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Thermophilic bacteria of the hot springs "Ashagi Istisu" and "Yukhari Istisu" of the Kalbajar region of the Republic of Azerbaijan

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The presented work is devoted to the study of the bacterial biota of the hot springs "Ashagi Istisu" (pH=8.0, t=64°C, mineralization is 6.7 g/l) and "Yukhari Istisu" (pH=9.0, t=71°C, mineralization is 4.3 g/l) of the Kalbajar region, characterized by a wide range of environmental conditions (pH, temperatures, chemical components, etc.). Samples of thermal waters were inoculated and colonies of thermophilic bacteria that grew on a solid medium of nutrient agar were isolated. Some colonies of thermophilic bacteria were isolated in pure culture and 6 strains were received (KA_1, KA_2, KA_3, KY_1, KY_2, KY_3). The cell morphology of these strains is represented by short and long gram-positive, spore - forming rods, varying in size. Colonies differ in color, shape, margin, consistency, size, etc. The morphology of cells and colonies of two strains (KA_1, KY_1) has been well studied, and the ratio of these strains to the pH and temperature of the medium was determined. It was found that strains KA_1, KY_1 actively grow in the temperature range 55-60°C, at pH=7.0-9.0. Based on morpho-cultural characteristics and some physiological properties, the strains were identified. The KA_1 strain was assigned to the genus Bacillus, and the KY_1 strain was presumably assigned to the Bacillus stearothermophilus species.

Keywords: *Hot springs, thermophilic bacteria, morpho-cultural characteristics*

INTRODUCTION

Kalbajar region is one of the most beautiful regions of Azerbaijan, distinguished by its climatic conditions and natural wealth. Kalbajar is an integral part of Azerbaijan, the highest mountainous region, located at the height of the Jamyshdag mountain (3724 m). In 1993 Kalbajar was occupied by Armenian armed bandit formations (Kamal, 2012). But, based on the trilateral agreement signed on November 10, 2020, the Kalbajar region again came under the control of Azerbaijan. Azerbaijani President Ilham Aliyev said, that the authorities will restore the Kalbajar region, which has come under the control of Baku according to the trilateral agreement on Karabakh including the "Istisu" resort zone. The Istisu spring is worthy of a separate mention the "living water" of the spring unique in its properties has cured and brought back to life millions of people (Гасанов, 1978). The spring is located on the slopes of mountain ranges in the west of Kalbajar, at an altitude of 2225 meters above sea level. The water here is hyperthermal, hydrocarbonate – sulphate – chloride - sodium, carbonated (Трешников, 1983). There are 12 water springs in total, which have a curative effect on internal and external human diseases. In 1980 a mineral water collection plant and a large resort were built here. In the village of Istisu in the former USSR, sanatoriums of All-Union significance worked which every year received 50.000 people. The water of the Istisu spring attracts the attention of scientists from all over the world. It was noted that in terms of its physical characteristics and composition this water is close to water from the Czech Republic (Karlovy Vary) and in some parameters the water has no analogues in the whole world (Ахмедова и др., 2016). The springs of Istisu with therapeutic and sickleological influence include Yukhari Istisu, Ashagi Istisu, Keshdag, Garasu, Tutkhun, Mozchay, which glorified Kalbajar (Гасанов, 1978). Istisu thermal springs also attract

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the attention of microbiologists due to their unique microbiota. It is known that hot springs are natural habitats for thermophilic microorganisms (Adiguzel et al., 2009, 2011; Khiyami et al., 2012; Verma et al., 2014; Akhmedova, 2021). Investigations of thermophilic microorganisms of the Istisu springs were systematically and fruitfully carried out many years ago (Əhmədova, 2007).

Knowledge of the qualitative composition of the microbiocenosis of hot springs is extremely important for the assessment and effective control of their condition while the study of the biological characteristics of the main representatives of microorganisms contributes to the knowledge of the thermophilic group (Əhmədova, 2007; Quliyeva, 2015).

Thermophilic microorganisms are one of the most practically significant groups of microorganisms since they have several properties such as thermal stability, resistance to ionizing radiation, fast cultivation time, wide distribution, etc. (Логинова и др., 1966; Ахмедова, 2007; Souza et al., 2001; Robb et al., 2008; Khalil, 2011). Therefore, the use of thermophilic microorganisms in various spheres of human activity is currently an urgent trend in microbiology (Burgess et al., 2010; Kawasaki et al., 2012; Aanniz et al., 2015; Baltaci et al., 2017).

