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ABSTRACT

of the dissertation for the degree
of Doctor of Philosophy

PHYSIOLOGICAL-BIOCHEMICAL REGULATION OF PEROXIDASE ENZYME SYNTHESE IN MICROMYSETS

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INTRODUCTION

Relevance and development of the topic. As we know, land is the most common place for species belonging to different taxonomic groups of living things, but the role of soils does not end by this process.Thus, it is known to perform many physical, chemical and biological informative functions. As a whole, the global function of soils is their impact on the atmosphere, hydrosphere, lithosphere and the Earth's biosphere in general, which allows us to emphasize that the sum of its ecosystem and biosphere functions plays an irreplaceable role in maintaining life on our planet. However, in recent years, the increasing burden of anthropogenic impact on the environment, especially soils, is characterized by intensive land use, which changes the nature of the process of natural soil formation, significantly loses humus layer, and so on. unpleasant processes occur. As a result, the agrophysical, physicochemical and biological properties of soils change, which ultimately leads to a decrease in soil fertility and a decrease in the productivity of crops grown there. Both of these are unacceptable against the background of the current food shortages in the world. Therefore, their elimination is one of the tasks facing various fields of science, especially biology.

From the point of view of solving these issues, it is necessary to look for informative parameters that more accurately reflect the condition of soils, from which point of view it is more important to assess the current ecological condition of soils "*according to biological indicators*".[1] "*The biological activity of the soil is determined by the processes associated with the overpopulation of both plants and soils, microorganisms (bacteria, fungi, protozoa, etc.), as well as their life activities.*"[2] Although all of these organisms have some biological activity, microorganisms, primarily microscopic fungi (micromycetes), have higher levels of activity. This gives us an opportunity to note that, their role of the soil in the formation process is higher, which has been repeatedly confirmed in "*conducted researches*"[3].

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[1] Dobrovolskaya T.G., Zvyagintsev D.G., Chernov I.Yu. et al., The role of microorganisms in the ecological functions of soils, Eurasian Soil Science. 2015. No. 9. S. 1087.

[2] Zvyagintsev D.G., Babieva I.P., Zenova G.M. Soil biology. Moscow: MGU Publishing House, 2005, 445 p

[3] Schulz, S., Brankatschk, R., Dümig, A. et al. The role of microorganisms at different stages of ecosystem development for soil formation, Biogeosciences, 2013, 10, 3983–3996

Only one factshould be mention that "*more than half of the biomass in the soil falls on fungi."*[4] However, certain aspects of the micromycetes' life activities on land have not been fully clarified.

However, certain aspects of the micromycetes life activities on land have not been fully clarified. One of such issues is related to the "*microbiological transformation of organic matter into humus*"[5] in the soil, which is the main process in the formation of soil fertility.It is true that some information has been collected over time on the general role of microorganisms in the process of soil formation, a number of issues have been clarified, but it is not enough to clarify this issue unequivocally. Thus, the conversion of organic residues into humus is a complex biochemical process, and its realization occurs due to "*functional microorganisms"*[6], as well as "*some enzymes related to oxyreductases accumulated in the soil"*[7]. At present, the mechanism of this process has not been fully clarified and there is no single view on it, the problem is the subject of discussion. In addition, the physiological and biochemical regulation of micromycetes and their enzyme synthesis, producers of phenoloxidases involved in the conversion of organic residues into humus, the specific gravity and role of individual groups in the degradation of lignin in soil biota, changes in anthropogenic pollution and micromycetes have many points which are overlooked. Influence of natural abiotic and biotic factors of the areas with specific ecological conditions on these issues are different, then the importance of conducting such kind of researches on this direction is not dautable.

Purpose and objectives. The purpose of this paper is to assess the physiological and biochemical regulation of peroxidase, as well as the synthesis of some oxidases in micromycetes isolated from clean and anthropogenically affected lands in different parts of Azerbaijan and their role in soil processes.

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[4]Polyanskaya, L.M., Yumakov, D.D., Tyugay, Z.N., Stepanov, A.L. The ratio of fungi and bacteria in dark humus forest soil // Soil Science, -2020, No. 9, -p.1094-1099)

[5]Eldor, A.P. The nature and dynamics of soil organic matter: Plant inputs, microbial transformations, and organic matter stabilization//Soil Biology & Biochemistry,2016,98 109-126

[6]Gerke, J. Concepts and misconceptions of humic substances as the stable part of soil organic matter: a review.//*Agronomy* 2018, *8*(5), 76; https://doi.org/10.3390/agronomy8050076

[7]Esiana B., Coates C., Adderley W. et al. Phenoloxidase activity and organic carbon dynamics in historic Anthrosols in Scotland, UK. PLOS ONE, 2021, 16(10): e0259205. https://doi.org/10.1371/journal.pone.0259205

According to the goal, it was considered necessary to solve the following tasks:

-Assessment of soil types distributed from different parts of Azerbaijan for peroxidase and polyphenol oxidase activity and fungal biota;

- Screening of selected strains from the sampled soils for peroxidase activity, as well as polyphenol oxidases and selection of active producers;

- Physiological and biochemical regulation of peroxidase synthesis in cultures selected as an active producer;

- Comparative evaluation of micromycetes isolated from clean and anthropogenically affected soils for peroxidase activity.

