ЕРЕВАНСКИЙ ГОСУДАРСТВЕННЫЙ УНИВЕРСИТЕТ
YEREVAN STATE UNIVERSITY

СТУДЕНЧЕСКОЕ НАУЧНОЕ ОБЩЕСТВО
STUDENT SCIENTIFIC SOCIETY

ISSN 1829-4367

СБОРНИК НАУЧНЫХ СТАТЕЙ СНО ЕГУ

МАТЕРИАЛЫ ЮБИЛЕЙНОЙ НАУЧНОЙ СЕССИИ, ПОСВЯЩЕННОЙ 95-ЛЕТИЮ ОСНОВАНИЯ ЕГУ

COLLECTION OF SCIENTIFIC ARTICLES OF YSU SSS

MATERIALS OF THE SCIENTIFIC SESSION DEDICATED TO THE 95TH ANNIVERSARY OF YSU

1.1 (4)

Естественные науки (Биология и химия)

Natural sciencies (Biology and Chemistry)

ЕРЕВАН - YEREVAN

ИЗДАТЕЛЬСТВО ЕГУ - YSU PRESS

2015

ԵՐԵՎԱՆԻ ՊԵՏԱԿԱՆ ՀԱՄԱԼՍԱՐԱՆ

ՈՒՍԱՆՈՂԱԿԱՆ ԳԻՏԱԿԱՆ ԸՆԿԵՐՈՒԹՅՈՒՆ

ISSN 1829-4367

ԵՊՀ ՈՒԳԸ ԳԻՏԱԿԱՆ ՀՈԴՎԱԾՆԵՐԻ ԺՈՂՈՎԱԾՈՒ

ԵՊՀ ՀԻՄՆԱԴՐՄԱՆ 95-ԱՄՅԱԿԻՆ ՆՎԻՐՎԱԾ ՀՈԲԵԼՅԱՆԱԿԱՆ ԳԻՏԱԿԱՆ ՆՍՏԱՇՐՋԱՆԻ ՆՅՈՒԹԵՐ

1.1 (4)

Բնական գիտություններ
(կենսաբանություն և քիմիա)

ԵՐԵՎԱՆ

ԵՊՀ ՀՐԱՏԱՐԱԿՉՈՒԹՅՈՒՆ

2015

Հրատարակվում է
ԵՊՀ գիտական խորհրդի որոշմամբ
Издается по решению Ученого совета ЕГУ
Published by the resolution of the Academic Council of YSU

Հրատ. պատասխանատու խմբագիր՝ ***Մ. Մալխասյան***

Հրատարակիչ՝ ԵՊՀ հրատարակչություն
Հասցե՝ ՀՀ, ք. Երևան, Ալ. Մանուկյան 1, (+374 10) 55-55-70, publishing@ysu.am
Հրատարակության նախապատրաստող ստորաբաժանում՝ ԵՊՀ ուսանողական գիտական ընկերություն
Հասցե՝ ք. Երևան, Ա. Մանուկյան 1, (+37460) 71-01-94, ssspub@ysu.am, sss@ysu.am
ԵՊՀ ՈՒԳԸ հրատարակումների կայք՝ ssspub.ysu.am

Gohar Vardanyan[1], Armine Margaryan[2], Hovik Panosyan
YSU, Faculty of Biology, [1]Master's Student, [2]Junior Researcher
Supervisor: PhD, Associate Professor H. Panosyan
E-mail: gohar.vardanyan.91@mail.ru

ISOLATION AND CHARACTERIZATION OF LIPASE-PRODUCING BACILLI FROM TATEV GEOTHERMAL SPRING (ARMENIA)

Introduction: Thermophilic microorganisms, thrived in extreme environments are interesting sources for the thermozymes, which are capable of catalyzing biochemical reactions at high temperatures. Thermozymes are generally superior to the traditional biocatalysts, because they produce proteins with unique properties and show reasonable activity even at 100°C in the presence of organic solvents and detergents [Antranikian, 2008]. Lipases are known as one of the most imperative biocatalysts in today's epoch and carry out novel reactions both in aqueous and nonaqueous environments [Sharma et al., 2013]. Microbial lipases form an important group of enzymes used in a variety of biotechnological applications, such as organic chemical processing, detergent formulation, synthesis of biosurfactants, agrochemical industry, paper manufacture, nutrition, cosmetics and pharmaceutical processing, esterification of the oil and in the production of bio diesel [Jaeger et al., 2002; Sharma et al., 2013].

