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Evaluation by the Oxidase Activity of Xylotropic Macromycetes Causing White Decay

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Abstract

In researches, were evaluated 103 strains of 25 species of xylotrophic micromycetes causing white decay which spreads in ecologically different regions of Azerbaijan for the synthesis of phenoloxidases (laccase, peroxidase, and ligninase). It was determined that strains belonging to species such as *Cerena unicolar, Pleurotus ostreatus, Schizophyllum commune* and *Trametes versicolor* are more actively synthesized phenoloxidase and this process generally occurs by the inductive way. Despite this, unlike from classic inductive enzymes basal level of phenoloxidases in the basidiomycetes is much higher, which can be characterized as the sign adaptation of xylotroph macromisets to live in woody substrates.

Keywords

Xylotrophic Macromycetes, White Decay, Phenol Oxidase, Unitary Theory

1. Introduction

As is known, mushrooms are one of the research objects in the center of special attention, such as biologically active substances, as well as the producer of enzymes which have a different effect [1]. So that, the synthesis of enzymes that catalyzes the degeneration of polymers such as cellulose, lignin, and hemicellulose which form in green biomass resulting from photosynthesis has increase interest to them [2] [3]. In this regard, the studies where an active producer of these enzymes was found, clarified a number of issues of enzyme synthesis [4] [5] and some results have even been applied to the industrial. However, fungi investigated in this direction constitute a small part of those known ones and

some issues related to the synthesis regulating of some enzymes have not been clarified yet [6] [7] as well as producers with high biological activity is in the minority today.

Mushrooms are a permanent component of every ecosystem where organic matter is present and carry out various functions there, more accurately are active participants of all processes (production, destructions, arrangements, and indications) taking place in the ecosystem [8]. The basis of the use of these mushrooms in practice stays their active participation in these functions [9]. In addition to the variety of functions carried out by mushrooms in nature, the ecological groups that they formed in nature are also characterized by a wide variety and they are some features directed to the practice are related to this grouping. One of the ecological groups of mushrooms is the xylotrophs, which their main habitat and feeding areas are woody plants (trees, as well as a number of shrubs) [2] [5] [10].

Although there is no accurate literature information about the number of xylotrophic macromycetes, can be noted that about 1000 species of mushroom are carried such features. Their main spreads areas are natural and artificial forests [11], green areas, gardens, and parks.

All of the above mentioned are found in the territory of the Republic of Azerbaijan and 212 species of xylotrophic macromycetes have been found in trees and shrubs at these areas [4] [12] [13]. Have been clarified some issues such as about synthesis of hydrolase and oxidase enzymes by some of the mushroom and regulation of synthesis in selected mushroom as an active producer. Be changeable of activity indicators in the level of strain, peculiar to the this or another producer, also takes part of this in a similar situation in the catalytic activity of these enzymes, makes necessary to study those mushroom. Taking this into account, the presented work was devoted to the evaluation of xylotroph macromycetes spread in the nature of Azerbaijan by the activity of enzymes such as phenol oxidase, more accurate laccase, peroxidase, and lignin. In research, the selection only phenoloxidase is related to the fact that, were widely conducted investigations on the study of xylotroph macromycetes which spread in Azerbaijan and compared to them phenoloxidase, especially ligninaza was not subject to special investigation.

2. Material and Methods

The research has begun from 2016 and the samples taken for research have covered all major geomorphological units (Greater Caucasus—GC, Kura Araz lowland—KA, Talish Mountains—TM and Small Caucasus—SG) of Azerbaijan (**Figure 1**).

Taking of sampling, initial passportization, and preparation for laboratory analysis was carried out according to known methods as well as those used in previous studies [4] [13]. Examples from the study areas area were taken from trees and arms only because of their vital shape. Sampling was carried out in accordance with the methods of the route and permanent observation areas (100 m × 100 m).