Research methods. Known methods widely used in microbiology, as well as in mycology and soil science,during obtaining samples fromfrom the study areas. Known biochemical methods were used to determine the enzymatic activity of soil samples, to separate and clean fungi from those samples, and to study the enzymatic activity of pure cultures. The accuracy of the devices and equipment used and the degree of purity of the reagents were also at the required level. In the study, all experiments were performed in 4 repetitions and the results were statistically processed. The ratio of the standard standard deviation to the mean value of repetitions (σ / Chor) was ≤ 0.05. This shows that the results obtained are accurate.

The main provisions of the dissertation submitted for defence:

- The main role in determining the level of activity of peroxidase, as well as polyphenol oxidases is not the difference in soil types, but the amount of humus in them;
- Although the amount of humus plays a key role in determining the level of activity of peroxidase and polyphenol oxidase, in all cases, anthropogenic influences cause the accumulation coefficient of humus (K) below the background;
- Fungi play a key role in the formation of peroxidase, polyphenol oxidase, as well as catalase activity in soils, in which the synthesis of the enzyme depends significantly on the form of nitrogen sources added to the environment;

- Anthropogenic impact leads to an increase in the activity of peroxidase in soils, which is the basis for confirming its active participation in the catalysis of the degradation of xenobiotics that cause soil pollution.

The scientific novelty of the research: In the research, different soil types in certain areas of the Republic of Azerbaijan were evaluated for both enzymatic activity (peroxidase and polyphenol oxidase), as well as peroxidase of micromycetes distributed in these soils, as well as synthesis of other oxidizing enzymes and regulation of the synthesis process.

It was found that in all soil types studied, there was a certain relationship between peroxidase activity and humus content, which is reflected in the

increase in peroxidase activity corresponding to the increase in humus content, but this is not characterized as a linear dependence. The opposite is observed in the activity of polyphenol oxidase. Although the amount of humus plays a key role in determining the level of activity of both peroxidase and polyphenol oxidase, in all cases, depending on anthropogenic influences, as well as the degree of soil culture, the value of humus accumulation factor (K) is lower than the background.

It was clear that micromycetes are widespread in the studied soils, including those that actively synthesize peroxidase, as well as polyphenol oxidase and catalase. There is a specific pattern in the synthesis of peroxidase in these fungi, as the addition of organic and inorganic nitrogen sources to the environment, separately and in various combinations, leads to an increase, decrease or complete cessation of peroxidase activity. On the other hand, micromycetes that synthesize peroxidase enzymes differ in both soil distribution and activity level, as the number of active strains of micromycetes distributed in forest objects varies from 10 to 26%, and the synthesis of peroxidase is inductive. occurs, but the inductor of each fungus is specific in a certain sense.

It was determined that the total peroxidase activity of micromycetes, which are recorded in anthropogenically affected soils, especially in oil-contaminated soils, is higher than in relatively clean soils. Therefore, the role of peroxidase synthesized by fungi in anthropogenically affected soils is determined by their more active participation in the degradation of xenobiotics that cause pollution.

Theoretical and practical significance of the research. The results obtained in the research are factual materials that serve to expand the information on the peroxidase enzyme activity of both soils and micromycetes distributed in the same soil**.**

For the first time, the coefficient of humus accumulation (Kr) for 8 soil types were calculated experimentally and the factors influencing its quantitative expression were determined, which will be useful in determining the nature of processes in soils and developing preventive measures for their restoration.

Publication, approbation and application of dissertation. 14 scientific works on the topic of the dissertation were published and dissertation materials were presented at the International scientific conference on "Actual problems of modern natural sciences" (Ganja, 2017), International scientific conference on "Actual problems of modern nature and economic sciences" (Ganja, 2018), International conference on "Microbiology and immunology: development prospects" (Ukraine R, Kiev, 2018), scientific conference on "Actual problems of modern biology"(Baku, 2019).

Organization where the dissertation work is performed. The dissertation was performed in the laboratory of experimental mycology of the Institute of Microbiology of ANAS.

Dissertation stucture and volume. The total volume of the dissertation is 137 computer pages, in total are 210,450 characters.

CHAPTER I
BIOLOGICAL ACTIVITY OF SOILS AND THE ROLE OF MICROMYCETES AND THEIR PHENOLOXIDASES IN ITS FORMATION

General description on biological components of soils had been givenin the 1.1. section of the dissertation. Section 1.2 provides information on the role and importance of the use of biological indicators in assessing the ecological condition of soils. Section 1.3 was based on analyzes and summarizes research work on soil enzymes and their impact on soil biological indicators . Section 1.4 of the dissertation analyzes the ecological functions of peroxidase, as well as phenoloxidases in the soil, the sources of their activity and the physiological and biochemical basis for regulating the synthesis of enzymes in these sources, touches on the issues important for solving the planned problems.

CHAPTER II
MATERIALS AND METHODS OF RESEARCH

2.1.General charachteristics of research areas

Samples for the study were taken mainly from relatively clean and diverse lands in the north-eastern part of Azerbaijan and the Absheron Peninsula, more precisely in the Great Caucasus, which were affected by both anthropogenic and man-made impacts. The samples were analyzed for enzymatic activity, fungal biota, as well as peroxidase and polyphenol oxidase activity of soil-derived fungi.

The study areas are mainly mountainous and plain, with widespread sandy, gray-brown, gray, light brown, chestnut and dark chestnut, brown meadow and meadow forest types. The study area also differs in climatic conditions, as the Absheron Peninsula is characterized as one of the driest regions in the Caucasus, while the north-eastern part of the Greater Caucasus differs in rainfall, number of sunny days, flora, and dominant soil type. features.