Thermostable lipases were purified and characterized from thermophilic isolates mainly belonging to genus *Bacillus* and related genera, which nowadays are used in industry [Sugihara et al., 1991; Fakhreddine et al., 1998; Kim et al., 2002; Rahman et al., 2007; Tayyab et al, 2011]. Thermophilic lipase-producing bacilli were isolated from various environments, but geothermal springs are usually their favorable habitats [Haki and Rakshit, 2003].

Therefore, the isolation and identification of new thermophilic bacilli from natural sources are quite beneficial in terms of discovery of novel thermostable lipases with high catalytic rates.

The aim of this study was to isolate and characterize thermophilic lipase-producing bacilli from Tatev geothermal spring (Armenia).

Materials and methods: *Sampling:* the location of Tatev geothermal spring was determined by GPS. Water temperature and pH were measured *in situ* using portative combined pH/EC/TDS/Temperature tester (HANNA HI98129/HI98130). Sediment samples of the studied spring were aseptically collected into sterile flasks and kept on ice until processed.

Enrichment and isolation: To isolate thermophile bacilli 1.0 g of sediment samples was suspended in 10 ml of sterile water and vortexed for 1 min. Supernatant was transferred to a glass tube with a screw cup and pasteurized at 80°C for 10 min in the water bath. 1.0 ml aliquots were placed on medium and incubated with shaking (120 rpm) overnight at 55 and 65°C. To enrich thermophilic bacilli we used medium with the following composition (%): peptone 1.0, NaCl 0.5, $CaCI_2 \cdot H_2O$ 0.01, tween 40 1.0, pH 7.4. Then 0.5 ml aliquots of appropriate dilution were placed on the same medium containing 1.5% agar and incubated overnight at 55 and 65°C. All obtained colonies were picked and purified by streaking on the same medium at least three times.

Phenotypic characteristics: Colonies were characterized regarding the form, elevation, margin and optical feature. Cells' morphology, sizes and motility were determined by a light microscope (Motic 10). Endospores were observed by Peshkov's staining method [Netrusov et al., 2005].

The growth temperature range was determined by incubation of isolates at the temperature from 40 to 70°C with5°C intervals. The optical density was measured hourly by spectrophotometric (Spectrophotometer Genesis 10S, Thermo Scientific, at λ=560 nm) during incubation. The pH dependence of growth was tested by incubating isolates at pH from 5 to 10.

Catalase and oxidase activities, anaerobic growth, reduction of nitrate to nitrite, Voges-Proskauer reaction, formation of dihydroxyacetone, utilization of citrate, hydrolyses of casein and starch were tested using commonly accepted methods [Gordon et al., 1973;Bergey's Manual of Systematic Bacteriology, 1986; Netrusov et al., 2005].

Lipase activity: Bacterial biomass was obtained by overnight incubation of isolates with shaking (120 rpm) at 65°C in the medium continuing 1% tween 40. The cultures were centrifuged (5000 g, 15 min) and obtained supernatant were used as source of crude enzyme. The lipase activity was measured by alkalimetric titration method [Lee and Rhee, 1993; Netrusov et al., 2005]. Reaction mixture containing 0.5 ml tween 40, 5.0 ml phosphate buffer (pH 7.0) and 1.0 ml of crude enzyme was incubated with shaking (120 rpm) at 65°C. The reaction was terminated every hour (during whole day) by adding 1 ml ethanol and titrated with 0.1N potassium hydroxide. One unit of lipase activity (U) was defined as the release of 1 μmol of fatty acid per min under mentioned conditions.

Results and discussion: Tatev geothermal spring is located at N 39°23.765′, E 46°15.482′, with temperature of 27ºC, pH 6.0 and conductivity of 2120 μSsm^{-1}. Two thermophilic-aerobic chemoorganotrophic lipase producing bacilli strains designed as TatevN5 and Tatev N6 were isolated from sediment samples of Tatev geothermal spring. The isolates formed light yellow/creamy, circular, smooth colonies with 0.5-2 mm diameter. Cells of both strains were Gram positive, motile rod shaped varying in length between 3.0 to 12 μm and in diameter between 0.3 to 0.7 μm. The cells were formed terminal swollen sporangia with ellipsoid endospores. Optimal temperature of growth was 65°C. The pH range for growth was from 6 to 9, with the optimum pH at 7. Catalase and oxidase tests were positive, while Voges-Proskauer test was negative. They did not use citrate, did not hydrolyze starch and casein, except the strain Tatev N 5, which hydrolyzed casein. Some phenotypic characteristics of the isolates are presented in the table 1.

