O‘ZBEKISTON MILLIY UNIVERSITETI XABARLARI, 2023, [3/1/1]
ISSN 2181-7324

BIOLOGIYA
http://journals.nuu.uz
Natural sciences

UDK: 579.475(3)

Nozim SHODIEV,
Master’s degree student of the Microbiology and Biotechnology Department
National University of Uzbekistan
E-mail: nozimbek.shodiyev@bk.ru
Sokhibjon ABDUSAMATOV,
PhD., National University of Uzbekistan
Kakhramon DAVRANOV,
Professor, Director of the Institute of Microbiology, Academic Science of Uzbekistan

Review based on associate professor Fayziyev Vahid Bakhromovich, Chirchik State Pedagogical Institute

ISOLATION OF THERMOPHILIC BACTERIA FROM OLTINSOY VILLAGE, NAVOIY REGION, UZBEKISTAN, AND THEIR ENZYMATIC ACTIVITIES

Annotation

In this research, we isolated thermophilic bacteria from the hot spring of Oltinsoy village which is located in the Navoiy region of Uzbekistan. We carried out some experiments on them such as learning their morphological structure and some physiological activities. We found optimal conditions for thermophilic bacteria growth and chose specific media for them. In general, our four isolates showed high activity in skimmed milk, which means they can produce protease enzymes.
Key words: Thermophilic bacteria, hot spring, protease, amylase, gelatinase, lipase .

ВЫДЕЛЕНИЕ ТЕРМОФИЛЬНЫХ БАКТЕРИЙ ИЗ СЕЛА ОЛТИНСОЙ НАВОИЙСКОЙ ОБЛАСТИ, УЗБЕКИСТАН, И ИХ ФЕРМЕНТАТИВНАЯ АКТИВНОСТЬ

Аннотация

В данном исследовании мы выделили термофильные бактерии из горячего источника поселка Олтинсой, расположенного в Навоийской области Узбекистана. Мы провели с ними некоторые эксперименты, такие как изучение их морфологической структуры и некоторых физиологических действий. Найдены оптимальные условия для роста термофильных бактерий и подобраны для них специфические среды. В целом четыре наших изолята показали высокую активность в обезжиренном молоке, что означает, что они могут продуцировать ферменты протеазы.
Ключевые слова: Термофильные бактерии, горячий источник, протеаза, амилаза, желатиназа, липаза.

O‘ZBEKISTONNING NAVOIY VILOYATI OLTINSOY QISHLOG‘IDAGI QAYNAR BULOQDAN TERMOFIL BAKTERIYALARINI AJRATIB OLISH VA ULARNING FERMENTATIV FAOLLIGI

Annotatsiya

Bu ilmiy ishimizda termofil bakteriyalarini Navoiy viloyati, Oltinsoy qishlog‘idagi qaynar buloqdan ajratib oldik. Bu maqolada bir nechta amalga oshirilgan tajribalarimiz yoritilgan bo‘lib, jumladan, biz ularning morfologik va fiziologik xususiyatlarini o‘rgandik. Oltinsoy qishlog‘idagi qaynar buloqdan ajratib olingan termofil bakteriyalari o‘sishi uchun eng qulay bo‘lgan ozuqa muhitini tanlab oldik. Bundan tashqari ularning fermentativ xususiyatini o‘rganish mobaynida bir nechta ferment faolliklari aniqlandi, natijada umumiy ajratib olingan 6 ta shtammdan 4 tasi qurutilgan sut substrati yordamida proteaza fermenti faolligi aniqlandi.
Kalit so‘zlar: Termofil bakteriyalar, qaynar buloq, proteaza, amilaza, jelatinaza, lipaza.

Introduction. Currently, thermophilic bacteria are the main source of some industries in Uzbekistan. They can be applied in different fields of science and learning their enzymatic activities may help us to conduct some experiments cheaper and more effectively. In Uzbekistan, there are some hot springs that have not been learned about yet. In these springs, it can be possible that find new strains of thermophilic bacteria.

Literature review. There have been several fascinating organisms which inhabit the earth over the last few decades that have intrigued scientists. The organisms are known as extremophiles because they thrive in habitats that are not suitable for other organisms [1]. Enzymes found in thermophiles are excellent for enduring and accelerating reactions at high temperatures[2].

Several biomolecules (exopolysaccharides, antimicrobials, biosurfactants) are produced by thermophiles that are of biotechnological and industrial interest [3]. It is necessary to study the thermophile ecosystem in order to understand its structure and composition, as well as to discover new taxa and determine how microorganisms interact with microbial communities in order to solve the problem. Thermophile biodiversity provides an overview of the potential [4].