Known strains of thermophilic bacteria are highly resistant to radiation and are widely used in practice due to this ability. An example is *Deinococcus radio*durans an extremophile that is one of the most radiation-resistant in the world, capable of surviving doses up to 10000 Gy. Due to this ability, it is used for bioremediation of radioactive waste (Mattimore et al., 1996; Lin et al., 1999; Brim et al., 2000; Makarova et al., 2001; Levin-Zaidman et al., 2003; Cox et al., 2005). Thermophilic anaerobic bacteria which have their powerful hydrolases are used in the production of ethanol which significantly speeds up the process since the stage of preliminary hydrolysis of raw materials is excluded and a high cultivation temperature increases the reaction rate (Захарук, 2018; Kublanov et al., 2009; Abdel-Banat et al., 2010; Tobler et al., 2011; Obeidat et al., 2012; Bakshaliyeva et al., 2019). Thermophiles are also used to accelerate chemical reactions in wastewater treatment at petrochemical enterprises in the disposal of animal corpses in thermal pits to ensure the biological safety of territories and the population of the Arctic zone (Ксенофонтов, 2010; Арьков, 2016; Золотухин, 2018; Tango et al., 2002). Some scientific works talk about the synthesis of vitamin B_{12} for the needs of animal husbandry using a mixed culture that includes thermophilic microorganisms (Evelyne et al., 1998; Martens et al., 2002). This makes it possible to talk about thermophiles as a component of drugs in pharmacology (Махмутова, 2017). Known scientific works talk about the extraction of copper in industrial reactors using thermophilic bacteria. Therefore, we can judge the use of thermophilic bacteria in the metal mining industry (Rodrigues et al., 2015).

Thermophilic bacteria are also used in nanobiotechnology in the green synthesis of metal nanoparticles. In practice, this method mainly produces silver nanoparticles, which are widely used in medicine and have antibacterial properties (Feng et al., 2000; Sharma et al., 2009; Sinha et al., 2009; Wei et al., 2015; Deljou et al., 2016; Gunashova et al., 2021).

The aforementioned unique properties of thermophilic microorganisms as well as many different spheres of human activity in which thermophilic microorganisms can be applied show how important it is for modern science to isolate and study thermophilic bacteria from their natural habitat-hot springs.

MATERIALS AND METHODS

For isolating the culture of thermophilic bacteria the object of the study was the hot springs "Ashagi Istisu" (pH=8.0, t=64°C) and "Yukhari Istisu" (pH=9.0, t=71°C) of the Kalbajar region. The material was collected from hot springs in April 2021, samples were obtained with the help of the military personnel of the Kalbajar region. The collected samples were stored at a temperature of +5°C, after which they were inoculated on a nutrient medium with meat-peptone agar. Sowing of natural material was carried out on an agar medium of the following composition: meat extract - 1g/l, peptone - 5 g/l, NaCl - 5 g/l, agar - 15 g/l. The cultivation was carried out at a temperature of 60°C for 4-5 days. To obtain a pure culture of thermophilic bacteria the Koch method was used as well as the mechanical method of repeated subcultivation on the medium of meat-peptone agar (Нетрусов, 2005). Study of the

morphology of isolated strains of bacteria by methods microscopy was performed using a light microscope (XSP-30 series microscope). The preparations were prepared using standard methods (Егорова, 1995; Ившина, 2014; Лавренчук и др., 2019).

The strains were also cultured on a meat-peptone agar medium supplemented with 0.2 g/l of yeast extract. Yeast extract was not required for growth, but the addition stimulated growth significantly.

To determine the ratio of strains to temperature cultivation was carried out in the temperature range 40-70°C using a set of thermostats set at different temperatures. The diameter of the grown colonies was used to judge the optimum temperature for the growth of strains. To determine the ratio of the isolated strains to pH the strains were inoculated on meat-peptone agar media with different pH values of the medium (6.0-10). The growth pH range was determined at the optimum growth temperature. The diameter of the grown colonies was used to judge the optimal pH value for a given strain (Теппер и др., 2004).

Some strains were identified based on morpho-cultural characteristics and some physiological properties (pH, t°C) (Логинова и др., 1996).

