BIODEGRADATION OF AROMATIC COMPOUNDS BY THE YEAST-LIKE FUNGUS Trichosporon cutaneum

S. A. Medvedeva, É. I. Kolomiets, I. A. Antipova, V. A. Babkin, S. Z. Ivanova, I. V. Volchatova, and T. V. Romanovskaya

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The biodegradation reactions of veratryl- and guaiacylpropane compounds modeling structural fragments of the lignin macromolecule by the yeast-like fungus Trichosporon cutaneum D-46 have been investigated. It has been shown that the enzyme complex of T. cutaneum is capable of catalyzing a series of reactions of the biotransformation of arylpropane substrates, namely: oxidation, the cleavage of the C_α–C_β bond of the propane chain, demethylation, and decarboxylation. The action of the enzymes was shown most actively in the case of substrates having an unsubstituted phenolic hydroxy group.

Specialists in various branches of the national economy are working on the problem of the rational utilization of technical lignins. However, many of the proposed methods of treating lignins are unsuitable because of the absence of a comprehensive solution satisfying both the technological and the ecological-economic aspects of the problem. In particular, the use of lignin wastes as a raw material for the microbiological industry is limited by the low efficiency of the process of biodegradation and requires a more careful approach to the selection of the destructive microorganisms. The yeast-like fungus Trichosporon cutaneum is capable of growing on various lignin-containing substrates – hydrolysis lignin (HL) and lignosulfonates, producing a nontoxic biomass possessing a phytoprotective and growth-stimulating action [1]. In contrast to basidial lignin-destroying fungi, T. cutaneum is characterized by a high rate of growth and is resistant to contamination by foreign microflora. These features provide the possibility of the practical use of the culture for obtaining biolignofertilizers and have served as a reason for a more intense study of its enzymatic activity and its realizable spectrum of chemical reactions.

The aim of this work consisted in an investigation of reactions leading to the biodegradation under the action of a culture of T. cutaneum D-46 of veratryl- and guaiacylpropane substrates possessing different degrees of oxidation of the C_α atom.

The biodegradation of veratrylpropan-1-ol (1) and of veratrylpropan-1-one (2) by the T. cutaneum culture took place equally slowly: on the sixth day of incubation, 44 and 49%, respectively, of the substrates remained in the culture filtrates (Figs. 1 and 2). The degree of oxidation of the C_α atom of the propane chain did not affect the rate of inclusion of the veratryl-substituted substrates in the metabolism of T. cutaneum. The biotransformation of veratrylpropan-1-ol (1) was accompanied by the oxidation of the C_α atom of the propane chain during the very first day of incubation, with the formation of veratrylpropan-1-one (2). On the second day, cleavage of the C_α–C_β bond was observed. The main degradation product proved to be vanillic acid (3). On the sixth day of incubation there was 11% of it in the culture filtrate. The qualitative composition of the metabolites and the dynamics of their appearance permits the following sequence of reactions brought about by T. cutaneum in the oxidation of substrate (1) to be proposed (Fig. 3, route A).

In view of the fact that not only the main identified metabolites, veratrylpropan-1-one (2) and vanillic acid (3), but also trace amounts of veratryl alcohol were detected, the possibility may be assumed of a parallel route for the biodegradation of veratrylpropan-1-ol (1), namely the direct cleavage of the C_α–C_β bond with the formation of veratryl alcohol

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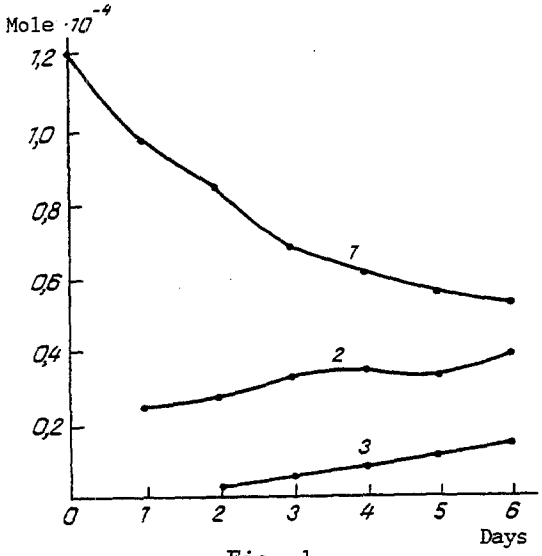