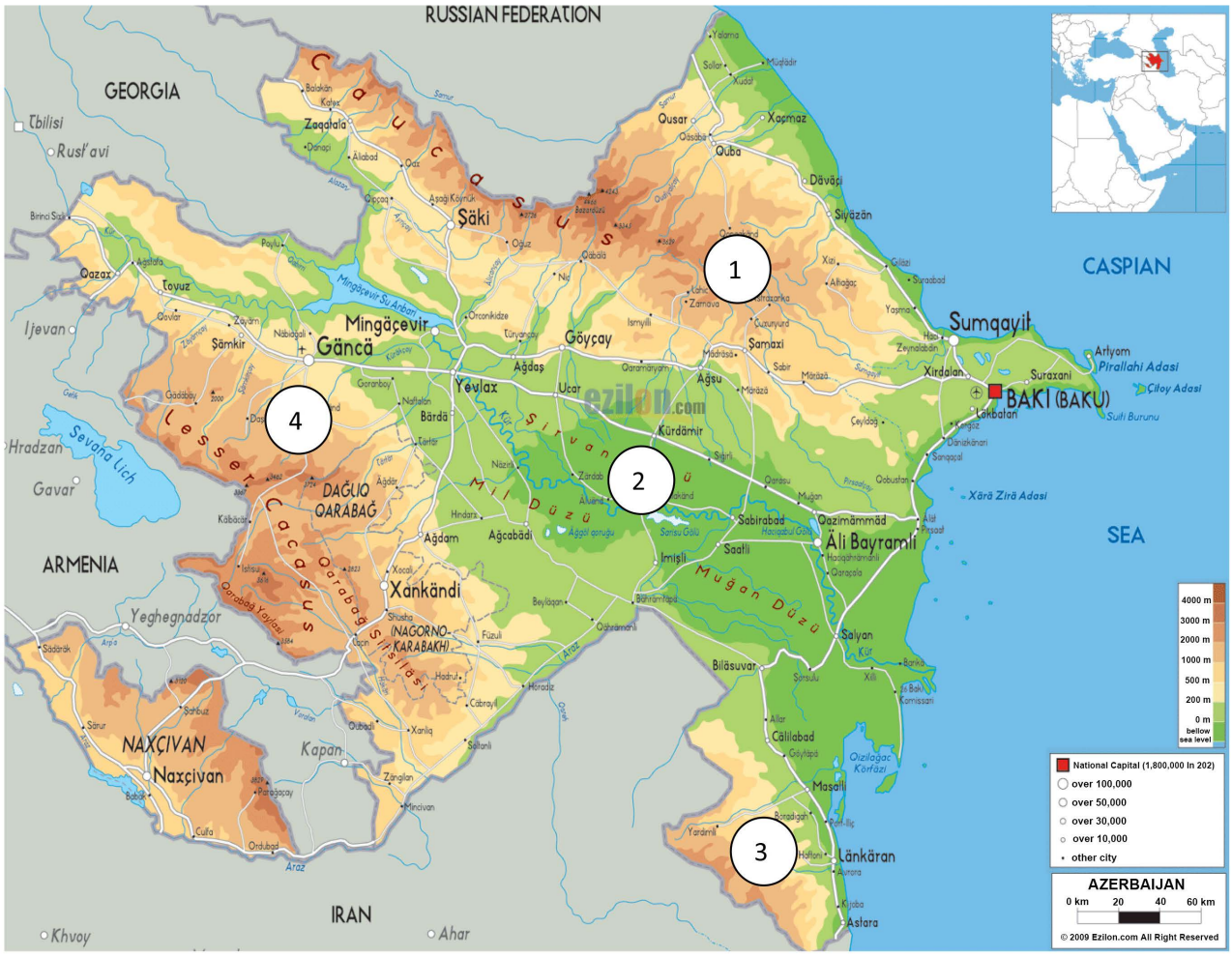

Figure 1. An overview of the surveyed areas. 1—Greater Caucasus; 2—Kura Araz lowland; 3—Talish Mountains and 4—Small Caucasus.

During the identification of mushrooms, the determinant prepared was used based on morphological, culturological, and physiological symptoms of mushrooms [14] [15].

During the cultivation of separated cultures, initial cultivation was carried out in a liquid nutrient medium, which content of the nutrient medium used for this purpose was: glucose—10; peptone—3; NH_4NO_3—1.5; NaCl—0.5; $MgSO_4 \cdot 7H_2O$; K_2HPO_3—0.4; $FeSO_4$—in small quantities, ordinary water—1 L. Sterilization condition—0.5 atm, 0.5 hours, acidity of the environment after sterilization 5.5 - 5.7. As the primary sowing material was used from quadratic particles taken from the colonia of mushroom cultures cultivated in a standard nutrient medium. The biomass obtained in this way is cut into small pieces by using a magnetic mixer is used as a sowing material.

