МРНТИ 579.672

[1]M.Zh. Kanzhigitova
[2]A.M. Esimova
[2]M.A. Dzhakasheva

[1]*South Kazakhstan medical Academy, Shymkent, Kazakhstan*
[2]*M. Auezov South Kazakhstan University, Shymkent, Kazakhstan*
(E-mail:molya_1503@mail.ru)

Search for promising protease producers used for molecular diagnostics

Annotation: *The article considers that the majority of biotechnologies in industry and agriculture are based on catalytic processes carried out by enzymes of microbial origin. Special importance is currently attached to enzyme preparations of proteolytic action, which is due to the significant opportunities for their multi-purpose use in various industries. Particularly relevant in recent years is the use of proteolytic enzymes in basic and applied medical research-the detection of vital proteins in the human body. In this paper, we selected the most promising strain - a producer of proteolytic enzymes. The activity of proteolytic enzymes formed by local strains isolated from natural sources (soil of the Turkestan region) was studied. Active producers of proteases are bacteria, microscopic fungi and actinomycetes. Microscopic fungi of the genus Aspergillus have the greatest ability to biosynthesize proteases. As a seed material, an aqueous spore suspension obtained after the growth of fungi was used. The choice of the producer strain is determined by its ability to provide sufficiently high levels of protease activity in the fermentation medium, the rate of formation of enzymes per unit mass of the substrate used, as well as the cost of the substrates themselves.*

Key words: *strains, proteases, cultivating, enzymes, proteins, screening.*

DOI: https://doi.org/10.32523/2616-7034-2020-133-4-16-21

Proteases are enzymes from the class of hydrolases that cleave the peptide bond between amino acids in proteins. They are one of the most important industrial enzymes [1].

The most promising sources of protease production are microorganisms. Active producers of proteases are bacteria, microscopic fungi and actinomycetes. The choice of the producer strain is determined by its ability to provide sufficiently high levels of protease activity in the fermentation medium, the rate of formation of enzymes per unit mass of the substrate used, as well as the cost of the substrates themselves [2].We can name hundreds of microorganisms belonging to various taxonomic groups that are used in the industrial production of proteases. They are most often related to childbirth *Bacillus, Aspergillus, Penicillium, Rizopus, Streptomyces, Pseudomonas, Streptococcus, Lactococcus, Aeromonas* инекоторыедругие [3-6].

Microscopic fungi of the genus have the greatest ability to biosynthesize proteases *Aspergillus: A. oryzae, A. flavus, A. foetidus, A. niger, A. terreusetc* [7-9].Thus, it was found that micromycetes, unlike bacteria, form not only serine and metal-dependent proteinases, but also carboxyl proteinases and a complex of peptidases. Fungi of the genus *Aspergillus* as important industrial microorganisms for the production of various enzymes were included in the list of safe cultures [10].

37 fungal strains isolated from various natural objects (soils, plant residues, etc.) using soil breeding or storage cultures were used as the objects of research [11-12]. Identification of the selected fungi before the genus was performed based on the results obtained by analyzing macro, micro morphological features, morphological features of conidial sporulation of the culture under study, and comparison with those presented in the determinants [10-12]. The selection of producing strains was carried out in two stages. At the first stage, a high-quality (Cup) method was used, which provides for growing crops on *agarized* selective nutrient media. The tested mushrooms were grown in Petri dishes on modified *Chapek* media with sodium *Caseinate* and kept in a thermostat at 30°C for 2 days. Colonies with the largest zones of hydrolysis (enlightenment) of the nutrient medium, protease activity was estimated by the ratio of the diameter of the enlightenment zones (d_{zones}) and the diameter of the colonies ($d_{colonies}$). Deep cultivation of fungi was carried out in 250 ml Erlenmeyer flasks with 50 ml of nutrient medium on a rocker (180-200 rpm) at 26-30°C for 4-7 days. The nutrient medium contained (g/l): rye bran-20.0; malt sprouts-5.0; NH4NO3 (medium # 1) - 1.0; KH2PO4-1.0; MgSO4×7H2O – 0.5; KCl – 0.5; FeSO4×7H2O – 0.01. *The initial pH of the nutrient medium is* 4.5.