Approximately 2% of all proteins in all organisms are proteases, also called peptidases, which play numerous essential roles. It is possible to divide peptidases into different clans and families by using the MEROPS database1, which provides a detailed classification of proteases [5].

Protease enzymes are produced commercially from plants, animals, and microbial sources. The advantage of microorganisms for protease enzyme production is that they can be easily cultured using established fermentation techniques in large numbers and produce a regular supply of the desired product. They can also be genetically manipulated more easily than plants and animals, which makes them an ideal source of protease enzymes [6].

Research Methodology. Our research started with taking samples from hot springs in the village of Oltinsoy, Khatirchi district, Navoiy region of Uzbekistan. In this case, water and sediment samples were taken from different parts of the hot springs (mainly the beginning of the spring, 1-2 and 3-4 meters away from the basin), measuring temperature and pH values. A total of three water samples and three sediment samples were collected from the hot spring using 1000 ml sterile thermal glass containers and sterile flasks.

We used Thermus 162 media to cultivate thermophilic bacteria and we prepared two 300 ml culture media (dis. water 300 ml, peptone 0.1% 300 mg, yeast extract 0.1% 300 mg, NaCl 0.1% 300 mg and salt buffer 300 ml): pH=8 and pH=6.4 ([7]) and a total of 6 isolates was put into 60^0 C for 3-4 days. After that, culture media was put on Petri dishes (20 ml) and bacteria were cultivated through a method of bacterial lawn to Petri. Then, our samples were put into a thermostat at 60^0 C for 3-4 days. Colony diameter, morphology, and colony structure were recorded after 5 days of isolates. Colonies of bacterial isolates are characterized by the following characteristics: color, shape, height, border, diameter, surface, transparency, and texture, and the isolates were stained by Gramm’s method for morphological analysis.

Enzymatic activities

Because of the importance to determine the enzyme activity of thermophilic bacteria, enzyme activity such as Amylase, Protease, Gelatinase, and Lipase was studied [8]. Initially, our isolates were grown in liquid nutrient media for 2-3 days and inoculated on substrates grown in solid nutrient media.

Amylase activity

Amylase assay was determined on 1% (w/v) starch agar medium. The starch hydrolysis was detected by flooding plates with Lugol’s iodine solution. The clear zone around the colony indicates a positive result [9].

Protease activity

The activity of protease enzymes was observed using Skim Milk Agar. Microbes were observed during protease activity in the medium and were incubated at a temperature of 50^0C for 24-72 hours. Protease activity is characterized by forming transparent zones around the colony of microorganisms [10].

Gelatinase activity

The gelatin hydrolysis assay was conducted according to the method of Frazier [11] using 0.4% gelatin-based agar. After incubation, the degradation of gelatin was seen as a clear zone around colonies, in the somewhat opaque agar. When the plate was flooded with a saturated aqueous solution of ammonium sulfate, a precipitate was formed that made the agar more opaque and enhanced the clear zones around the colonies [12].

Lipolytic activity

Lipolytic activity was detected according to the diffusion agar method on Tween based medium containing 1% of the tween (20 or 80), 10 g Difco Bacto-peptone, 5 g NaCl, 0.1 g CaCl2.1H2O, 30 g agar-agar, and 1 L distilled H2O (pH 7.4). A well-visible halo or turbid around the colonies indicated lipolysis [13].

Analysis and results. A total of 14 strains were isolated from the Oltinsoy hot spring, but only 6 isolates showed high growth, and isolation and purification procedures were carried out with both water and sediment samples. We learned colony diameter, morphology, and colony structure were recorded after 5 days of isolates. Colonies of bacterial isolates are characterized by the following characteristics: color, shape, height, border, diameter, surface, transparency, and texture.

When our isolates grew after incubation, the morphological appearance of our isolates was yellow, they grow widely. According to microscopic analysis (figures 1 and 2), these thermophilic bacteria are related to the Bacillus genera. Furthermore, Gramm's method stained the isolates in our next experiments. It was observed that 3 out of 6 isolates were gram-positive and the remaining 3 were gram-negative.

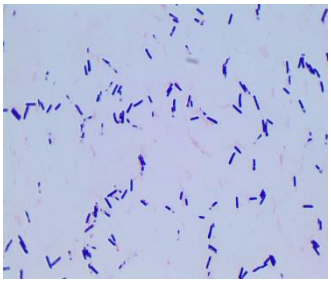

Figure 1. ***Bacillus sp*** **(57^0 C water sample)**

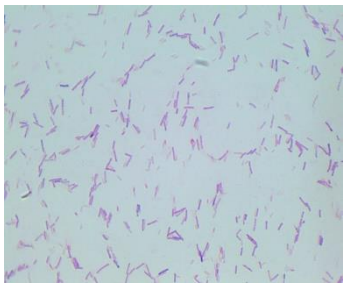

Figure 2. *Bacillus sp* **(60^0 C sediment sample)**

The next experiment was the activity of thermophilic bacteria on different substrates such as skimmed milk, starch, gelatin, and tween 80. According to enzymatic activity reactions, only four strains were gotten from 57^0 C sediment and water, 60^0 C sediment, and water parts of the hot spring. We can see our 4 strains show the high result for some enzymes. According to these results, strains 57^0 C and 60^0 C showed good results on skimmed milk and they can produce protease enzymes. However, they did not give any activities against starch, gelatin, and tween 80.