2.2.Methods used for the analysis of samples taken for research

Soil samples taken from the selected sites covered depths of 0-20 cm. In total, more than 350 samples of 8 soil types (sandy, gray-brown, gray, light

brown, chestnut, dark brown (clike a chestnut colour), brown meadow and meadow forest) were taken during the research, and the seasonal factor was taken into account.

A suspension (diluted up to 100 times) prepared from soil samples was used to determine the activity of enzymes in the soil and to separate the fungi. Standard nutrient media (SQM) such as solidified bleached malt juice (CAS), Chapek medium (CM), potato agar (CA) and Saburo agar (SA) were used to bring the mushrooms to pure culture. *"Separation, purification and storage of fungi from soil samples to the methods adopted in microbiology"* [8]And the purity of the obtained culture was monitored under a microscope (MBI-7).Identification of pure fungal cultures was carried out using "determinants based on cultural-morphological characteristics of fungi".[9,10]

The amount of humus in the soil was determined according to the "*known method of Tyurin"*.[11]

The activity of peroxidase and other oxidases (polyphenol oxidase and catalase) in soils was determined according to the "*standard methods"*[12] used in the works of F.Kh. Khaziyev and the activity was expressed in mg of purpurgalli per 1 g of soil.

"*Standard methods and approaches"*[13,14] used in the work of various authors were also used in the study of physiological and biochemical features of the optimization of the environment and the regulation of the synthesis process for the synthesis of peroxidase and other oxidases in fungal strains selected as active producers.

The following "*formula*"[15]was used to calculate the humus ratio:

Kr =(S/D) x100,

here, Kr- humus ratio, S - activity of polyphenoloxidase, D- activity of peroxidase.

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[8] Netrusov AI, Egorova MA, Zakharchuk LM and others. Workshop on microbiology. M: Publishing House "Academy", 2005 , 608p.

[9] Sutton D., Fothergill A., Rinaldi M. Determinant of pathogenic and conditionally pathogenic fungi / - Moscow: Mir, 2001, 486p.

[10] Kirk, P. M. Cannon P. F., Minter D. W. et al.Dictionary of the fungi. -UK, 2008, 747 p.

[11] http://agrohimija.ru/agrohimicheskie-metody/438-opredelenie-gumusa-pochvy-po-metodu-iv-tyurina-chast-1.html)

[12] Khaziyev, F.Kh. Methods of soil enzymology / F.Kh. Khaziyev. – M.: Hauka, 2005. – 250 p

[13] Muradov, P.Z. Changes in the activity of hydrolase and oxidases in the process of biodegradation of plant wastes: dr.in bio. .... abstract of the dissertation. / - Baku, 2004. -45 p.

[14] Chemeris, O. V., Kuptsova, Yu. G., Boyko M. I. Influence of various sources of carbon nutrition on the synthesis of milk-clotting proteinases by strains of the fungus Irpex lacteus / / Problems of ecology and nature conservation of the technogenic region. – 2018. No. 1–2, - p.117-123

[15] Chunderova, A.I. Whiteness of polyphenol oxidases and peroxidases in late-podzolic soils.// Soil science, -1970, № 7, -p.22-28.

At least 4 repetitions were used in both the sampling and laboratory experiments, and the results were processed "statistically"16.

CHAPTER III
ASSESSMENT OF DIFFERENT SOIL TYPES IN AZERBAIJAN DUE TO ACTIVITY OF PHENOLOXIDASES

3.1.Peroxidase and polyphenol oxidase activity of different types of soils in Azerbaijan

In order to clarify the enzymatic activity observed in soils, the analysis of samples taken from different soil types was carried out and it became clear from the results that they also have peroxidase and polyphenol oxidase activity, but the activity indicators for each soil type are different (Fig. 3.1.1). As can be seen, the highest activity of peroxidase is found in sandy soils, and the lowest activity is found in brown meadow soils. In general, the difference between the maximum and minimum values of peroxidase activity between the studied soil types is 2.3 times. The highest activity of polyphenol oxidase, which is a reflection of peroxidase by function, is found in meadow forests, and the lowest in sandy soils.

In this case, the difference between the minimum and maximum activity of the enzyme is 3.1 times. In other words, although there is no linear relationship between peroxidase and polyphenol oxidase for the soil types studied, there is an inverse relationship. When characterizing the activity of peroxidase and polyphenol oxidase in terms of the amount of humus in the soil type studied, it is clear that the amount of humus also plays an important role in the formation of the level of activity of these enzymes, but this is more pronounced in polyphenol oxidase activity. Thus, depending on the amount of humus in the soil types, the quantitative indicator of polyphenol oxidase activity is either high or low. More precisely, the high A similar situation is not observed in the activity of peroxidase, ie the activity of peroxidase does not change according to the increase or decrease of humus, either in a straight line or in the opposite direction. The fact that there is a certain dependence on polyphenol oxidase, which is not always observed in relation to peroxidase, we think that the functions of the enzyme peroxidase are more specific than polyphenol

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[16]Кобзарь, А. И. Прикладная математическая статистика / А. И. Кобзарь - Москва: ФИЗМАТЛИТ, - 2006, - 816