As a seed material, an aqueous spore suspension obtained after the growth of fungi on a supporting medium for 14 days at 24-26 °C was used. At the end of cultivation, the biomass was separated by filtration, and the culture liquid filtrate was used for analysis. The total proteolytic activity was determined by a modified Anson method [13] in which a 2% solution of bovine hemoglobin denatured with urea was used as a substrate. As a unit of proteolytic activity, the amount of the enzyme was taken, which in 1 minute under experimental conditions (30-50°C, pH 5.0) catalyzes the transition to the state of such an amount of hemoglobin that contains 1^{mmol} of tyrosine (1^{mmol} of tyrosine is 0.181 mg), which is not precipitated by a 5% solution of trichloroacetic acid. The experimental results presented in this paper represent the average values of 3-5 experiments. For statistical processing of the obtained data, the Microsoft Excel computer program was used.

To increase the effectiveness and reduce the time of detection of biologically active compounds, the selection of the screening method is relevant when searching for producers of any groups of substances of natural origin. The criterion for evaluating the decay of a substance, sample, or material can be the formation of colonies of microorganisms and the appearance of lysis zones. In microbiological practice, the screening method is used to detect the presence of enzymes by seeding on Petri dishes and in test tubes.

The literature presents Express methods of selection of microorganisms-producers of proteases on nutrient media containing various protein substrates: meat-peptone gelatin (MPG) «column» in a test tube, rolled horse or bovine serum, milk, blood agar. To detect proteolytic activity, a modified CAPEC medium with sodium Caseinate was used. Microorganisms with proteolytic activity formed lysis zones on the medium with casein, forming a transparent area around the colonies.

Using the above-mentioned Express method, the ability of 37 *micromycetes* isolated from the environment was analyzed. The ability to produce proteases was detected in 11 cultures, of which 7 strains were related to the genus *Aspergillus*, 2 – *Actinomyces*, 2 – *Trichoderma*, The relationship of the diameters of lysis areas and diameters of colonies made up: of 1.02– 2,03. The most active protease-producing strains were selected based on the maximum values of the d zone / d ratio of the colony (table 1).

Table 1 – Comparative analysis of collection strains-producers of proteases

№	Strains	Samplinglocation	d_{zones}/ $d_{colonies}$	KOE/ г×10-6
1	*A. flavus*	Tolebi district, vill. Tasaryk, serozem	1,06±0,05	16,1
2	*A. foetidus 1*	Kazygurt district, village. Rabat, serozem	1,02±0,05	15,4

3	*A. orizae 1*	Arys, vill. Montaitas, Sierozem	1,92±0,04	17,4
4	*A. niger 1*	Saryagash district, village Tabolina, the Sierozem	2,03±0,03	8,7
5	*A.orizae2*	Tulkibas district, village of Yntymak, Sierozem	1,55±0,04	11,4
6	*A. niger 2*	Tolebi district, Lenger city, the Sierozem	1,68±0,04	9,3
7	*Tr. viride*	Maktaral district, zhetisay city, Sierozem	2,00±0,04	15,3
8	*Tr. koningii*	Shymkent, Enbekshidistrict, Sierozem	2,02±0,05	15,5
9	*A. foetidus2*	Tulkibas district, village of Yntymak, Sierozem	1,84±0,05	11,5
10	*Actinomyces ther movulgaris*	Shymkent, al-Farabidistrict,Sierozem	1,93±0,05	10,4
11	*Actinomyces fradiae*	Saryagash district, Derbesek village, Sierozem	1,85±0,04	10,6

This method made it possible to quickly differentiate colonies with proteolytic activity. The largest diameter of clarification around the colonies were the following strains: *A. niger 1*, isolated from the gray-earth soil of Saryagash district, Tabolino village; *Tr. koningii*, isolated from the soil of Enbekshinsky district, Shymkent and *Tr. viride,* isolated from the soil of Maktaralsky district, Zhetysay. However, for quantitative determination of protease activity, it is necessary to screen the most active variant during deep cultivation of microorganisms, which served as the next stage of work

