Advanced Studies in Biology, Vol. 15, 2023, no. 1, 129 - 135
HIKARI Ltd, www.m-hikari.com
https://doi.org/10.12988/asb.2023.91697

Evaluation of Micromycetes Isolated from Oil-Contaminated Soils for Lipolytic Activity

Konul F. Bakshaliyeva [1,2], Agil A. Ahmedli [1] and Guler M. Seyidova [3]

[1] Institute of Microbiology of the Ministry of Science and Education of the Republic of Azerbaijan, Baku, Azrbaijan

[2] Scientific Research Institute of Fruit and Tea Cultivation of the Ministry of Agriculture of the Republic of Azerbaijan, Guba, Azerbaijan

[3] Azerbaijan Medical University, Baku, Azerbaijan

Abstract

In the conducted studies, 116 strains of micromycetes were isolated from the soil contaminated with oil to different degrees in the Republic of Azerbaijan 41,4% of which belonged to *Aspergillus, Pencillium* and *Mucor*. Screening of all isolated strains for lipolytic activity revealed that 67,2% of them had lipolytic activity, and the level of activity is characterized by different indicators, even at the level of the same genus. As a result of the screening, 3 strains with the highest activity were selected, and the medium was optimized for them to synthesize the enzymes with lipolytic activity maximally.

Keywords: oil-contaminated soils, micromycetes, lipolytic enzymes, active producers

Introduction

The production of biological catalysts, that is enzymes, is one of the leading areas of modern biotechnology and preparations produced in this field are expanding both in terms of volume and field of application [1]. The reason for this is that the enzymes have a high activity of protein nature, lack of toxic effect and wide distribution in nature [19]. Organizing the production of widely used enzymes provides an opportunity to significantly modify, intensify and improve

existing technologies or, in principle, allows to create new highly efficient processes. All this makes it possible to note that the production of enzyme preparations is a promising area of biotechnology, and that it is gradually developing and expanding. From this point of view, the production of enzyme preparations widely used in various fields of national economy is of great interest. One of such enzymes is lipolytic enzymes Lipase, which plays an important role in lipid metabolism, is one of the unique groups of enzymes (EC 3.1.1.3) [22] so that the reactions catalyzed by them occur at the boundary of heterogeneous phases (for example, oil: water) [2, 22]. This increases the theoretical interest in their research and expands the boundaries of the fields of application.

Taxonomically, wide groups of organisms have the ability to synthesize lipase [5, 8-10, 18]. So, both animals and plants, as well as prokaryotic and eukaryotic microorganisms have it. Nevertheless, microorganisms as producers of lipolytic enzymes are considered more promising, and bacteria and fungi are the source of most of the enzyme preparations with lipolytic effect currently produced on an industrial scale [15, 21]. The ability of one or another producer to synthesize lipolytic enzymes can vary quantitatively even within a species, and this makes it urgent to keep in mind the possibility of finding new strain-producers as an urgent issue.

The richness of the nature and ecological diversity of the Republic of Azerbaijan has made it possible for microorganisms to spread widely in its territories and involve them in various types of research. Research aimed at elucidating the species composition, ecology, physiological-biochemical and biotechnological characteristics of microorganisms, primarily fungi, showed the presence of promising producers of biologically active substances of various purposes among them [3, 16]. From this point of view, the study of microorganisms spreading in Azerbaijan as producers of lipolytic enzymes cannot be considered comprehensive therefore, there is no systematic research in this direction. It manifests itself more clearly in relation to fungi.

Therefore, in the presented work, the evaluation of the potential of fungi distributed in oil-contaminated soils in terms of lipolytic activity and the selection of active producers was set as a goal.

