

Dioxygenases Activity of Wood-destroying White-rot Fungi, Isolated from Woody Biogeocenosis

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ABSTRACT: Dioxygenases are enzymes which catalyse the activation of dioxygen from the atmosphere and use it to effect a wide variety of remarkable reactions, including the reaction of aromatic ring cleavage. In this work we studied the ability of wood-destroying white-rot fungi to dioxygenase synthesize. Dioxygenases activity of filamentous cultures of 26 strains of 14 species, relevant to 10 genera of wood-destroying white-rot fungi, isolated from Tugay forests of Azerbaijan, were studied. Pyrocatechase and metapyrocatechase activity of the studied fungi was absent. 20 strains showed gentisinate-1,2-dioxygenase activity, 13 out of which showed protocatechuate-3,4-dioxygenase and protocatechuate-2,3-dioxygenase activity. Apparently, these wood-destroying white-rot fungi are able to split aromatic ring, having a side group. Six strains of fungi *Ganoderma lipsiense* BDU 18 and BDU 62, *Phellinus igniarius* BDU 53 and BDU 154, *Schizophyllum commune* BDU 12, *Trametes pubescens* BDU 32 haven't shown activity of dioxygenases. Probably, they perform degradation of the lignin molecule to oligomers and monomers without further splitting of aromatic ring.

KEYWORDS: Wood-destroying fungi, dioxygenases activity, splitting, aromatic ring.

I. INTRODUCTION

Production of saccharine matter from plant substrates by means of enzymatic way is still vital [11,18,19]. Natural cellulose containing materials, as a rule, possess low reactivity because of high concentration of non-cellulose substances, especially lignin that complicates realization of enzymic hydrolysis [1,8,16]. In this connection, it is important to study possibility of prevention of inhibitory action of lignin by means of preliminary biodelignification of plant substrates, for the purpose of deeper enzymic hydrolysis of cellulose component. A special attention is given to biodelignification of lignocellulose substrates by using of wood-destroying white-rot fungi, that decompose lignin component of timber [7, 9,16].

Ligninase system of wood-destroying fungi consists of multienzyme complex that affect polymeric molecule of native lignin, performing its initial decomposition. They include laccase, tyrosinase, peroxidase and manganese depending peroxidase. A number of research works was dedicated to the study of lignin complex enzymes. In the issue, it comes out that wood-destroying fungi possess a great number of phenoloxidase and peroxidase, a part of which takes part in depolymerization of lignin [5, 9,13,22].

As monomers of lignin are aromatic compounds (for example, coniferyl alcohol), complete decomposition of lignin must occur together with the cleavage of aromatic ring. It is known that the breakup of aromatic ring is performed by dioxygenases, that attach two molecules of oxygen to substrate [12,17]. Existence of some dioxygenases of wood-destroying fungi was shown in a number of research works [2, 10, 15, 21].

The main aim of the given research paper was to study dioxygenases activity of wood-destroying white-rot fungi, isolated from Tugay forests of Azerbaijan.

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II. MATERIALS AND METODS

Filamentous cultures of 26 strains of wood-destroying white-rot fungi, isolated from Tugay forests of Azerbaijan, were used as an object of research: *Bjerkandera fumosa* (Pers.:Fr.)P.Karst, *Fomes fomentarius* (L.:Fr.)Gill., *Ganoderma lipsiense* (Batsch.)G.F., *Lenzites betulina*(Fr.)Fr. , *Panus tiqrinus* (Fr.)Sing., *Phellinus igniarius* (L.:Fr.)Quel., *Pleurotus ostreatus* Jac.:Fr.)Kumm.,*Schizophillum commune* Fr.,*Trametes cervina* (Schwein.) Bress., *T. gibbosa* (Pres.)F.r, *T. hirsuta* (Wulfen:Fr.) Pilat, *T. ochracea* (Pers.) Gibb .et . Ryvarde n,*T. versicolor* (L.:Fr.)Pilat. Fungal cultures were kept in wort-agar medium (4-5 points), pH 5,0 and replanted once in six months. In order to study enzymatic activity fungi were cultivated in submerged culture on shaker 180 rpm during 96 hours at 28° and 37°C in the medium with the following composition (g/l): wheat bran -15; NH₄NO₃ -1,5; KH₂PO₄ – 0.4; NaCl-0,5; MgSO₄ - 7H₂O- 0,5; pH-5.0-5,5. As an inoculum was used three-day fungi mycelium, crushed in a magnetic stirrer for 15 minutes.