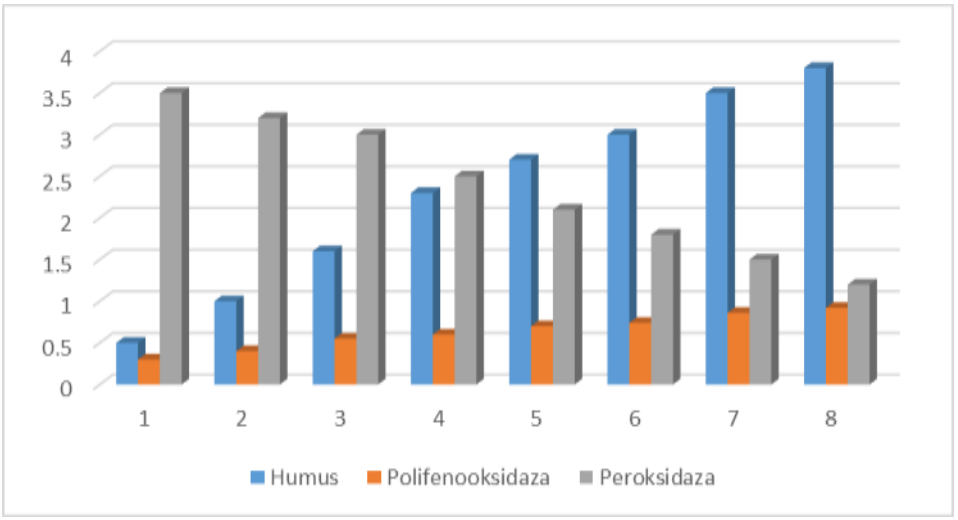

Figure 3.1.1. Effect of humus content on different soil types on the activity of polyphenol oxidase and peroxidase enzymes (spring)

1.Sandy; 2. Gray-brown; 3. Gray; 4. Light brown; 5.Meadow; 6. Brown; 7. Brown meadow; 8. Meadow forest

oxidase, and therefore the increase in humus affects its activity differently. Because "*humus is a complex, heterogeneous component*"[17]. In this complexity, it would be logical to look for the reason for the difference in the effect of enzyme activity on the amount of humus.

According to the results of the study, when calculating the humus coefficient (Kr), it became clear that the increase and decrease in its value is the opposite of peroxidase, ie as the peroxidase activity increases, the humus factor decreases, which is slightly different in forest-meadow soils. coefficient in this variant is slightly different from the others. Thus, the coefficient of humus calculated per unit of peroxidase1 activity of different soil types is 2.5%, in gray-brown soils - 3.9%, in gray soils - 6.1%, in light brown soils - 9.6%, in meadow steppe soils - 15, 7%, 22.8% in chestnut soils, 38.2% in brown meadow soils and 23.0% in meadow forest soils.

In samples taken from the same soil types in the autumn, the situation in the spring is almost repeated with some quantitative differences. For example, in

[17]Ivanov A. L., Kogut B. M., Semenov VM and others. Development of the doctrine of humus and soil organic matter: from Tyurina and Vaxman to our days // Bulletin of the Institute of Soil VV Doguchaeva, 2017, v.201, p.1-36)

spring, the activity of peroxidase in gray-brown soils is 3.2, while in autumn it is 1.33 times lower. This figure is 1.36 times in gray soils. Similar indicators for polyphenol oxidase enzyme activity is 1.25 times lower in autumn in gray-brown and gray soils. This is the case in all variants, ie the activity of both peroxidase and polyphenol oxidase in autumn is lower in all soil types than in spring. The different dependence on enzymes observed in different soil types, both in spring and autumn, is due to the fact that the enzymes whose activity is determined have a different share in the formation of humus (polyphenol oxidase) and its mineralization (peroxidase).

It should be noted that land is a major source of food that plays an important role in meeting people's food needs, and for this reason people use it extensively in their economic activities. This type of approach also causes different changes in those lands. These changes are naturally reflected in both biological and physical-chemical properties of soils. Taking this into account, research has been conducted to clarify the nature of the changes in enzymatic activity depending on the involvement of different soil types in the studies. It became clear from the results that the difference between the activity of the enzymes studied depends on the involvement of the soil in economic activities, in other words, the degree of agro-technical maintenance (Table 3.1). As can be seen, regardless of the degree of culture, the activity of polyphenol oxidase increases with increasing humus content, while that of peroxidase decreases. In short, the amount of humus in the soil plays a key role in changing the activity of enzymes.

Shedule 3.1

The effect of soil culture on the amount of humus and the activity of enzymes

Degree\level of soil cultivation	Humus,%	Polyphenol oxidase	Peroxidase
		purpurgallin, mg	
Raw (forest)	3,8	0,80	2,0
Weak	4,5	0,83	1,8
Middle	5,1	0,86	0,8
Good	5,8	0,90	0,5

One of the most pressing problems of modern times is the expansion of human economic activities, which intensifies human intervention in nature, the consequences of which are almost always negative, and its prevention is becoming an important issue. Taking this into account, the enzymes studied were also characterized in this aspect, and for this purpose, soils (oil-contaminated, irrigated, urban and relatively clean soils) that differed in the source of pollution and the nature of anthropogenic impact were used. From the analysis of the samples, it became clear that the difference in the amount of humus in the soils exposed to different anthropogenic influences is decisive in

terms of the effect on the activity of enzymes, but there are also changes due to the nature of the anthropogenic impact. Thus, the activity of peroxidase in oil-contaminated soils is relatively high compared to other soils. can also be characterized as a factor that has a more negative effect on the accumulation of humus.