Material and methods

Samples for research were taken from oil-contaminated soils in the Absheron Peninsula of the Republic of Azerbaijan. The samples were taken from the soil depth of 0-20 cm. A 10% suspension were prepared from the samples taken, transferred directly and by dilution (10 and 100 times) to the nutrient medium, and pure cultures were obtained [14, 17]. The purity of the cultures was monitored with a microscope (OMAX 40X-2500X LED Digital Lab Trinocular Compound Microscope). During taken to the pure culture of fungi as a nutrient medium were used saburo agar, agarized malt juice, and agarized Czapek. The identification of the fungal strains according to the genus composition was carried out based on the determinants [6-7, 11-12, 20]. During the screening of the

obtained pure cultures for lipolytic activity, the cultivation was carried out for 5 days under deep cultivation conditions (200 cycles/min) at a temperature of 28^0C in liquid Czapek medium, its composition (g/l): sucrose– 20, $NaNO_3$ – 3; K_2HPO_4 – 1; $MgSO_4$ – 0,5; KCl – 0,5; $FeSO_4$ – 0,01.

As a source of lipolytic enzymes were used the culture solution (from the liquid that remains after the separation of the processed biomass) of this or that fungi formed in Çapek medium for 5 days. As a substrate during enzyme activity was used a 40% emulsion of vegetable oil (olive oil) prepared in 2% polyvinyl alcohol [13]. The reaction mixture consists of 5 ml of emulsion, 4 ml of phosphate buffer (pH=7) and 1 ml of culture solution, which is incubated at 37^0C for 1 hour. At the end of the period, 30 ml of ethanol is added and the solution is titrated with NaOH (0.05N) in the presence of 1% phenolphthalein. As a control instead of CS or ES were used solution added 1 ml distilled water.

Lipase activity (LS) is calculated according to the following formula:

$$LS = AT \times (50/B)$$

here A – titer differences of experimental and control solutions, T – titer of alkali, B – amount of enzyme (g/ml^3).

As a unit of activity is accepted amount of enzyme hydrolyzing 1 μmol of oleic acid from a 40% olive oil emulsion at pH 7.0 and 37^0C for 1 hour. Enzyme activity was expressed in $\mu mol.min^{-1}ml^{-1}$ (IU/ml).

Results obtained

116 cultures were isolated from 60 soil samples taken from the research areas (from the oil-contaminated parts of the territories located in the Absheron valley of the Republic of Azerbaijan). From the data on the distribution of isolated cultures, becomes clear that most of them belong to the genera *Aspergillus*, *Pencillium* and *Mucor* (tab. 1). Thus, the 41,4% of the total registered strains belongs to these 3 genera. It should be noted that the mentioned genera are involved in the formation of the mycobiota of the nature of Azerbaijan, especially in the areas affected by anthropogenic influences, with more species[4].

At the next stage of research, the isolated strains were evaluated for lipolytic activity, and from the obtained results, was determined that not all of the fungi strains(32,8%) have extracellular lipolytic activity (tab.2). As it can be seen, the minimum quantitative indicator of the distribution of strains with lipolytic activity by genus was 33.3%, and the maximum indicator was100%. However, there is no clear correlation between this indicator and the level of activity. Thus, while the maximum indicator of activity is observed in the strain belonging to the genus of *Aspergillus*, the number of strains with lipolytic activity belongs to the strains belonging to the genus of *Mortierella*. The activity indicators of some strains belonging to the genus of *Artabotrys, Penicillium* and *Rhizopus* is also high compared to fungi belonging to the genus of *Mortierella.* Despite the above, at the end of this stage of research, it was considered appropriate to select 3 strains as active producers. These are: *Aspergillus sp.-17*, *Pencillium sp. -81* and *Rhizobus*

sp.-94. The only reason for making such a choice was that the activity indicators of the mentioned strains was higher than all the remaining strains.