Table 1.

Phenotypic characteristics of isolates

Characteristics	Strains	
	Tatev N5	Tatev N6
Cell size	0.4-0.65×4-12 μm	0.3-0.6×3-7 μm
Optimal growth temperature (°C)	65	65
pH	7.0	7.0
Catalase	+	+
Oxidase	+	+
Nitrate reduction	+	-
Citrate utilization	-	-
Voges-Proskauer test	-	-
Formation of Dihydroxyacetone	-	-
Hydrolysis of		
Starch	-	-
Casein	+	-

Following the criteria of Bergey's Manual of Determinative Bacteriology the isolates were identified as *Geobacillus* species.

The logarithmic phase of growth for both strains was started after 2 hours and extended to the 10 hours of incubation at optimal temperature and pH (65°C, 7.0) (Fig. 1.). The culture liquid from late logarithmic growth phase was centrifuged and supernatant was used as a crude enzyme for lipase activity assay.

The strains showed high lipases activity during long incubation period at 65°C (Fig. 2). The high lipase activity of *Geobacillus* sp. Tatev N5 was observed after 5 hours of incubation and it was 70.3 U/ml. The activity of thermostable lipase from *Geobacillus* sp. Tatev N6 was observed even after 7 hours of incubation and was 1.15 time higher than the lipase activity of *Geobacillus* sp. Tatev N5 (Fig. 2).

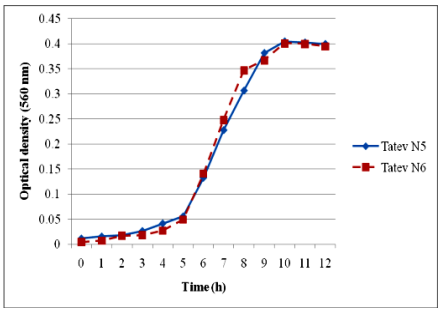

Figure 1. *Growth of the strains Geobacillus sp. Tatev N5 and Tatev N6.*

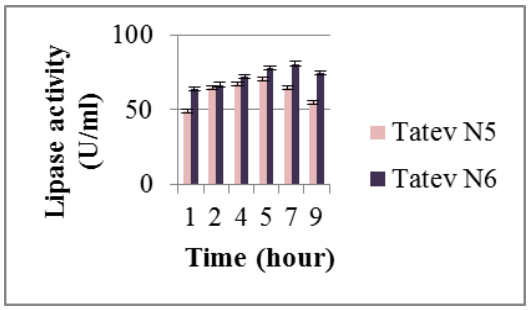

Figure 2. *Lipase activity of the strains Geobacillus sp. Tatev N5 and Tatev N6.*

We have concluded that the termostability and high activity of lipase from *Geobacillus* strains could be prospective for various biotechnological applications.

References

1. **Antranikian G.,** Industrial relevance of thermophiles and their enzymes. In: Robb F. et al. (Eds) Thermophiles-biology and technology at high temperatures, CRC Press, Boca Raton, 2008, pp. 113-160.
2. **Bergey's** Manual of Systematic Bacteriology, In: Endospore-forming Gram-Positive Rods and Cocci (Peter H.A.). Williams & Willkins, Baltimore-Hong Kong-London-Sydney. 2, 1986, pp. 1104-1139.
3. **Gordon R., Haynes W., Pang Ch. N.,** The genus *Bacillus,* United Stated Departmant of Agriculture, Handbook 427, 1973, p. 283.
4. **Fakhreddine L., Kademi A., Ait-Abdelkader N., Baratti J.,** Microbial growth and lipolytic activities of moderate thermophilic bacterial strain, Biotechnol. Lett., 20 (9), 1998, pp. 879-883.
5. **Jaeger K., Eggert T.,** Lipases for biotechnology. Current Opinions of Biotechnology, 13 (4), 2002, pp. 390-397.
6. **Haki G., Rakshit S.,** Developments in industrially important thermostable enzymes: a review. Bioresour. Technol., 89 (1), 2003, pp. 17-34.
7. **Kim H.K., Chio H.J., Kim M.N., Sohin S.B., Oh T.K.,** Expression and characterization of Ca^{2+} independent lipase from *Bacillus pumilus* B26, J. Biochem. Biophys. Acta., 11 (83), 2002, pp. 205-212.
8. **Lee S.Y., Rhee J.S.,** Production and Partial Purification of a Lipase from *Pseudomonas putida* 3SK, Enzyme and Microbial Technology, 15, 1993, pp. 617-623.
9. **Netrusov A.,** Guide to Practical Training on Microbiology, Academy Publishing, Moscow, RF., 2005, p. 608 (Russian).
10. **Rahman R., Leow T., Salleh A., Basri M.,** *Geobacillus zalihae* sp. nov., a thermophilic lipolytic bacterium isolated from palm oil mill effluent in Malaysia. BMC Microbiology, 7 (77), 2007, pp. 1-11.
11. **Sharma R., Thakur V., Sharma M., Birkeland N.,** Biocatalysis through thermostable lipases: adding flavor to chemistry. Chepter 34. In: Shatyanarayana T., Littlechild J., Kawarabayasi Y. (Eds). Thermophilic microbes in environmental and industrial biotechnology: *Biotechnology of thermophiles*, Springer, New York, 2013, pp. 905-927.