Taking into accout beeing of the humus coefficient is a useful indicator for predicting the productivity of different soil types, its calculation was also considered expedient for the soil types studied by chapters(tabl. 3.2). As can be seen, Kr is observed in the highest meadow forest soils and the lowest in sandy soils. Despite the fact that the quantitative indicators given in the Kr table

Table 3.2.

Changing of humus accumulation coefficient in different soil types in Azerbaijan due to the seasons

Soil types	Humus	Autumn	Winter	Spring	Summer	Annual average
Sandy	0,52	6,23	5,32	8,6	10,24	7,6
Gray-brown	0,88	13,3	10,74	12,5	14,22	12,7
Gray	1,42	20,9	16,42	18,3	20,68	19,1
Light brown	2,34	29,4	22,44	24,0	28,72	26,1
Meadowsteppe	2,46	39,3	28,82	33,0	36,42	34,4
Chestnut	3,10	63,64	34,42	41,1	45,48	46,2
Brown meadow	3,20	102,8	50,48	57,3	62,12	68,2
Meadowforest	3,42	160,0	40,60	46,0	50,82	74,4

differ from each other, ie the difference between the maximum and minimum indicators is 9.8 times, Kr calculated for 1% of humus is 13.5 for sandy, gray-brown, gray, meadow steppe and chestnut soil types. Varies between 14.9, ie in the form of an almost similar expression. This indicator is 11.2 in light brown soils.The calculated value for brown meadow and meadow forest lands is also very close (21.3 and 21.8), but 1.43-1.95 times higher than similar indicators of other soil types. In our opinion, this is due to the fact that these soil types are rich in organic matter, including humus, as well as plants in this soil type. Thus, the amount of organic matter that reaches the end of each year at the end of the growing season, as well as the amount of organic matter entering these soils in accordance with the spring period, is higher than in other soil types.In this case,

in light brown soils, a single amount of Kr is observed to deviate slightly from the above. The real reason for this can be explained in detail by the results of future research, but it can be assumed that this is due to the chemical properties of the organic substances it contains.

It would also be appropriate to mention one issue related to Kr calculated for the 8 soil types sampled in the studies. This ratio, calculated for the first time for Azerbaijani lands, can also be considered valuable in terms of forecasting the future productivity of these lands, as well as reducing the volume of research work in this area. Therefore, their systematization according to the calculated Kr for different soil types was also considered expedient and for this purpose a 4-point scale was proposed:

1. It was considered expedient to include lands that fall into this category and are considered to have low productivity, and it was assumed that Kr ≤10% was in the soil types corresponding to this type.
2. This includes soil types that are considered to have sufficient productivity, for which the location between 20% ≤Kr> 10% should be accepted.
3. This group includes soils with good productivity, for which the formula 40% ≤Kr> 20% should be used.
4. The productivity of this category of soils should be considered high and for them the formula Kr> 40% should be considered acceptable.

According to the proposed scale, if we evaluate the soil types sampled in the course of research, it becomes clear that sandy soils are 1st, gray-brown and gray soils are 2nd, light brown and meadow steppe soils are 3rd, chestnut, brown meadow and meadow forest soils. and can be referred to the 4th group.

CHAPTER IV

PEROXIDASE ACTIVITY OF MICROMYCETES DISTRIBUTED IN DIFFERENT SOILS OF AZERBAIJAN AND ITS PHYSIOLOGICAL-BIOCHEMICAL REGULATION

4.1. Isolation of micromycetes from different soils and their screening for peroxidase activity

Issues such as the source of enzymatic activity specific to soils is mainly soil-dwelling organisms, primarily fungi and bacteria, the next stage of the study clarified the issues related to changes in the activity of peroxidase and polyphenol oxidase on micromycetes isolated from the studied soils are highlighted.

For this purpose, 87 strains of micromycetes from different soil types selected as the object of research were cultured and identified as species. So, the strains isolated during the study belonged to 19 species(*Aspergillus fumigatus, A.nidulans, A.niger, A.oryzae A.ustus, Fusarium moniliforme, F.solani Penicillium adametzii, P.citrinum, P.expansum, P.frequentans, P.funiculosum, P.implicatum, P.jensenii, P.melinii, P.rubrum, P.thomii, P.wortmanii və Trichoderma viride*), which 5 species.

Screening for isolated strains for peroxidase as well as catalase activity revealed that only 18.4% of them had peroxidase activity and 42.5% had catalase activity. Of the strains with peroxidase activity, 4 were *Aspergillus* (yield 17.4% by genus), 4 were *Fusarium* (40.0%), 7 (14.3%) were *Penicillium*, and 1 (100%) was *Trichoderma*. belongs to the species of the genus. Therefore, the oxidizing potential of fungi recorded in the studied soils differs according to the fungal biota.

While conducting the research, 100 strains of 32 more species from forest objects, forest soils and rhizosphere of trees were evaluated for daperoxidase activity. It became clear that the trees, their stumps, the drying parts of the trees, and so on. Among the strains isolated from the material, the ones with peroxidase activity are more (26% of the total strains), the specific gravity of the peroxidase-active strains from the rhizosphere of trees is slightly lower (25%), among the strains isolated from forest soils the total is 10%. These differences are based on the different chemical composition of forest objects.