Table 1. Distribution by genus of fungi taken to the pure culture

№	Genus	Number of strains, units	Total share, %
1	*Alternaria*	4	3,4
2	*Arthrobotrys*	6	5,2
3	*Aspergillus*	14	12,1
4	*Chaetomium*	4	3,4
5	*Cladosporium*	6	5,2
6	*Fusarium*	16	13,8
7	*Mortierella*	5	4,3
8	*Mucor*	18	15,5
9	*Pencillium*	16	13,8
10	*Rhisobus*	14	12,1
11	*Stachybotrys*	3	2,6
12	*Trichoderma*	6	5,2
13	*Ulocladium*	4	3,4
Total		116	100

Table 2. Evaluation of isolated fungal cultures for lipolytic activity

№	Genus	Number of strains, (have activity), units	Total share, %	Activity (IU/ml)
1	*Alternaria*	4(2)	50	5450-6340
2	*Arthrobotrys*	6(4)	66,7	7320-8430
3	*Aspergillus*	14(11)	78,5	5330-11300
4	*Chaetomium*	4(3)	75	2200-4530
5	*Cladosporium*	6(4)	66,7	1700-3420
6	*Fusarium*	16(8)	50	2100-4500
7	*Mortierella*	5(5)	100	3670-7960
8	*Mucor*	18(15)	83,3	2560-7950
9	*Pencillium*	16(10)	62,5	3420-10170
10	*Rhisobus*	14(10)	71,4	4680-10220
11	*Stachybotrys*	3(2)	66,7	1560-4560
12	*Trichoderma*	6(2)	33,3	2340
13	*Ulocladium*	4(2)	50	3200
Total		116(78)	100(67,2)	1560-11300

During the optimization of the environment for the maximum synthesis of lipolytic enzymes in the strains selected as active producers, it became clear that although they differ from each other in terms of the quantitative indicator of some components, they can show their biosynthetic abilities to the maximum in generally similar environments(tab. 3). As can be seen, as a result of optimization, the activity level in all three strains increases by 1.12-1.23 times compared to the medium used during screening. After the optimization of the medium, when the activity indicator is compared with the known producers of lipolytic enzymes, it is clear that the strains selected as active producers in the studies, primarily *Aspergillus sp.-17*, do not lag behind them, and opens up new perspectives for future practical use.

Table 3. Optimum environmental parameters for fungi strains selected as active producers

Producent	Carbon source(g/l)	Nitrogen source (in % by nitrogen)	Cultivation temperature, ^{0}C	Initial pH	Culti-vation time, hours	Gro-wth effect (%)
Aspergillus sp.-17,	Sucrose (21,0)	$NaNO_3$(0,038)	30	6,7	120	23
Pencillium sp. -81	Sucrose (19,0)	$NaNO_3$(0,040)	28	6,7	120	18
Rhisobus sp.-94	Sucrose (18,0)	$NaNO_3$(0,040)	28	6,7	110	12

Thus, as a result of the conducted research, 116 micromycete strains were isolated from the oil-contaminated soils of Azerbaijan, among which there are also active producers of lipolytic enzymes, which are promising for practical purposes in the future.

References

[1] J.L. Adrio, A.L. Demain, Microbial enzymes: tools for biotechnological processes, *Biomolecules*, **4** (2014), no. 1, 117-139.
https://doi.org/10.3390/biom4010117

[2] S. Ali, S.A. Khan, M. Hamayun, I-J. Lee, The Recent Advances in the Utility of Microbial Lipases: A Review, *Microorganisms*, **11** no. 2, (2023) 510.
https://doi.org/10.3390/microorganisms11020510

[3] K.F. Bakhshaliyeva, N.R. Namazov, A.R. Hasanova et al., Assessment of the prospects of studying and using mushrooms of Azerbaijan as effective producers

of biologically active substances, *Periódico Tchê Química*, **17** (2020), no. 34, 403-411. https://doi.org/10.52571/ptq.v17.n34.2020.427_p34_pgs_403_411.pdf

[4] K.F. Bakshaliyeva, N.R. Namazov, S.M. Jabrailzade et al., Ecophysiological Features of Toxigenic Fungi Prevalent in Different Biotopes of Azerbaijan, *Biointerface Research in Applied Chemistry*, **10** (2020), no. 6, 6773 – 6782. https://doi.org/10.33263/briac106.67736782

[5] S.M. Basheer, S. Chellappan, P.S. Beena, Lipase from marine Aspergillus awamori BTMFW032: production, partial purifcation and application in oil efuent treatment, *New Biotechnol.*, **28** (2011), no. 6, 627–38.