The activity of phenoloxidases was determined by spectrophotometrically way. In the course of the study, to determining the activity of peroxidase and laccase as a substrate uses 1% hydroxynone solution and the activity of the enzymes is determined by the optical density change in the 240 nm wavelength

after adding a cultural solution (CS) to that substrate. When determining peroxidase activity in addition to reaction mixture is added H_2O_2. During the determination of ligninase activity was used 2% veratryl alcohol. During determining the acellular and intracellular activity of enzymes were used the approach used in our previous work.

During the determination of fermentative activity, the amount of proteins was performed spectrophotometric way [16].

All experiments were performed in 4 - 6 replicates and the obtained results were statistically processed [17]. In all cases, the results corresponding to the formula of $m/M = P \leq 0.05$ were considered honest and included in the dissertation. Here, M—is the average price of repetitions, m—is mid-square dislocation mid-square displacement and P—is confidence level.

3. The Results and Discussion

To achieve the goals of the study were taken to the pure culture xylotrophic macromycetes that spreads in natural forest ecosystems located in different ecological areas of Azerbaijan and was created their collection. As a result, was created a collection from 103 strains which their characteristic by species specificity, area distribution, and substrate affiliation are shown in **Table 1**. As see, the registered strains belong to 25 species of xylotrophic macromycetes but there is a certain difference with the ability to spread in ecologically different regions of Azerbaijan and substrate attitude. True, although most of them belong to evritropes, among of them are meets conditional stenotrophs.

In connection with the question about the table should be noted one issue. As seen, for the isolate of strains were used mainly from the fruit body of xylotrophic macromycetes cause white decay in natural conditions. The reason for this is characterized by the ability to the active realization of degradation of both cellulose and lignin in natural conditions of species xylotrophic macromycetes cause white decay. More specifically, due to the fact that both oxidases and hydrolases involved in their enzyme system, the strains needed for the research were separated from them.

From the studies conducted to assess the ability of the isolated strains to synthesize phenoloxidases became clear that, all of the tested strains have the ability to effectively synthesize the enzymes studied in this or that extent (**Table 2**), but they differ from each other by the level of activity. For example, if the activity of laccase, peroxidase, and ligninase in the mushroom *Ganoderma applanatum S*-17 was 70.6, 50.3 and 58.9 ui/ml, respectively, the similar indication in the *Lentinus tigrinus FS*-28 was 60.5, 45.3 and 54.5 ui/ml. Similar differences are also observed among other fungi.

Despite these differences, the activity calculated based on single biomass in mushrooms such as *Cerena unicols*, *Pleurotus ostreatus*, *Schizophyllium commune* and *Trametes versicolor* is higher than other mushrooms. For example, in *Bjerkandera adusta P*-40 10.2 g/l, the activity of laccase, peroxidase and ligyninase is respectively 85.1 ui/ml, 62.2 ui/ml and 58.3 ui/ml. The unit of enzyme

Table 1. Species belonging spreads area and substrate relations of strains taken to the pure culture.

№	Fungi species which was isolated strains	The number of isolated strains	Geomorphological units in which the species spread and relation to the substrate
1	*Armillaria mellea*	4	GC, SC, TM, eurytrophy
2	*Bjerkandera adusta*	5	GC, SC, TM, eurytrophy
3	*Cerena unicolar*	5	GC, SC, TM, eurytrophy
4	*Daedalea quersina*	3	GC, SC, TM, conditionally stenotroph
5	*Daedaleopsis confragosa*	3	GC, SC, TM, conditionally stenotroph
6	*Flamulinna velutipes*	3	GC, SC, TM, conditionally stenotroph
7	*Fomes fomentarius*	5	GC, SC, TM, KA, eurytrophy
8	*Ganoderma applanatum*	5	GC, SC, TM, KA, eurytrophy
9	*G.lucidum*	4	GC, TM, eurytrophy
10	*Heteroporus pergamenus*	3	GC, SC, TM, KA, eurytrophy
11	*Lentinus strigosus*	4	GC, SC, TM, eurytrophy
12	*L.tigrinus*	3	GC, SC, TM, eurytrophy
13	*Lenzitez betulina*	4	GC, SC, TM, eurytrophy
14	*Phellinus igniarus*	5	GC, SC, TM, KA, eurytrophy
15	*Ph.pomaceus*	4	GC,SC,TM,KA, conditionally stenotroph
16	*Ph.torulosus*	3	GQ, TM, şərti stenotrof
17	*Pycnoporus cinnabarinus*	3	GC, TM, eurytrophy
18	*Pleurotus ostreatus*	5	GC, SC, TM, KA, eurytrophy
19	*Polyporus suqamozus*	5	GC, SC, TM, eurytrophy
20	*Pseudotrametes gibbosa*	5	GC, SC, TM, eurytrophy
21	*Schizophyllium commune*	5	GC, SC, TM, KA, eurytrophy
22	*Stereum gausapatum*	3	GC, TM, conditionally stenotroph
23	*St.hirsitum*	4	GC, SC, TM, KA, eurytrophy
24	*Trametes hirsuta*	5	GC, SC, TM, KA, eurytrophy
25	*T.versicolor*	5	GC, SC, TM, KA, eurytrophy

