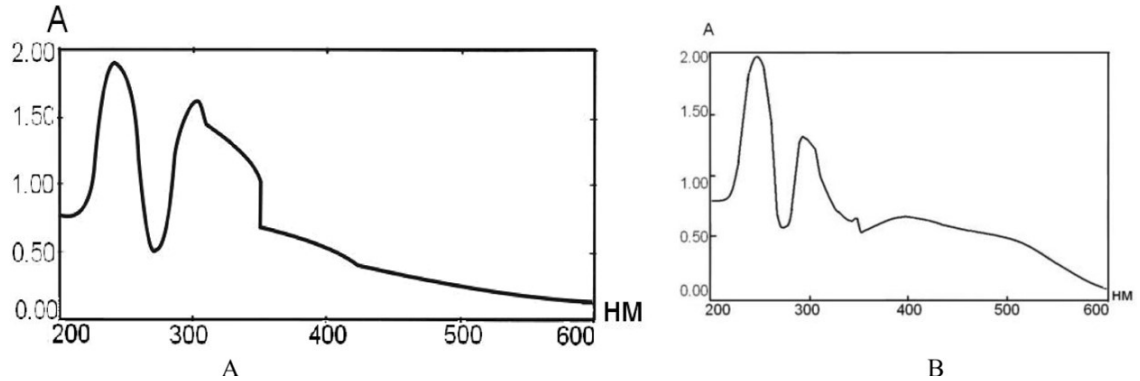

Figure 6. Reaction spectra of 6-APC modification: A - without the use of a mediator (60 minutes of incubation); B - using the mediator 4-hydroxy-TEMPO (15 min incubation).

hydroquinone under the action of *LS* 1566 laccase for 2 hours. This may indicate the formation of heteromolecular compounds of the antibiotic.

To increase the efficiency of the 6-APA modification process, we have optimized the conditions of this reaction. A significant formation of new products was observed already after 60 minutes of incubation; a further increase in the reaction time did not lead to the formation of new compounds, but the accumulation of already formed products took place. A change in the absorption spectrum is observed at 250 nm after 30 min of incubation (see Figure 6).

One factor that makes it possible to expand the bed specificity of laccases is the use of mediators. The mediator is oxidized by laccase to a stable radical, which is an oxidizing agent of the substrate (Wang et al., 2018; Lee et al., 2019). The presence of mediators significantly hastens the oxidation of pentachlorophenol, anthracene, and other polycyclic hydrocarbons, textile dyes by laccases of basidiomycetes. The inclusion of mediators (vialuronic acid, 4-hydroxy-TEMPO, etc.) in the 6-APA modification reaction did not affect the formation of new compounds but significantly accelerated the reaction up to 15 minutes.

4. Conclusion

The data obtained indicate that purified enzyme preparations of the laccase of the fungus *LS* 1566 are promising enzymes for the transformation of penicillin antibiotics with the formation of at least three products identified by TLC (R_f - 0.18; 0.15; 0.11; 0.07).

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