Dioxygenases activity was determined in cell-free extracts of fungi. Cell-free extracts were gained by grinding the washed by 0.05M phosphate buffer solution (pH 4.7) biomass on tissue microblender RT-2 at 5000 rpm (3 times 3 min each with cooling). Homogenate was centrifuged 20 min at 10000 rpm and supernatant was used as a cell-free extract. Determination of enzyme activity was carried out spectrophotometrically by using Specord UV vis (German). The system for determination of pyrocatechase (catechol-1,2-dioxygenase, EC 1.13.11.1)

contained: 0.1 M catechol- 50 mkl, 0,002 M etilendiamin-tetrachloroacetic acid – 100 mkl, cell-free extract -100-400 mkl and the volume was adjusted to 3 ml by means of 0.01 M phosphate buffer (pH 7.5). The measurements were carried out at λ max 250 nm by increase the optical density [12]. The activity of metapyrocatechase (catechol- 2,3-dioxygenase, EC 1.13.11.2) was evaluated by the increase of optical density at λ max 375nm [14].The activity of protocatechuate-3,4-dioxygenase (EC 1.13.11.3) was determined by the a decrease of optical density at λ max of 290 nm [6]. The activity of protocatechuate - 2,3 - dioxygenase (EC 1.13 11.8) was evaluated by the increase of optical density at λ max 375nm [3].The activity of gentisinate -1.2- dioxygenase (EC 1.13.11.4) was estimated by increase of the a absorption of λ max at 334nm[4].Activity of all dioxygenases was expressed in nanomoles of reaction product that formed in 1 min, based on 1 mg of protein (unit= nmol/min/mg protein). The protein content in the cell-free extract was determined spectrophotometrically [20].

III. RESULTS AND DISCUSSIONS

The study of the activity of aromatic ring cleaving enzymes (dioxygenases) has shown that in cell-free extracts of all the studied fungi, pyrocatechase (PC) and metapyrocatechase (MPC) are absent. With the exception of *Bjerkandera fumoza* BDU 60 and BDU 63 strains of wich have shown lowermost activity of ortho- cleaving enzym (pyrocatechase) of aromatic ring (table).

Table . Dioxygenases activity of wood-destroying basidial white- rot fungi

FUNGI	Enzyme activity, nmol / min /mg of protein				
	PC	MPC	P-3,4-D	P-2,3-D	G-2,3-D
<i>Bjerkandera fumosa</i> BDU 60	1,8±0,06	0,0	18,7±0,4	12,6±0,6	37,5±1,2
<i>B. fumosa</i> BDU 63	1,4±0,01	0,0	22,4-0,5	11,1±0,1	44,2±0,1
<i>Fomes fomentarius</i> BDU 31	0,0	0,0	0,0	0,0	3,2±0,07
<i>Ganoderma lipsiense</i> BDU 18	0,0	0,0	0,0	0,0	0,0
<i>G.lipsiense</i> BDU 62	0,0	0,0	0,0	0,0	0,0
<i>Lenzites betulina</i> BDU 33	0,0	0,0	0,0	0,0	12,4±0,5

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<i>L.betulina</i> BDU 110	0,0	0,0	0,0	0,0	8,8±0,3
<i>Panus tigrinus</i> BDU 45	0,0	0,0	6,4±0,2	2,1±0,6	33,±1,2
<i>P.tigrinus</i> BDU 43	0,0	0,0	2,3±0,1	1,3±0,04	28,2±1,2
<i>Phellinus igniarius</i> BDU 53	0,0	0,0	0,0	0,0	0,0
<i>Ph. igniarius</i> BDU154	0,0	0,0	0,0	0,0	0,0
<i>Pleurotus ostreatus</i> BDU 22	0,0	0,0	0,0	±	10,8± 0,5
<i>Pl. ostreatus</i> BDU 44	0,0	0,0	±	±	14,5± 0,6
<i>Schizophillum commune</i> BDU12	0,0	0,0	0,0	0,0	0,0
<i>Trametes cervina</i> BDU 21 7,8±0,3	0,0	0,0	0,0	0,0	7,8±0,3
<i>T. cervina</i> BDU26	0,0	0,0	0,0	0,0	5,3±0,2
<i>Trametes gibbosa</i> BDU 7	0,0	0,0	12,4±0,3	11,0±0,3	14,6±0,6
<i>T. gibbosa</i> BDU 19	0,0	0,0	14,8±0,6	8,4±0,4	18,4±0,8
<i>T.hirsuta</i> BDU 8	0,0	0,0	37,6±1,1	24,1±1,2	58,5±2,4
<i>T.hirsuta</i> BDU 14	0,0	0,0	29,8±1,2	32,8±2,2	48,9±2,2
<i>T. ochracea</i> BDU 6	0,0	0,0	28,3±0,6	18,1±0,8	127,8±4,2
<i>T.ochracea</i> BDU 16	0,0	0,0	18,9±0,7	10,7±0,4	118,4±3,7
<i>T.pubescens</i> BDU 32	0,0	0,0	0,0	0,0	0,0
<i>T. versicolor</i> BDU 21	0,0	0,0	6,4±0,2	2,2±0,04	42,2±1,9
<i>T.versicolor</i> BDU 38	0,0	0,0	8,2±0,4	4,1±0,2	41,8±2,0
<i>T.versicolor</i> BDU 88	0,0	0,0	4,1±0,1	6,9±0,2	33,3±1,3