According to the results of the two-stage selection, 36 out of 187 strains of 48 species were found to have peroxidase activity, which is 19.5% of the total strains. The taxonomic relevance of strains with peroxidase activity was as follows:*A.tenius* – 7, *A.niger* – 3, *A.ustus* – 1, *B.maydis* -1, *B.sorokiniana* - 1,*Botrytrichum piluliferum* -1, *C.herbarum* – 3, *F.moniliforme* -2, *F.oxysporum* – 2, *F.solani* -2,*G.candidum* – 2,*Neospermospora avenae – 1,Paecilomyces variotii* -1, P. *adametzi* -1, *P.citrinum* -1, *P.cyclopium* - *1, P.funiculosum* -2, *P.wortmanii* – 3, *T.viride* -1.

Both quantitative and qualitative methods were used to determine the peroxidase activity of strains of fungi isolated from different locations. It became clear from the results that the fungal strains with peroxidase activity differ in quantitative indicators of the level of enzyme activity (Table 4.1). As can be seen, the highest activity is found in the strain specific to the fungus *A.tenuis*. In general, the general level of peroxidase activity of strains belonging to this type of fungus is higher than others. The lowest peroxidase activity is observed in strains of fungi such as *A.ustus, T.viride, P.wortmanii and P. funiculosum*, and their activity level does not exceed 2.0 bv / ml, which is the highest activity of the strain. compared to 2.9-3.4 times less.

Shedule 4.1

Characteristic of isolated fungal strains due to the peroxidase activity

№	The type of strain	The number of strain	Activity (bv/ml)
1	*A.tenius*	7	4,4-5,8
2	*A.niger*	3	2,1-2,7
3	*A.ustus*	1	1,7
4	*B.maydis*	1	4,1
5	*B.sorokiniana*	1	3,8
6	*Botrytrichum piluliferum*	1	3,8
7	*C.herbarum*	3	3,7-4,9
8	*F.moniliforme*	2	4,3-4,5
9	*F.oxysporum*	2	4,0-4,3
10	*F.solani*	2	2,6-3,0
11	*G.candidum*	2	4,4-4,9
12	*Neospermospora avenae*	1	4,5
13	*Paecilomyces variotii*	1	2,1
14	P. *adametzi*	1	3,1
15	*P.citrinum*	1	2,7
16	*P.cuclopium*	1	3,3
17	*P. funiculosum*	2	2,0-3,0
18	*P.wortmanii*	3	1,8-2,8
19	*T.viride*	1	1,9

Due to the fact the peroxidase activity of strains of this or that species differs both within the species were considered satisfactory expedient to make the next selection by strains, and 7 *Alternaria tenius,* 1 *Bipolarismaydis,* 1 *B.sorokiniana*, 1 *Botrytrichum piluliferum*, 20 strains belonging to 3 *Cladosporium herbarum*, 1 *Fusarium moniliforme*, 1 *F. oxysporium*, 2 *Geotrichum candidum,* 1*Neospermospora avenae,*1 *Penicillium funiculosum* and 1*P.wortmanii* species were selected. The activity level of peroxidase for the selected strains ranged (changed) from 3.7 to 5.8 bv / ml.

1.2. **Optimization of the environment for the synthesis of peroxidase in micromycetes selected as an active producer of peroxidase**

In order to clarify this issue, carbon and nitrogen sources, cultivation temperature, initial pH, method and duration of preparation of planting material,

etc. were selected for the selected strains. were conducted according to the parameters and first of all researches on selection of the nutrient medium itself were carried out. For this purpose, 3 environments (Chapek environment, environment with specific root composition and oak root) were used. Although all of the isolated strains have the ability to grow in the above-mentioned nutrient medium, their synthesis of peroxidase, or more precisely their peroxidase activity, is explained by a different situation. Thus, it was clear from the results that the activity of peroxidase was not observed in the culture solution (CM) of 19 out of 20 active strains tested during cultivation in synthetic Chapek medium. Second, activity is observed in 7 strains in the nutrient medium prepared from the root of the oak plant, and peroxidase activity is recorded in all strains only when cultures are grown in a specific root medium.Although changes in nutrient media do not affect fungal growth, their effect on peroxidase synthesis can be explained by the presence of compounds that stimulate or induce both fungal growth and enzyme synthesis in a specific root nutrient medium. On the other hand, the fact that peroxidase is recorded in some fungal strains in nutrient media, and not in others, suggests that the synthesis of peroxidase in fungi varies from species to species. For this reason, the specific nutrient content was selected as the optimal nutrient medium, and the effect of the nitrogen source was studied in that nutrient medium.

Studies of nitrogen sources have shown that the activity of peroxidase synthesis depends significantly on the form of nitrogen compounds added to the environment. Each strain of the fungus has its own regularity. Thus, the addition of ammonium sulfate and ammonium nitrate, as well as urea and asparagine to the environment in some (*Alternaria tenuis*VI-8, *Geotrichum candidum* VI-1, *Bipolaris maydis* VI-16, *Botrytrichum piluliferum* VI-3 to increase the activity of peroxidase in peroxidase 4) causes a decrease and even a complete cessation (*Alternaria tenius* VI-9*).*

Thus, high activity of peroxidase in micromycetes occurs mainly during the combined use of ammonium nitrate and urea.