[6] V.I. Bilay, E.Z. Koval, *Aspergilli.-Kyiv*: Naukova Dumka, 1988.

[7] V.I. Bilay, *Fusarium.* - *Kyiv*: Naukova Dumka, 1977

[8] P. Chandra, R.S. Enespa, K.A. Pankaj et al., Microbial lipases and their industrial applications: a comprehensive review, *Microb Cell Fact.*, **19** (2020), 169. https://doi.org/10.1186/s12934-020-01428-8

[9] T.M. Costa, K.L. Hermann, M. Garcia-Roman et al., Lipase production by Aspergillus niger grown in diferent agroindustrial wastes by solid-state fermentation, *Brazil J. Chem. Eng.*, **34** (2017), no. 2, 419–427. https://doi.org/10.1590/0104-6632.20170342s20150477

[10] A.F. de Almeida, S.M. Tauk-Tornisielo, E.C. Carmona, Acid lipase from Candida viswanathii: production, biochemical properties, and potential application, *Bio Med. Res. Int.*, **2013** (2013), 1–10. https://doi.org/10.1155/2013/435818

[11] M.B. Ellis, J.P. Ellis, *Microfungi on Land Plants. An identification Handbook*, -London, Helm, 1987.

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

[13] Laboratory workshop on the technology of enzyme preparations. -M: Legkaya and food industry, 1980

[14] Methods of experimental mycology./Under. ed. Bilay V.I. Kyiv: Naukova Dumka, 1982

[15] D.B. Mendes, F.F.D. Silva, P.M. Guarda et al., Lipolytic Enzymes with Hydrolytic and Esterification Activities Produced by Filamentous Fungi Isolated

from Decomposition Leaves in an Aquatic Environment, *Enzyme Res.*, **2019** (2019), 8182425. https://doi.org/10.1155/2019/8182425

[16] N.R. Namazov, Species Composition of Fungi Participate in the Formation of Mycobiota of Some Essential Oil Plants Included in the Flora of Azerbaijan, *Int. J. Curr. Mikrobiol. App. Sci.*, **7** (2018), no. 11, 167-187. https://doi.org/10.20546/ijcmas.2018.711.211

[17] A.İ. Netrusov, M.A. Egorov, L.M. Zakharchuk et al., *Workshop on Microbiology*. - M.: Publishing house "Academy", 2005.

[18] V. Neugnot, G. Moulin, E. Dubreucq, F. Bigey, The lipase/acyltransferase from Candida parapsilosis: molecular cloning and characterization of purifed recombinant enzymes, *Eur. J. Biochem.,* **269** (2002), no. 6, 1734–1745. https://doi.org/10.1046/j.1432-1327.2002.02828.x

[19] R.K. Robinson, Enzymes: principles and biotechnological applications, *Essays Biochem*, **59** (2015), 1–41. https://doi.org/10.1042/bse0590001

[20] D. Satton, A. Fotergil, M. Rinaldi, Determinant pathogenic and opportunistic fungi – M.: Mir, 2001

[21] İ.E. Sharapova Influence of the composition of nutrient media on the productivity and biological activity of the strain of the entomopathogenic fungi Beauveria bassiana, *Agricultural science of the Euro-North-East*, **22** (2021), no. 6, 918-927. https://doi.org/10.30766/2072-9081.2021.22.6.918-927

[22] J.E. Yu, S.Y. Han, B. Wolfson, Q. Zhou The role of endothelial lipase in lipid metabolism, inflammation, and cancer, *Histol Histopathol*, **33** (2018), no. 1, 1-10.

Received: June 27, 2023; Published: June 12, 2023