12. **Sugihara A., Tani T., Tominaga Y.,** Purification and characterization of a novel thermostable lipase from *Bacillus* sp., J. Biochem., 109 (2), 1991, pp. 211-216.
13. **Tayyab M., Rashid N., Akhtar M.,** Isolation and identification of lipase producing thermophilic *Geobacillus* sp. SBS-4S: Cloning and characterization of the lipase, Journal of Bioscience and Bioengineering, 111 (3), 2011, pp. 272-278.

Գոհար Վարդանյան, Արմինե Մարգարյան, Հովիկ Փանոսյան

ՏԱԹԵՎԻ ԵՐԿՐԱՋԵՐՄԱՅԻՆ ԱՂԲՅՈՒՐԻՑ (ՀԱՅԱՍՏԱՆ) ԼԻՊԱԶ ԱՐՏԱԴՐՈՂ ԲԱՑԻԼՆԵՐԻ ՄԵԿՈՒՍԱՑՈՒՄԸ ԵՎ ԲՆՈՒԹԱԳՐՈՒՄԸ

Բանալի բառեր՝ ջերմասեր բացիլներ, ջերմակայուն լիպազ, Geobacillus

Տաթևի երկրաջերմային աղբյուրից (Հայաստան) մեկուսացվել են Tatev N5 ու Tatev N6 անվանակոչված երկու ջերմասեր լիպազ արտադրող բացիլների շտամներ: Մեկուսացված բացիլները ֆենոտիպական հատկանիշների հիման վրա նույնականացվել են որպես *Geobacillus* ցեղի տեսակներ: *Geobacillus* sp. Tatev N5 և Tatev N6 շտամների չմաքրված ֆերմենտների ակտիվությունները օպտիմալ ջերմաստիճանի և pH-ի պայմաններում (65°C, pH 7) կազմել են 70.3 և 80.7 Մ/մլ համապատասխանաբար:

Гоар Варданян, Армине Маргарян, Овик Паносян

ИЗОЛИРОВАНИЕ И ХАРАКТЕРИСТИКА БАЦИЛЛ-ПРОДУЦЕНТОВ ЛИПАЗ ИЗ ГЕОТЕРМАЛЬНОГО ИСТОЧНИКА ТАТЕВ (АРМЕНИЯ)

Ключевые слова: термофильные бациллы, термостабильная липаза, Geobacillus

В статье рассматриваются две термофильные липазЫ, продуцирующие бациллы, выделенные из геотермального источника Татев (Армения), обозначенные как Tatev N5 и Tatev N6. На основании фенотипических признаков изоляты идентифицированы как представители рода *Geobacillus*. Липазная активность штаммов *Geobacillus* sp. Tatev N5 и Tatev N6, определенНая при оптимальных значениях температуры и pH (65°C, pH 7), составляла 70.3 и 80.7 Е/мл, соответственно.

Gohar Vardanyan, Armine Margaryan, Hovik Panosyan

ISOLATION AND CHARACTERIZATION OF LIPASE-PRODUCING BACILLI FROM TATEV GEOTHERMAL SPRING (ARMENIA)

Keywords: thermophile bacilli, thermostable lipase, Geobacillus

Two thermophilic lipase-producing bacilli strains designed as Tatev N5 and Tatev N6 were isolated from Tatev (Armenia) geothermal spring. Based on phenotypic characteristics isolates were identified as species of genus *Geobacillus*. The lipase activities of the *Geobacillus* sp. Tatev N5 and Tatev N6 at optimal growth temperature and pH (65°C, pH 7) were 70.3 and 80.7 U/ml, correspondingly.