In order to obtain peroxidase as an adaptive exoenzyme, the effect of specific substrates on the synthesis of the enzyme peroxidase was studied on cultures studied in previous experiments. Six active strains that develop well in a synthetic environment were selected for the experiment. These were the following cultures: *Penicillium funiculosum*VI-1, *Bipolaris maydis*VI-1, *Fusarium oxysporium*VI-1, *Cladosporium herbarum*VI-1, *Geotrichum candidum* VI-1 and *G.candidum* VI-2.

The mushrooms were grown in a 250 ml Erlenmeyer flask in a 100 ml nutrient medium. Peroxidase activity and culture growth were monitored for 5, 10, and 15 days. The effect of inductors, as a rule, manifests itself in the fact that this or that substrate is the only source of carbon or nitrogen. In our experiments, a mineral nutrient medium containing 1% sucrose iron and manganese

containing the first 1% of the fungi was used. Inductors introduced into the environment as the main source of carbon - peroxidase-specific substrates of oxidation - pyrogallol, tannin, gal acid, guaiacol and ascorbic acid - were added. An environment containing the full norm of sucrose was taken as control.

Experiments have shown that peroxidase induction is not observed, although fungi grow in the environment. It has been established that in addition to specific substrates, a light source of easily assimilated carbon must be sufficient to support the synthesis of peroxidase. (Table 4.1). Experiments 20

Table 4.1

G.candidum VI-1 strain of sugar introduced into the environment effect on the development and activity of peroxidase formation

Experiment	In the 10th day		In the 15th day		In the 20th day		Dry weight of all mycelium		pH
	D*	A	D	A	D	A	mq/100 ml	Control %	
Control	3**	0	3	0	3	0	43,6	100	6,5
Sucrose (saccharose)	3	1	3	1	3	0	103,6	236,2	6,03
Oak bark decoction + sucrose medium	4	5	5	5	5	5	370,5	849,7	5,39
Oakbarkdecoction	3	0	3	0	3	0	47,7	109,3	6,29

Note: * - D- development, A- Activity; **-Obtained results are statistically developed:m ≤ 4,8%

It was carried out by adding sucrose to the environment during the day. Decreased sugar concentration led to a decrease in enzyme activity. That is why in subsequent experiments, specific substrates of peroxidase oxidation were introduced into the medium with a complete norm of sucrose (30 g / l). At the same time, oak bark and 0.1% pyrogallol were used as substrates in practice.It became clear from the results that the substrates used have a different effect on the growth of the fungus and the synthesis of peroxidase (tab. 4.2). For example, all substrates added to *G.candidum* VI-1 strain (except guaiacol) induce peroxidase, so that when oak bark decoction is added to the culture fluid, the enzyme activity is 42 times higher than the control, and when tannin is added, it is 8 times higher.

Table 4.2.

Dependence of peroxidase synthesis on specific substrates in fungi

Experience	*G.candidum* VI-1			*P.funiculosum* VI-1			*B.maydis*VI-1		
	Mitsel		Peroxi-dase activity	Mitsel		Peroxi-dase activity	Mitsel		Peroxi-dase activity
	mg/100mlenvironment	%		mg/100 ml environment	%		mg/100 ml environment	%	
Control	107,6	100	0,02	299,2	100	–	99	100	
Oak bark	300,9	279,7	0,84	194,2	64,9	–	553,3	554,5	–
Tannin	90,4	84,0	0,16	196,0	65,5	–	607	608,8	–
Galicacid	90,4	84,0	0,09	395,3	132,1	–	643,0	654,3	–
Gvayakol	35,5	32,9	–	95,2	31,8	–	107,4	107,7	–
Pirogallol	90,5	84,3	0,04	288,1	96,3	–	775,1	776,6	–
Ascorbicacid	107,9	100,3	0,04	679,5	227,1	–	582,6	583,7	–
Experience	*F.oxysporum* VI-1			*C.herbarum* VI-1			*G.candidum* VI-*2*		
	Mitsel		Peroxi-daseactivity	Mitsel			Mitsel		Peroxi-dase activity
	mg/100 ml environment	%		mg/100 ml environment	%	Peroxi-dase activity	mg/100 ml environment	%	
Control	130,8	100	0,04	130,8	100	–	91,4	100	–
Oak bark	226,7	142,5	0,04	226,7	173,3	–	108,1	118,2	–
Tannin	279,8	149,2	–	279,8	213,9	0,16	123,5	135,1	–
Galicacid	245,5	211,4	–	245,5	187,7	–	72,4	79,2	–
Gvayakol	–	–	–	–	–	–	74,4	81,4	–
Pirogallol	117,4	259,7	–	117,4	89,7	–	136,7	149,5	–
Ascorbicacid	324,1	252,1	0,04	324,1	247,8	–	82,1	89,8	–

It should be noted that the tested cultures change the pH of the medium in the direction of acidity and alkalinity during the growth process, regardless of the initial level. Thus, the pH of the culture medium of *G.candidum* VI-1 strain varies between 5.1-6.2, but in *F.oxysporum* VI-1 strain it varies between 3.1-4.7. Only under control and when guaiacol is introduced into the environment does the pH of the medium change sharply towards alkalinity.

Once an appropriate substrate has been selected that has a positive effect on the growth and activity of each strain, it is necessary to determine the optimal conditions for peroxidase induction. Therefore, further research has been conducted on these issues. For this purpose, three concentrations of each substrate - 0.1, 0.01 and 0.001% were taken. In most cases, low concentrations were more effective. Thus, the maximum induction of peroxidase at a concentration of 0.001% of tannin and pyrogallol is recorded in the strain of *G. candidum* VI-1.