Note: GC—Greater Caucasus; KA—Kura Araz lowland; TM—Talish Mountains and SG—Small Caucasus.

activity in 1 g of biomass is respectively 8.34 ui/ml, 6.10 ui/ml and 5.72 times. The analogous indicator for *T.versicolor R*-24 is 9.47 ui/ml, 6.45 ui/ml and 8.65 ui/ml. For this reason, they were selected as active producers for further research. Interestingly, all these noted mushrooms are closely related to ecological-trophic relationships and are characterized mainly as polytrophc. More intensively the synthesis of phenoloxidase by polytrophic mushroom may be related with one of the indicators of their high adaptability.

From the carried out of research related to defining factors affect to the enzyme synthesis in selected strains, first of all, to clarify the impact of the carbon source, became clear that studied phenoloxidases by nature of synthesis carry the

Table 2. Evaluation of the strains used in the studies by the oxidase activity (iu/ml).

	Fungi species which was isolated strains	Biomass q/l	Laccase	Peroxidase	Ligninase
1	*Armillaria mellea*	6.8 - 9.7	62.2 - 71.2	45.7 - 51.3	50.2 - 56.2
2	*Bjerkandera adusta*	8.2 - 10.2	75.8 - 85.1	50.3 - 62.2	54.8 - 58.3
3	*Cerrena unicolar*	8.8 - 10.1	87.7 - 98.6	55.8 - 66.3	59.8 - 68.9
4	*Daedalea quersina*	7.7 - 9.2	66.0 - 75.1	45.3 - 50.8	53.7 - 60.4
5	*Daedaleopsis confragosa*	6.7 - 7.4	65.4 - 78.6	46.8 - 54.3	49.2 - 57.3
6	*Flamulinna velutipes*	8.1 - 9.1	58.1 - 68.3	37.0 - 48.3	45.7 - 59.6
7	*Fomes fomentarius*	8.7 - 10.4	64.2 - 84.9	45.5 - 59.3	63.8 - 84.8
8	*Ganoderma applanatum*	6.9 - 9.3	62.2 - 70.6	47.1 - 53.3	52.1 - 60.6
9	*G.lucidum*	7.5 - 8.6	56.2 - 65.1	32.0 - 45.9	40.9 - 56.7
10	*Heteroporus pergamenus*	7.7 - 9.2	57.1 - 64.5	32.4 - 43.3	40.8 - 55.1
11	*Lentinus strigosus*	7.9 - 9.1	53.2 - 65.3	32.1 - 48.9	41.1 - 55.7
12	*L.tigrinus*	6.9 - 8.8	57.8 - 64.5	42.5 - 50.3	52.5 - 56.5
13	*Lenzitez betulina*	5.5 - 7.7	54.3 - 64.3	31.5 - 47.2	39.9 - 58.3
14	*Phellinus igniarus*	6.1 - 7.8	54.0 - 66.4	34.6 - 47.5	40.3 - 55.4
15	*Ph.pomaceus*	7.1 - 8.1	51.9 - 60.5	30.9 - 47.2	39.8 - 58.7
16	*Ph.torulosus*	7.0 - 9.1	53.2 - 64.2	32.1 - 46.2	40,9 - 55.9
17	*Pycnoporus cinnabarinus*	5.1 - 6.1	51.2 - 62.3	30.5 - 45.4	39.3 - 57.4
18	*Pleurotus ostreatus*	7.8 - 9.5	84.5 - 94.1	50.3 - 64.2	47.5 - 67.1
19	*Polyporus suqamozus*	6.8 - 8.9	72.7 - 80.6	41.8 - 50.3	50.3 - 58.1
20	*Pseudotrametes gibbosa*	8.6 - 9.8	63.2 - 85.4	46.5 - 58.3	60.7 - 73.5
21	*Schizophyllium commune*	7.4 - 8.8	78.4 - 92.8	50.8 - 60.3	60.2 - 79.5
22	*Stereum gausapatum*	7.6 - 8.9	60.2 - 67.5	38.9 - 44.7	43.5 - 49.5
23	*St.hirsitum*	7.2 - 9.0	58.2 - 69.8	35.8 - 47.7	44.3 - 47.9
24	*Trametes hirsuta*	8.3 - 10.2	74.2 - 90.2	50.2 - 62.3	67.3 - 78.3
25	*T.versicolor*	8.7 - 10.1	77.1 - 95.6	58.9 - 65.1	63.1 - 88.2