RESULTS AND DISCUSSION

As a result of the study, 3 strains of thermophilic bacteria were obtained from a water sample taken from "Ashagi Istisu" (KA_1, KA_2, KA_3) and 3 strains from a water sample from "Yukhari Istisu" (KY_1, KY_2, KY_3). The cell morphology of the isolated strains is represented by short and long gram-positive, spore-forming rods, varying in size. Differences in cultural characteristics are noted. Colonies are mainly cream and beige in color, small, large, and medium-sized, round, irregular, and rhizoidal in shape, also differing from each other in the consistency, at the edges, in surface structure, etc. So far, only 2 of the isolated strains (KA_1, KY_1) have been well studied, so we will show the morpho-cultural traits and physiological properties (pH, t°C) of these two strains. The morphological and cultural characteristics of the KA_1 and KY_1 strains are shown in Fig.1 and Fig. 2, respectively (Table1).

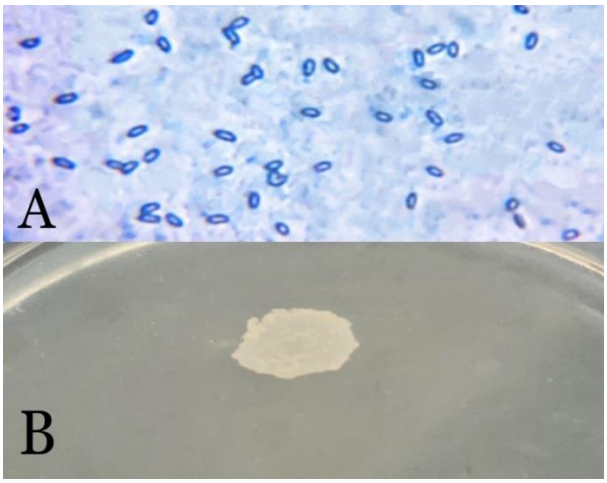

Fig. 1. A: Morphology of cells of the KA_1 strain under a microscope (magnification x1000); **B:** Morphology of the KA_1 strain colony (cultivation was carried out at 60°C, within 2 days)

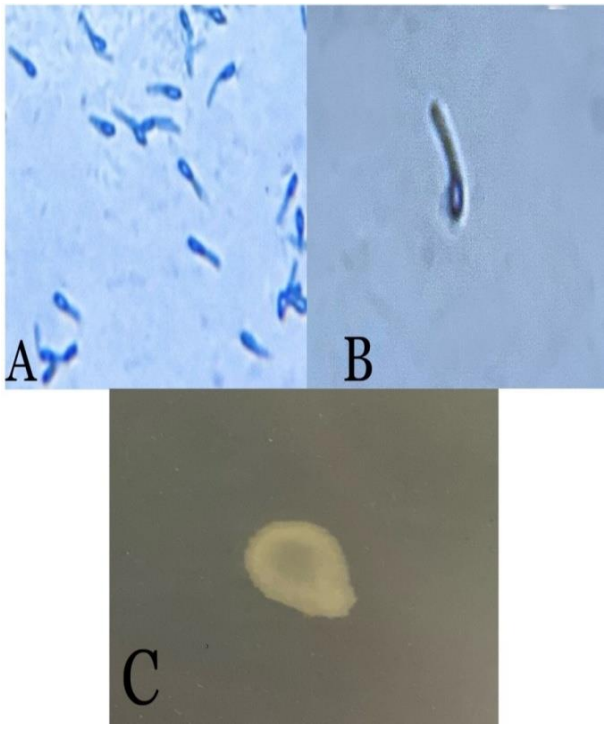

Fig.2. A: Morphology of cells of strain KY_1 under a microscope (magnification x1000); **B:** single cell of strain KY_1 (magnification x100); **C:** Colony morphology of strain KY_1

Table 1. Morpho-cultural characteristics of KA_1 and KY_1 strains.

Strains	Cultural traits					Morphology of cells	
	Shape	**Size, cm**	**Color**	**Margin**	**Elevation**	**Shape**	**Motility, size, micrometer (μm)**
KA_1	Round	1	Cream	Undulate (wavy)	Raised	Rods	Motile 0.5-0.6x0.9-1.5
KY_1	Irregular	1.5	Beige	Undulate (wavy)	Convex	Rods (with terminal spores)	Motile 0,3-1,2 x 1,5-4

Were studied some physiological properties (pH, t°C) of the KA_1 and KY_1 strains. It was found that strains KA_1 and KY_1 actively grow in the temperature range 55-60°C, at a pH value (7.0-9.0). The strains were identified based on morpho-cultural characteristics and some physiological properties. The KA_1 strain was assigned to the genus Bacillus, and the KY_1 strain was presumably assigned to the Bacillus stearothermophilus species.