Thermophilic bacteria in the hot springs of the Navoiy region have not been learned yet. Some species of thermophilic bacteria are unknown. Our upcoming research will be dedicated to sequencing these bacteria’s 16S r-RNK and their protease usage in

Figure 3. Protease activity

different fields of industries in our country.

Conclusion. In this experiment we found a strain that has high protease enzyme activity and the next conducting experiments will be dedicated to analyze a type of protease and finding out the strain of taxa through sequencing their nucleic acid.

REFERENCES

1. Rampelotto P., “Extremophiles and Extreme Environments,” Life, vol. 3, no. 3, pp. 482–485, Aug. 2013, doi: 10.3390/life3030482.
2. Amanuel M.G., Nancy L.M.B., Sylvester E.A., Tadesse M., and Hamadi I.B., “Thermotolerant bacteria of biotechnological potential from hot springs in Eritrea,” Afr. J. Microbiol. Res., vol. 12, no. 22, pp. 512–524, Jun. 2018, doi: 10.5897/AJMR2017.8626.
3. Wang J., Salem D.R., and Sani R.K., “Extremophilic exopolysaccharides: A review and new perspectives on engineering strategies and applications,” Carbohydr. Polym., vol. 205, pp. 8–26, Feb. 2019, doi: 10.1016/j.carbpol.2018.10.011.
4. Li L.and (Sam) Ma Z., “Global Microbiome Diversity Scaling in Hot Springs With DAR (Diversity-Area Relationship) Profiles,” Front. Microbiol., vol. 10, p. 118, Feb. 2019, doi: 10.3389/fmicb.2019.00118.

A. Barrett J., Rawlings N. D., and O‘Brien E. A., “The MEROPS Database as a Protease Information System,” J. Struct. Biol., vol. 134, no. 2–3, pp. 95–102, May 2001, doi: 10.1006/jsbi.2000.4332.

5. G. R., B. Q., K. S., and C. B., “An overview on fermentation, downstream processing and properties of microbial alkaline proteases,” Appl. Microbiol. Biotechnol., vol. 60, no. 4, pp. 381–395, Dec. 2002, doi: 10.1007/s00253-002-1142-1.
6. Degryse E., Glansdorff N., and Pirard A., “A comparative analysis of extreme thermophilic bacteria belonging to the genus Thermus,” Arch. Microbiol., vol. 117, no. 2, pp. 189–196, 1978, doi: 10.1007/BF00402307.
7. Benammar L.et al., “Diversity and enzymatic potential of thermophilic bacteria associated with terrestrial hot springs in Algeria,” Braz. J. Microbiol., vol. 51, no. 4, pp. 1987–2007, Dec. 2020, doi: 10.1007/s42770-020-00376-0.
8. Marteinsson V., Birrien J.-L., Jeanthon C., and Prieur D., “Numerical taxonomic study of thermophilic Bacillus isolated from three geographically separated deep-sea hydrothermal vents,” FEMS Microbiol. Ecol., vol. 21, no. 4, pp. 255–266, Dec. 1996, doi: 10.1111/j.1574-6941.1996.tb00122.x.
9. Alnahdi H.S., “Isolation and screening of extracellular proteases produced by new Isolated Bacillus sp,” J. Appl. Pharm. Sci., Sep. 2012, doi: 10.7324/JAPS.2012.2915.
10. Frazier WC., “A Method for the Detection of Changes in Gelatin Due to Bacteria: Two Plates,” J. Infect. Dis., vol. 39, no. 4, pp. 302–309, Oct. 1926, doi: 10.1093/infdis/39.4.302.
11. Gopinath S.C.B., Hilda A., and Anbu P., “Extracellular enzymatic activity profiles in fungi isolated from oil-rich environments,” Mycoscience, vol. 46, no. 2, pp. 119–126, Apr. 2005, doi: 10.1007/S10267-004-0221-9.
12. Sierra G., “A simple method for the detection of lipolytic activity of micro-organisms and some observations on the influence of the contact between cells and fatty substrates,” Antonie Van Leeuwenhoek, vol. 23, no. 1, pp. 15–22, Dec. 1957, doi: 10.1007/BF02545855.