Fig. 1

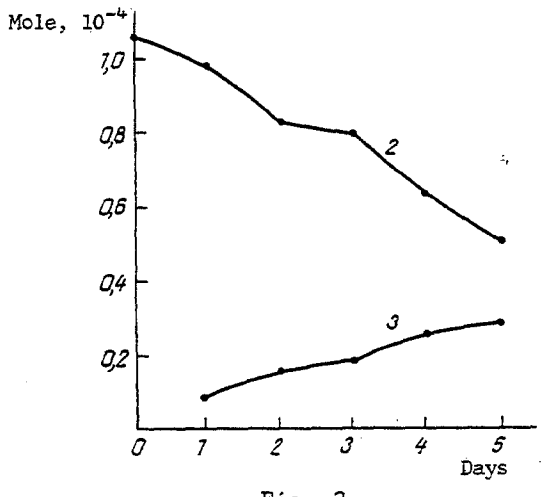

Fig. 2

Fig. 1. Amounts of veratrylpropan-1-ol (1) and the metabolites veratrylpropan-1-one (2) and vanillic acid (3) in a T. cutaneum culture filtrate.

Fig. 2. Amounts of the substrate veratrylpropan-1-one (2) and the metabolite vanillic acid (3) in a T. cutaneum culture filtrate.

(5) and its subsequent oxidation and demethylation to vanillic acid (3) (route B). However, the reactions observed with veratrylpropan-1-one (2) confirmed the probability of route A (Fig. 3).

The biotransformation of guaiacylpropan-1-ol (6) took place considerably faster (Fig. 4). As early as the fifth day of incubation only trace amounts of the substrate remained in a culture filtrate.

The biodegradation of guaiacylpropan-1-ol (6), just like that of veratrylpropan-1-ol (1), took place through the oxidation of the C_α atom with the formation of guaiacylpropan-1-one (7) (Fig. 5). However, the dominating compound among the metabolites of guaiacylpropan-

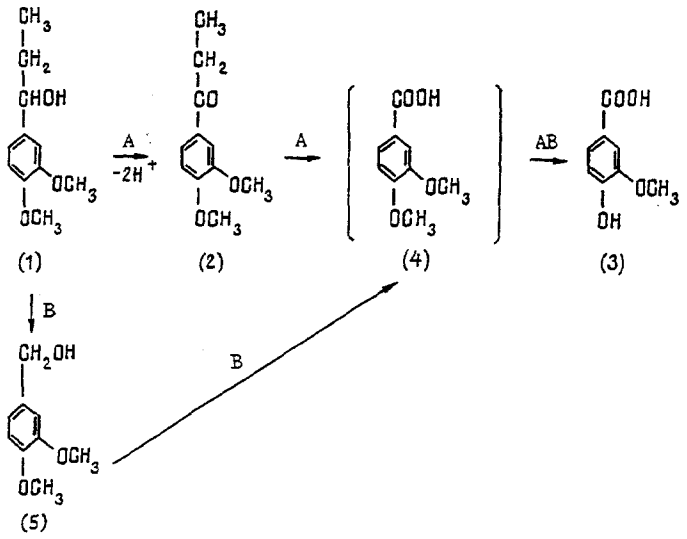

Fig. 3. Possible routes in the degradation of veratrylpropan-1-ol (1) under the action of T. cutaneum. The meanings of 1, 2, and 3, are the same as in Fig. 1; 4) veratric acid; 5) veratryl alcohol.

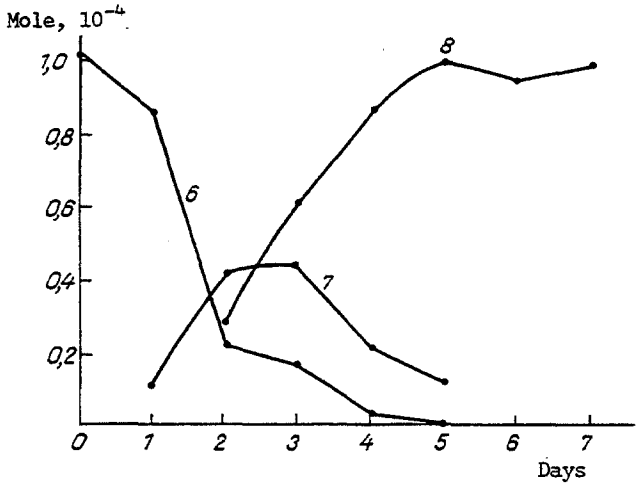

Fig. 4. Amounts of guaiacylpropan-1-ol (6) and of the metabolites guaiacylpropan-1-one (7) and methoxyhydroquinone (8) in a culture filtrate of Trichosporon cutaneum.