In order to obtain directed biosynthesis of proteolytic enzymes and increase the productivity of protease-producing strains, the influence of natural nutrient medium was studied, taking into account the physiological needs of the producers. When selecting the components of the natural nutrient medium, the fact that starch and proteins are favorable for the active formation of proteases was taken into account, that is, the main components of the medium should be grain raw materials. We conducted a search for grain raw materials that have a low cost. Such a source in the Turkestan region may be rye used for feeding livestock. Rye *(Secalecereale)* is a cereal crop, its advantages are simplicity of processing, low requirements to soils and fertilizers, as well as good frost resistance. This culture most fully corresponds to the natural and climatic potential of the main zones of the country, including Central Asia and southern Kazakhstan. High adaptive capacity of rye, stability of yield and green mass, agrotechnical significance as a good precursor put it at the moment among the most important agricultural crops. Studies have shown that the greatest biosynthesis of proteases is detected in the medium on 3-4 days of cultivation. The level of protease synthesis by the selected cultures in this case was 7.6-18.4 u / ml (table 2). The maximum level of biosynthesis of proteolytic enzymes was characterized by A. *niger* 1-18.4 u/ml.

Table 2 – biosynthesis of proteolytic enzymes by selected strains during deep cultivation

№	Producerstrain	Biomass, mg / ml	PS, units / ml	The time of growth, t
1	*A. niger 1*	4,8	18,4	48
2	*Tr. viride*	3,2	7,6	72
3	*Tr. koningii*	2,5	9,6	72

Thus, because of a step-by-step two-stage screening of proteolytic enzyme producers, the A. *niger* 1 strain was selected among the strains isolated from the soils of the Turkestan region. As the most active and promising protease producer, the selected strain can be used to create a domestic biotechnology for obtaining protease enzyme preparation in fundamental and applied medical research-the detection of vital proteins in the human body.

Список литературы

1. Поляков В.А., Римарева Л.В. Концентрированные ферментные препараты для спиртовой промышленности // Ликероводочное производство и виноделие – 2000. -№ 9. - С.6-8.
2. Лукерченко В.Н. Новое в организации биохимических процессов при производстве спирта. // Ферментная и спиртовая промышленность.-2002. № 1. С. 32-33.
3. Котов В.Б., Коновалов С.А. О возможности прямой диссимиляции аминокислот дрожжами // Ферментная и спиртовая промышленность -1965. №2. - С. 9-15.
4. Методы экспериментальной микологии / В.И. Билай [и др.]; под ред. В.И. Билай. – Киев: Наук. думка, 1973. 242 с.
5. Веселов И.Я., Канн А.Г., Грачева И.М. Синтез аминокислот и образование высших спиртов при брожении // Ферментная и спиртовая промышленность.-1964. -№ 8. - С. 7 -9.
6. Маринченко В.А., Смирнов В.А., Устинников Б.А. Технология спирта - М.: Легкая и пищевая технология, -1981. 416 с.
7. Авдеев А.Н., Носовская Л.Т., Лаптева Н.К. Технологическая оценка зерна ржи перспективных сортов как сырья для производства крахмала //Хранение и переработка сельхоз сырья. — 2003. -№3, С.66-67.
8. Яровенко В. Технология спирта. - М.: Колос. -1999. 464 с.
9. Неклюдов А.Д., Федорова Н.В. и др. Влияние аминокислот и полипептидов на процесс автолиза биомассы Sacch. cerevisiae// Прикладная биохимия и микробиология – 1994. - Т. 30 - №1. С. 127-131.
10. Полуянова М.Т., Устинников Б.А. Потери углеводов при сбраживании высококонцентрированного сусла // Ферментная и спиртовая промышленность – 1975. - № 5. С.22-24.
11. Яковлева М.Б.. Карпенко М.Л. Козельцев В.Л. Метод выделения грибов, обладающих протеолитической активностью. // Выделение, идентификация и хранение микромицетов и других микроорганизмов (сб. статей). Вильнюс. -1990. С. 101−192.
12. Практикум по микробиологии: учеб.пособие для студ. высш. уч. заведений / А.И. Нетрусов [и др.]; под ред. А.И. Нетрусова. – М.: Издательский центр «Академия», 2005. – 608 с.
13. Сапунова Л.И., Лобанок А.Г., Казакевич И.О., Евтушенков А.Н. Чашечный метод скрининга микроорганизмов – продуцентов ксилоизомеразы // Микробиология. – 2004. - № 1. - С.126-132.