CONCLUSION

Isolation and study of thermophilic bacteria from their natural habitat, hot springs, is one of the most urgent problems in modern science due to the widespread use of these bacteria in various fields of human activity, also in nanobiotechnology, in the green synthesis of metal nanoparticles. This paper investigates the isolation of thermophilic bacteria from the hot springs of the Kalbajar region, "Ashagi Istisu" and "Yukhari Istisu". Only 6 strains of thermophilic bacteria were isolated, 2 of which are well studied and the morpho-cultural characteristics of these strains have been described. The ratio of these strains to the pH of the medium and temperature was also determined, and the range of optimal values of pH and temperature for the growth of these strains was established. Based on the above studies the strains were identified and assigned to the genus Bacillus. Research in this direction continues.

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Azərbaycan Respublikasının Kəlbəcər rayonunun "Aşağı İstisu" və "Yuxarı İstisu" su mənbələrinin termofil bakteriyaları

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Təqdim olunan elmi işdə Kəlbəcər rayonunun ekoloji şəraiti ilə (pH, temperatur, kimyəvi komponentləri və s.) səciyyələnən "Aşağı İstisu" və "Yuxarı İstisu" termal su mənbələrinin bakteriyal biotası öyrənilmişdir. Termal su nümunələri ətli-peptonlu aqar qidalı mühitində becərilmiş, inkişaf edən koloniyalar təmiz kulturaya çıxarılaraq 6 termofil bakteriya ştamı alınmışdır (KA_1, KA_2, KA_3, KY_1, KY_2, KY_3). Bu ştamların hüceyrələrinin

morfoloji görünüşü bir birindən ölçüləri ilə fərqlənən qısa və uzun çöp şəkilli olub, spor əmələ gətirmək qabiliyyətinə malik olublar. Koloniyaları formasına, ölçüsünə, quruluşuna və s. kultural əlamətlərinə görə bir- birindən fərqlənirlər. KA_1, KY_1 saylı ştamlar morfo-kultural əlamətlərinə görə daha geniş öyrənilməklə yanaşı, onların mühitin temperaturuna və pH-na münasibəti də araşdırılmışdır. Müəyyən olunmuşdur ki, ştamların optimal inkişaf temperaturu 55-60ºC, pH=7.0-9.0 arasında tərəddüd edir. Aparılan tədqiqatlar nəticəsində KA_1, KY_1 ştamları identifikasiya edilmişdir. Belə ki, KA_1 ştamı Bacillus cinsinə aid edilmiş, KY_1 ştamının isə Bacillus stearothermophilus növünə aid olduğu ehtimal edilmişdir.

Açar sözlər: *Termal su mənbələri, termofil bakteriyalar, morfo-kultural əlamətlər*

Термофильные бактерии водных источников «Ашагы Истису» и «Йухары Истису» Кельбаджарского района Азербайджанской Республики

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В работе представлены данные по исследованию бактериальной биоты термальных водных источников «Ашагы Истису» (pH=8.0, t=64^{0}C, уровень минерализации-6.7 г/л) и «Йухары Истису» (pH=9.0, t=71^{0}C, уровень минерализации-4.3 г/л) Кельбаджарского района, характеризующихся широким диапазоном экологических условий (pH, температур, химических компонентов и др.). Был сделан посев образцов термальных вод и выделены колонии термофильных бактерий, выросших на твердой среде из мясо-пептонного агара. Некоторые колонии термофильных бактерий были выделены в чистую культуру, в результате чего получили 6 штаммов (KA_1, KA_2, KA_3, KY_1, KY_2, KY_3). Морфология клеток этих штаммов представлена короткими и длинными грамположительными, спорообразующими палочками, варьирующими в своих размерах. Колонии отличаются между собой по цвету, форме, краям, консистенции, размерам и т.д. Морфология клеток и колоний двух штаммов (KA_1, KY_1) хорошо изучены, а также определено отношение этих штаммов к pH и температуре среды. Установлено, что штаммы KA_1, KY_1 активно растут в интервале температур 55-60ºC, при значениях pH =7.0-9.0. На основе морфокультуральных признаков и некоторых физиологических свойств, штаммы были идентифицированы. Штамм KA_1 был отнесен к роду Bacillus, а штамм KY_1 предположительно отнесли к виду Bacillus stearothermophilus.

Ключевые слова: *Термальные источники, термофильные бактерии, морфо-культуральные признаки*