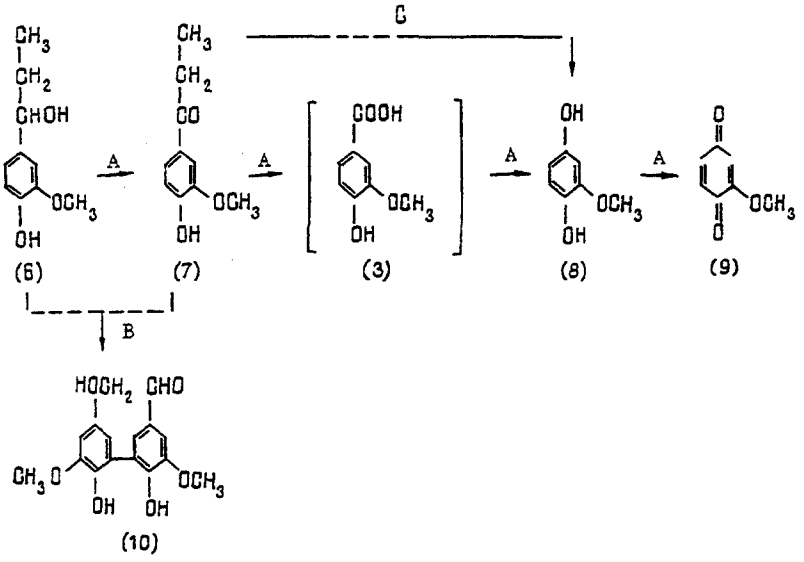

Fig. 5. Possible routes of the degradation of guaiacylpropan-1-ol (6) under the action of Trichosporon cutaneum. The meanings of 3, 6, 7, and 8 are the same as in Fig. 1: 9) methoxyquinone; 10) dimer.

1-ol (6) proved to be methoxyhydroquinone (8), which was not detected in the degradation of veratrylpropan-1-ol (1). On the sixth day its yield amounted to 91%. Minor metabolites were isolated individually and were accumulated with the aid of preparative column chromatography. One of them was identified by HPLC and mass spectrometry as methoxyquinone (9), and for a second one we chose structure (10) on the basis of mass-spectrometric results.

The composition of the metabolites shows that the biodegradation of guaiacyl-substituted substrates takes place through the cleavage of the C_α–C_β bond, which is realized equally readily for compounds containing C_α–OH (6) and for those containing C_α=O (7) (route A, Fig. 5). The subsequent degradation of the C_6–C_1 intermediates formed may be accompanied by decarboxylation (route A) or by condensation to form a C–C bond (route B). However, the suggested route B cannot be regarded as having been established, since we have been unable to identify the intermediate metabolites leading to the formation of the biphenyl (10). It is not excluded, either, that the biphenyl (10), like the methoxyquinone (9) may be formed as a secondary product in the process of chromatographic isolation.

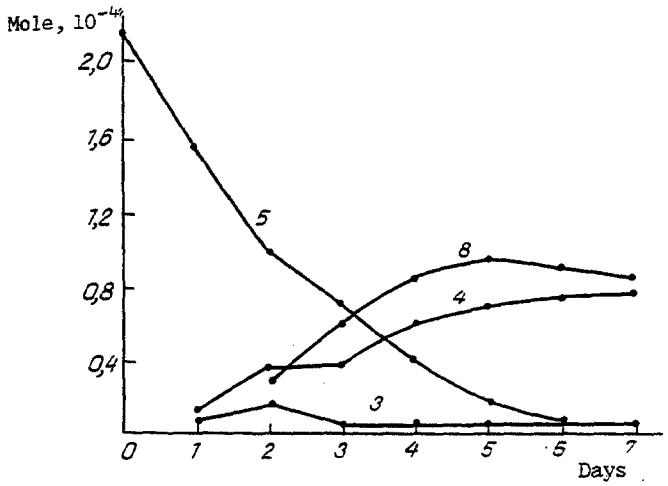

Fig. 6. Amounts of the substrate veratryl alcohol (5) and metabolites in a culture filtrate of Trichosporon cutaneum. The meanings of 4 and 8 are the same as in Figs. 3 and 5.