М.Ж. Канжигитова[1], А.М. Есимова[2], М.А. Джакашева[2]

[1]Южно-Казахстанский медицинский академия, Шымкент, Казахстан

[2]Южно-Казахстанского университета имени М.Ауэзова, Шымкент, Казахстан

Поиск перспективных продуцентов протеаз, применяемых для молекулярной диагностики

Аннотация. В статье рассматривается что в основе большинства биотехнологий в промышленности и сельском хозяйстве лежат каталитические процессы, осуществляемые ферментами микробного происхождения. Особое значение в настоящее время придается ферментым препаратам протеолитического действия, что обусловлено значительными возможностями их многоцелевого применения в различных отраслях. Особо актуальным в последние годы применение протеолитических ферментов в фундаментальных и прикладных медицинских исследованиях - обнаружения жизненно важных белков в организме человека. В данной работе отобран наиболее перспективный штамм-продуцент протеолитических ферментов. Исследованы активности протеолитических ферментов, образуемых местными штаммами, выделенных из природных источников (почвы Туркестанской области). Активными продуцентами протеаз являются бактерии, микроскопические грибы и актиномицеты. В наибольшей степени способность к биосинтезу протеаз обладают микроскопические грибы рода Aspergillus. В качестве посевного материала использовали водную споровую суспензию, полученную после роста грибов. Выбор штамма продуцента определяется его способностью обеспечить достаточно высокие уровни активности протеаз в ферментационной среде, скоростью образования ферментов с единицы массы используемого субстрата, а также стоимостью самих субстратов.

Ключевые слова: штаммы, протеазы, культивирование, ферменты, белки, скрининг.

[1] М.Ж. Қанжігітова, [2] А.М. Есимова, [2] М.А. Джакашева
[1]Оңтүстік Қазақстан медицина академиясы, Шымкент, Қазақстан
[2]М. Әуезов атындағы Оңтүстік Қазақстан университеті, Шымкент, Қазақстан

Молекулярлық диагностикада қолданылатын перспективалы протеаздың продуценттерін іздеу

Аңдатпа. Мақалада өнеркәсіпте және ауыл шаруашылығында көптеген биотехнологиялық әдістер микробтық тектес ферменттер жүзеге асыратын каталитикалық процестерге негізделуі қарастырылады. Қазіргі уақытта протеолитикалық әсер ететін ферменттік препараттарға ерекше мән беріледі, бұл әртүрлі салаларда оларды көп мақсатты қолданудың нақты шамадағы мүмкіндіктеріне байланысты. Соңғы жылдары протеолитикалық ферменттерді іргелі және қолданбалы медициналық зерттеулерде қолдануадам ағзасындағы өмірлік маңызды ақуыздарды анықтау аса өзекті болып табылады. Бұл жұмыста ең келешегі бар штамм-протеолитикалық ферменттердің продуценті іріктеледі. Табиғи көздерден (Түркістан облысының топырағынан) бөлінген жергілікті штаммдармен түзілетін протеолитикалық ферменттердің белсенділігін зерттеу қарастырылады. Протеиназдың белсенді продуценттері бактериялар, микроскопиялық саңырауқұлақтар және актиномицеттер болып табылады. Протеаз биосинтезге қабілеттілігі жоғары дәрежеде Aspergillus текті микроскопиялық саңырауқұлақтар бар. Егістік материал ретінде саңырауқұлақтардың өсуінен кейін алынған сулы споралардан құралған суспензия қолданылады. Продуцент штаммын таңдау оның ферментациялық ортада протеаз белсенділігінің жеткілікті жоғары деңгейін қамтамасыз ету қабілетімен, пайдаланылатын субстрат массасының бірлігінен ферменттердің пайда болу жылдамдығымен, сонымен қатар, субстраттардың өз құнымен анықталады.

Түйін сөздер: штаммдар, протеаздар, культивирлеу, ферменттер, ақуыздар, скрининг.