Note: PC- pyrocatechase; MPC- metapyrocatechase; P-3,4-D- protocatechuate- 3,4-dioxygenase ; P-2,3-D - protocatechuate -2,3- dioxygenase ; G -2,3 -D- gentisinate - 2,3- dioxygenase

The activity of protocatechuate-3,4-dioxygenase (P-3,4-D) and protocatechuate-2,3- dioxygenase (P-2,3-D) was observed in *Bjerkandera fumosa* BDU 60 and BDU 63, *Trametes gibbosa* BDU 7 and BDU 10, *T. hirsuta* BDU 8 and BDU 14, *T. ochracea* BDU 6 and BDU 16, *T. versicolor* BDU 21 and BDU 38, *Panus tigrinus* BDU 42 and BDU 45. The highest activity of both enzymes was displayed by strains of *Trametes hirsuta*, the P-3,4-D и P- 2,4-D activity of which were, accordingly, 1,7-2,6 and, 9-3,0 times higher than *B. fumosa*, 4,6 – 11,7 and 3,5-14,9 times higher than *T. versicolor*, 1,3-2,5 and 1,3-3,1 times higher than *T. ochracea*, 5,9-21,0 and 11,5-25,2 times higher than *P. tigrinus*, 2,5-3,9 and 2,2- 3,9 times higher than *T.gibbosa* (table).

Gentisinate - 2,3- dioxygenase (G-2,3-D) activity was observed in most of the fungi and high activity was noted in *B.adusta*, *T. hirsuta*, *T. versicolor* and *P. tigrinus* . According to the availability of dioxygenases, all the fungi are subdivided into four groups: those having PC, P-3,4-D, P-3,4-D and G-2,3-D activity (*B. fumosa* BDU 60 and BDU 63); those having P-3,4-D, P-2,3-D and G-2,3-D activity (*T. gibbosa* BDU 7 and BDU19, *T.hirsuta* BDU 8 and BDU 14, *T. ochracea* BDU 6 and BDU 16, *T. versicolor* BDU 21 , BDU 38 and BDU 88, *P. tigrinus* BDU53 and BDU 154); those having only G-2,3-D activity (*F. fomentarius* BDU 31, *L. betulina* BDU 33 and BDU110, *P. ostreatus* BDU 22 and BDU 19, *T. cervina* BDU 21 and BDU 26); those not having activity of enzymes of aromatic ring cleavage (*G.lipsiense* BDU 18 and BDU62 *Ph. igniarius* BDU53 and BDU154, *Sch.commun*BDU12, *T. pubescens* BDU 32).

Majority of active fungi had higher G-2,3-D activity degree than that of other dioxygenases. So, G-2,3-D activity of *B. fumosa* BDU 60 and BDU 63 was 13,4-31,5; 1,9-2,0 and 3,0-4,0 times higher than that of PC, P-3,4-D и P -2,3-D activity, accordingly. G-2,3-D activity of *P.tigrinus*, *T. hirsuta*, *T. ochracea* and *T.versicolor* was 1,6-12,2 and 1,5-21,7

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times higher than that of P-3,4-D and P-2,3-D activity, accordingly. P-3,4-D, P-2,3-D and G-2,3-D activity degree of strains of *T.gibbosa* did not show any essential difference.

It is important to mention that fungi studied in the given research work are known wood -destroying basidial white-rot fungi and they are able to decompose lignin [8]. Absence of aromatic ring cleavage enzyme activity in *G. lipsiense*, *Ph.igniarius*, *Sch. Commune*, evidently indicates that these fungi perform destruction of lignin molecules to oligomers and monomers without splitting of aromatic ring. *B. fumosa*, *P. tigrinus*, *T.gibbosa*, *T.hirsuta*, *T. ochracea*, *T.versicolor* are able to split aromatic ring, which has a side group (for instance, -COOH; -CH₂COOH and etc.). *F. fomentarius*, *L. betulina*, *Pl.ostreatus* and *T. cervina* perform splitting of aromatic ring only by means of gentisic acid (table).

It is known that coniferyl alcohol, sinapic alcohol and coumaric alcohol. As monomers of lignin have a side group in an aromatic ring [9]. That is possibly why the studied fungi do not possess pyrocatechase and metapyrocatechase activity, splitting aromatic ring of catechol, not having a side group.

IV. CONCLUSION

Thus, *G. lipsiense* BDU 18 and BDU 62, *Ph. igniarius* BDU 63 and BDU154, *Sch. commune* BDU12, *T. pubescens* BDU 32 wood-destroying white-rot fungi, known as lignin-destroying fungi, are not able to split the aromatic ring. It seems to be that these fungi perform destruction of lignin molecules to oligomers and monomers with no further splitting of aromatic ring. Majority of the studied white rot fungi had an activity of only P-3,4-D, P-1,2-D and G-2,3-D enzymes. Therefore, these fungi are able to decompose lignin with further splitting of aromatic ring, having a side group.

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