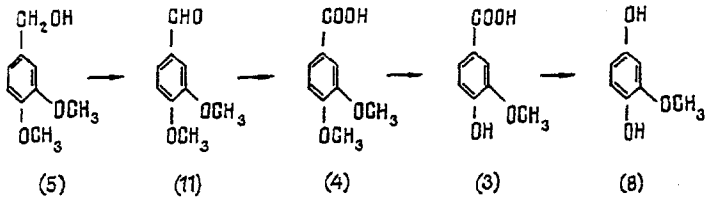

Fig. 7. Possible routes in the degradation of veratryl alcohol under the action of Trichosporon cutaneum. The meanings of 3, 4, and 8 are the same as in Fig. 3; 11) veratraldehyde.

The absence of vanillic acid (3) from among the metabolites of guaiacylpropan-1-ol (6) permits the assumption of one more route of the degradation of a phenylpropane structure — alkylphenyl cleavage (route C, Fig. 5).

As is known, the rupture of the C_α–C_β bond, alkylphenol cleavage, is a function of lignase — a complex of enzymes capable of catalyzing the degradation of lignin [2-4]. One of the tests for lignolytic activity is the oxidation of veratryl alcohol (5) to veratraldehyde (11) [5]. To check the possibility that T. cutaneum synthesizes a ligninase, we studied the biodegradation of veratryl alcohol (5).

It was found that veratryl alcohol (5) was readily included in the metabolism of T. cutaneum, and on the fourth day only 18% of it remained in the culture filtrate (Fig. 6). The main metabolites were identified as veratric acid (4), methoxyhydroquinone (8), and vanillic acid (3). Veratraldehyde (11) was detected in the culture filtrate only in small amounts. This made it possible to assume that the ligninase activity of T. cutaneum is very low. However, the actively occurring cleavage of the C_α–C_β bond indicated that either the enzyme catalyzing this reaction does not test for veratryl alcohol or the enzyme complex of T. cutaneum also contains an oxidase which very rapidly oxidizes the veratraldehyde (11) formed to veratric acid (3).

It may be assumed that the biotransformation of veratryl alcohol takes place as the series of successive oxidation, demethylation, and decarboxylation reactions shown in Fig. 7.

The results obtained permit us to consider that for the substrates veratrylpropan-1-ol (1), veratrylpropan-1-one (2), and guaiacylpropan-1-ol (6) (Fig. 5) biodegradation by route A (Figs. 3 and 5), i.e., through the stage of the formation of the acid (3) with its subsequent decarboxylation, is the most probable. It is interesting to note that demethylation takes place only at the level of C_α-carboxyl-containing compounds.

EXPERIMENTAL

A culture of Trichosporon cutaneum D-46 was maintained on wort-agar medium with the addition of hydrolysis lignin (0.5-1%). For inoculation, the culture was grown in Czapek medium (pH 5.0-5.5) containing 0.25% of added glucose with constant stirring at T = 30°C for 24 h.

In each of a number of Erlenmeyer flasks, 0.6-1.0 mg of the substrate was dissolved in 40 ml of Czapek medium and, after the addition of 4 ml of inoculate (10%), incubation was carried out as described above. Samples with a volume of 600-800 µl were taken every day, centrifuged (10,000g), and analyzed by reversed-phase HPLC on a Milikhrom chromatograph. The column (2 × 64 mm) was filled with the sorbent Silasorb-5-C_{18} (Lachema). The volume of sample added to the column was 20 µl, and mixtures of water and methanol were used for elution, which was conducted in a gradient regime with increasing concentrations of methanol: 5% methanol – 300 µl; 20% – 500 µl; 40% – 300 µl; 70% – 1000 µl. A pH of the eluent of 3.5 was created with phosphate buffer solution. The rate of flow of the eluents was 100 µl/min. The components of the mixture were detected at λ 280 nm. The qualitative analysis of the compounds was done by comparing their RTs with those of standard specimens and from spectral ratios, and amounts were calculated from the areas of the peaks on the chromatograms.

The preparative isolation of the products of biodegradation was carried out on a column (0.5 × 16 cm) filled with Kieselgel 60 (Merck, 70-230 mesh). The eluent consisted of mixtures of acetone and hexane the proportion of acetone in which ranged from 0 to 10%. The fractions isolated were analyzed on a LKB-2091 chromato-mass spectrometer with the use of a capillary column 30 m long containing the phase SE-30. The temperature was programmed from 100°C at the rate of 4°C/min. The ionization energy was 70 eV.

Mass spectrum of (10), m/z (%): 304 (M^+ 26), 276(16), 275(100), 273(12), 181(9), 180(9).

Mass spectrum of (9), m/z (%): 138 (M^+ 57), 110(30), 108(49), 95(28), 83(31), 69(100), 54(50).

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