References

1. Polyakov V.A. Rimareva L.B. Kontsentrirovannyye fermentnyye preparaty dlya spirtovoy promyshlennosti [Concentrated enzyme preparations for the alcohol industry] Liquor production and wine-making, 9, 6-8. (2000).
2. Lukerchenko V.N. Novoye v organizatsii biokhimicheskikh protsessov pri proizvodstve spirta [New in the organization of biochemical processes in the production of alcohol], Fermentnaya i spirtovaya promyshlennost, Moskow, 1, 32-33, (2002).
3. Kotov V.B., Konovalov S.A. O vozmozhnosti pryamoy dissimilyatsii aminokislot drozhzhami [About the possibility of direct dissimilation of amino acids by yeast], Fermentnaya i spirtovaya promyshlennost, Moskow, 2, 9-15. (1965).
4. V.I. Bilay et al. Metody eksperimentalnoy mikologii [Methods of experimental mycology], Kiev: Nauk. Dumka, 1973, 237- 242 pp.
5. Veselov I.Ya., Cannes A.G., Gracheva I.M. Sintez aminokislot i obrazovaniye vysshikh spirtov pri brozhenii [The synthesis of amino acids and the formation of higher alcohols during fermentation], Fermentnaya i spirtovaya promyshlennost, Moskow, 8, 7 -9, (1964).
6. Marinchenko V.A., Smirnov V.A., Ustinnikov B.A. Tekhnologiya spirta [Alcohol technology], M: Legkaya i pishevaya promyshlennost', 1981, -416 p.
7. Avdeev A.N., Nosovskaya L.T., Lapteva N.K. Tekhnologicheskaya otsenka zerna rzhi perspektivnykh sortov kak syr'ya dlya proizvodstva krakhmala [Technological evaluation of rye grain of promising varieties as raw materials for starch production], Storage and processing of agricultural raw materials, 3, 66 - 67. (2003).
8. Yarovenko B. Tekhnologiya spirta [Technology of alcohol], M .: Kolos, 1999, 464 p.
9. Neklyudov A.D., Fedorova N.V. et al. Vliyaniye aminokislot i polipeptidov na protsess avtoliza biomassy Sacch. cerevisiae [Effect of amino acids and polypeptides on the autolysis of Sacch biomass. Cerevisiae], Applied Biochemistry and Microbiology, 30(1), 127-131, (1994).
10. Poluyanova M.T., Ustinnikov B.A. Poteri uglevodov pri sbrazhivanii vysokokontsentrirovannogo susla [Loss of carbohydrates during the fermentation of highly concentrated wort] Fermentnaya i spirtovaya promyshlennost, Moskow, 5 , 22-24. (1975).
11. Yakovleva M.B. Karpenko M.L. Kozeltsev V.L. Metod vydeleniya gribov, obladayushchikh proteoliticheskoy aktivnost'yu [Method for isolating fungi with proteolytic activity], Isolation, identification and storage of micromycetes and other microorganisms (collection of articles). Vilnius. 5,101–192. (1990).

12. A.I. Netrusova Praktikum po mikrobiologii [Workshop on Microbiology], textbook for students. Higher student institutions / M.: Publishing Center «Academy», 2005, 608 p.

13. Sapunova L.I., Lobanok A.G., Kazakevich I.O., Evtushenkov A.N. Chashechnyy metod skrininga mikroorganizmov – produtsentov ksiloizomerazy [The Cup Method for Screening Microorganisms Producing Xylisomerase], Microbiology, 1, 126-132. (2004).

Сведения об авторах:

***Қанжігітова М.Ж.* – корреспонденция үшін автор,** «Биотехнология» мамандығының 2 курс докторанты, М. Әуезов атындағы Оңтүстік Қазақстан мемлекеттік университеті, Шымкент, Қазақстан.

Есимова А.М. – химия ғылымының кандидаты, доцент, М. Әуезов атындағы Оңтүстік Қазақстан мемлекеттік университеті, Шымкент, Қазақстан.

Джакашева М.Ә. – PhD, М. Әуезов атындағы Оңтүстік Қазақстан мемлекеттік университеті, Шымкент, Қазақстан.

***Kanzhigitova M.Zh.* – corresponding author,** «Biotechnology» PhD 2-year doctoral student. M. Auezov South Kazakhstan state University named after, 5 Tauke-Khan street, Shymkent, Kazakhstan.

Esimova A.M. – Ph.D. Candidate of Chemical Sciences, Associate Professor, M. Auezov South Kazakhstan state University, G. ilyaeva 8, Shymkent, Kazakhstan.

Dzhakasheva M.A. – Ph.D., M. Auezov South Kazakhstan State University, G. Ilyaev St.8, Shymkent, Kazakhstan.