Note: iu—International unit.

feature of inductive enzymes. So that, inclusion to the medium compounds holding in the composition lignin formed connections, but are characterized by relatively small degrees of polymerization is causes to raise the activity of all three enzymes this or that degree, which is clearly seen to the data given in the example of mushroom Sch.commune S-21 (**Table 3**). Compared with control, adding as the only carbon source to the medium of several substrates (Wood scrap, clean lignin, cornhorse, etc.) containing lignin in all the cases it leads to an increase in the overall activity of all three enzymes (total acellular and intracellular activity), which is inherent to inductive enzymes [18]. However, this process has slightly different shades than the synthesis of classic inductive enzymes. Thus, by adding any carbon source to the nutrient medium, meets

Table 3. The impact of carbon sours on the synthesis of phenoloxidases in the active producers.

Carbon sources	Laccase		Peroxidase		Ligninase	
	A[1]	B[2]	A	B	A	B
Cerena unicolar M-16						
Hidroxinon	22.1	14.5	10.8	7.5	19.5	14.4
Wood crumbs	32.3	19.5	15.0	10.7	23.5	17.4
Lignin (isolated from rye plant)	43.0	28.5	24.6	14.4	32.4	22.5
Lignin (isolated from ordinary pine)	30.0	20.5	15.4	8.4	23.2	14.7
Cornhorse	49.4	36.4	32.9	18.7	35.1	25.1
Wheat bran	54.3	43.7	32.3	19.5	40.1	27.8
Mannit	44.8	30.4	27.8	16.5	19.7	12.3
Corn extract	47.5	32.1	22.4	13.7	34.7	24.1
Saccharose	18.2	10.1	8.9	5.4	7.0	5.0
Glucose (control)	17.2	9.6	8.2	5.1	6.0	4.2

Note: A—intracellular activity; B—extracellular activity.

with the activity of enzymes. If always observed those activities consider as basal level inherent to inductive enzymes, then the basal level in xylotroph micromycetes is not in the amount to be determined by force as in classical inductive enzymes. That is, in xylotroph micromycetes, inductive synthesis has its own shades, which in our opinion, is due to their adaptability to living in woody substrates. More precisely, xylotrophic macromycetes have gained the character presence of an enzyme that initially initiates the degradation of the lignocellulose complex in the enzyme system and is secreted out of the cell. Since those situation has been observed in other mushrooms it can be noted as a feature peculiar to xylotrophic macromycetes.

4. Conclusion

Thus, from the carried out of research became clear that white decay cause species of xylotrophic macromycetes spread in the ecologically different regions of Azerbaijan can actively synthesize the phenoloxidases involved in the degradation of lignin which finds its highest point in the strains of *Cerena unicolar, Pleurotus ostreatus, Schizophyllium commune* and *Trametes versicolor*. While synthesis of phenoloxidases occurs in an inductive way, in contrast to classic inductive enzymes, in basidiomycetes basal levels are significantly higher which can be characterized as a sign to adaptation living in woody substrates in xylotrophic macromycetes.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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