Maximum induction of peroxidase at a concentration of 0.01% of tannin and galic acid is observed in *P.funiculosum* VI-1 strain. Low concentrations of specific substrates were more effective in *F.oxysporium* VI-1 strain. No peroxidase inducers were found between the substrates tested in the other two cultures.

Studies have shown that induction of peroxidase is possible in most strains, and this can occur in the presence of specific substrates - tannin, gallic acid, pyrogalol, ascorbic acid and oak bark decoction.

As such induction has a specific character, the most effective inductor has been identified for each strain. The main feature of peroxidase induction in these strains is that this process takes place in the presence of an easily assimilated carbon source in the environment, and the inductors act as inhibitors of fungal growth. Thus, the dependence of peroxidase induction on specific substrates in the fungal strains we studied was revealed.

As a result of research, it can be concluded that the role of peroxidase in micromycetes is in the neutralization of toxins, more precisely in xenobiotics, and the induction process is a manifestation of the body's self-defense reaction.

FINAL ANALYSIS OF RESEARCH

Soils are the system of the physicochemical and biological components, and for one reason or another, the biological components are the first to react to changes in soils. More precisely, the biological components of soils are more sensitive to the effects on soils, and therefore their study is one of the current areas of research to prevent undesirable changes in soils from this or that impact and to restore its previous state.

As a rule, the assessment of soils for biomonitoring is carried out either by soil microorganisms or by soil enzymes whose source they are. Taking this into account, in the presented work, soil types such as Sandy, Gray-brown, Gray, Light brown, Gray-meadow, Chestnut, Brown-meadow and Meadow-forest, which are spread in certain territories of the Greater Caucasus part of the Republic of Azerbaijan, It was considered expedient to evaluate microorganisms isolated from soils for their enzymatic activity. It was considered expedient to use mainly peroxidase, as well as polyphenol oxidase and catalase as soil enzymes, and fungi as soil microorganisms.

Based on the above mentioned issues, first of all, studies aimed at assessing the soils in the Greater Caucasus of the Republic of Azerbaijan for peroxidase activity were conducted and allowed to obtain the following results.

RESULTS

1. Inversely between the activity of peroxidase and the amount of humus in the assessment of enzymatic activity of soil types such as Sandy, Gray-brown, Gray, Light brown, Gray-meadow, Chestnut, Brown-meadow and Meadow-forest, distributed in certain areas of the Republic of Azerbaijan in the Greater Caucasus, with polyphenol oxidase[2, 12, 13].
2. Although the amount of humus plays a key role in determining the activity of both peroxidase and polyphenol oxidase, it has been found that in all cases, depending on anthropogenic influences, the value of Kr is lower than the background, and oil pollution has a stronger effect than [3, 9-10, 12].
3. For the first time in Azerbaijan, humus accumulation coefficients were calculated and studied experimentally for soil types such as Sandy, Gray-brown, Gray, Light brown, Gray-meadow, Chestnut, Brown-meadow and Meadow-forest, depending on local specific conditions. A 4-point scale was developed to assess biological productivity and those soil types were assessed accordingly. It was found that the amount of humus and the deviation of the pH of the medium from the norm have a more serious impact on the formation of the quantitative expression of the humus coefficient[5, 13].
4. It has been studied that fungi have a special role among the main sources of enzymatic activity in soils, and among them are active producers of peroxidase, as well as polyphenol oxidase and catalase. Thus, 36 out of

187 strains of 48 species of fungi registered in the study are able to actively synthesize peroxidase, in which the level of activity of the enzyme peroxidase significantly depends on the form of nitrogen compounds added to the environment. Each type of fungus has its own regularity, as the addition of organic and inorganic nitrogen sources to the environment separately and in various combinations leads to an increase in peroxidase activity in some, a decrease in others, and a complete cessation in others. Quantitative indicators of decline and increase are formed depending on both the biological characteristics of fungi and the nature of nitrogen sources[1, 7, 8, 11, 14]

5. Micromycetes synthesizing the enzyme peroxidase have been found to differ in both soil distribution and activity level, as the number of active micromycetes in the soil varies from 10 to 26%, and the distribution of peroxidase varies from 10 to 26%. The synthesis takes place inductively, but the inductor of each fungus is in a sense specific[4, 11, 13].
6. It was determined that the total peroxidase activity of micromycetes, which was recorded in anthropogenically affected soils, especially in oil-contaminated soils, was higher than in relatively clean soils. For this reason, the role of peroxidase synthesized by fungi in anthropogenically affected soils is characterized by a more active participation in the degradation of xenobiotics that cause pollution[4, 6, 12].

PRACTICAL RECOMMENDATIONS

1. To assess the general condition of soil types in the conditions of Azerbaijan and its biological productivity, the humus coefficient should be determined for each soil and it is better to have less than 10% Kr, between 10-20%, between 20-40% And more than 40% should be valued as high-yielding land.

2. When estimating the biological productivity of different soil types based on the proposed assessment, samples should be taken from the relatively stable parts of the soil type.

THE LIST OF SCIENTIFIC WORKS PUBLISHED ON THE TOPIC OF THE DISSERATATION

1. Isayeva V.K., Gasimova S.Y., Babayeva I.Kh., Aliyeva L.A. Influence of nitrogen source on peroxidase activity of micromycetes fungi //

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