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ISOLATION AND CHARACTERIZATION OF THERMOPHILIC BACTERIA FROM ZHARKENT GEOTHERMAL HOT SPRING

Abbreviations: CMC – carboxymethylcellulose; g/l – gram per liter.

The aim this study was the isolation and characterization of thermophilic bacteria from geothermal hot spring in Zharkent town, Almaty region, Kazakhstan. Thermophilic bacteria are less studied but important group of microorganisms due to their ability to produce industrial enzymes. Eight bacterial isolates were characterized by morphological, microscopic, biochemical, and physiological characteristics. Eight bacterial isolates were isolated which capable growing at 81°C. The isolates were screened for amylase, protease, lipase and cellulose activity. The seven from eight isolates tentatively as Thermus sp. by morphology, biochemistry and physiological characteristics and one as Bacillus sp.

The study confirmed that the isolates from geothermal hot spring Zharkent to be a true thermophile and could be a source of thermostable enzymes which can be exploited for industrial applications; four isolates (AW4, AW5, AW7, AW8) from eight showed good enzymatic characterization according to the results of their cultivations on solid medium. Isolation procedures were first carried out under various combinations of culture conditions; temperature (50, 60, 70, 80 and 90°C), different media (for amylase, cellulase, lipase producer), various pH (5, 6, 7, 8 and 10). Results indicated a high phenotype diversity and hydrolase enzymes activity, which encourages future studies to explore further industrial and environmental applications.

Key words: thermophiles, Zharkent geothermal hot spring, amylase, lipase, isolates.
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Жаркент геотермальный горячий источник

Целью данного исследования было выделение и характеристика термофильных бактерий из Жаркентского геотермального горячего источника.

Сокращения и обозначения: КМЦ – карбоксиметилцеллюлоза; г/л – грамм на литр.

Выделение и характеристика термофильных бактерий из Жаркентского геотермального горячего источника

Целью данного исследования было выделение и характеристика термофильных бактерий из Жаркентского геотермального источника в г. Жаркенте Алматинской области, Казахстан.

Термофильные бактерии являются менее изученными, но важной группой микроорганизмов из-за их способности продуцировать ферменты, представляющие интерес для промышленности. Было выделено восемь бактериальных изолятов, способных к росту при 81 °C. Изоляты подвергли скринингу на активность расщепления амилазы, протеазы, липазы и целлюлозы в качестве источников углерода. На основании изучения морфологических, биохимических и
Isolation and Characterization of Thermophilic Bacteria from Zharkent geothermal hot spring

физиологических признаков семь из восьми изолятов предварительно были отнесены к роду Thermus sp., а один изолят к роду Bacillus sp.

Исследование подтвердило, что изоляты из горячего геотермального источника Жаркент являются истинными термофилами и могут быть источниками термостабильных ферментов, которые можно использовать для промышленного применения; четыре изолята (AW4, AW5, AW7, AW8) из восьми показали хорошие ферментативные характеристики по результатам их культивирования на (агаризованной) твердой среде. Процедуры выделения были сначала выполнены в различных комбинациях условий культивирования; температуры (50, 60, 70, 80 и 90ºC), различные среды (для амилазы, целлюлазы, продукта липазы), различные значения рН (5, 6, 7, 8 и 10). Результаты свидетельствуют о фенотипическом разнообразии и способности к синтезу гидролазных ферментов, что стимулирует будущие исследования.

Ключевые слова: термофилы, Жаркентский горячий геотермальный источник, амилаза, липаза, изоляты.

1. Introduction

Geothermal areas considered the source of the main habitats of thermophilic microorganisms [1]. Geothermal features are not common ecological features; they occur in clusters, in a few widely separated locations of the world where the conditions are right for their occurrence. Due to this specific nature of the geothermal sources, hot springs are available in a few areas only. The best recognized areas and the most studied are in Iceland, United States, New Zealand, Japan, Italy, Indonesia, Central America, and Central Africa [2–4]. The attractive feature of hot water resources is the ecology with its variety of the organisms [5] and the molecular strength of its components [6].

Only a small fraction of the microorganisms found in a natural habitat can be cultivated under laboratory conditions and subsequently isolated. The knowledge of environmental microbial diversity has been largely aided by the development of culture-independent molecular phylogenetic techniques [7–10].

Thermophilic microorganisms have gained worldwide importance due to their tremendous potential to produce thermostable enzymes (such as amylases, cellulases, chitinases, pectinases, xylanases, proteases, lipase, and DNA polymerases) that have wide applications in pharmaceuticals and industries [11]. Among these commercially important enzymes are the protease enzymes: alkaline protease possesses the property of a great stability when used in detergents and protease enzymes have found applications in bioindustries such as washing powders, food industry, leather processing, and pharmaceuticals and for studies in biology. Moreover, cellulase enzymes showed great commercial potential for the production of glucose feedstock from agricultural cellullosic materials [13] and in the production of bioethanol and value-added organic compounds from renewable agricultural residues [14]. Various enzymes have significance in applications in bioindustries; for example, protease and amylases are used together in many industries such as the food industry, detergent industries, and pharmaceuticals [12].

Thermophiles can be categorized into moderate thermophiles (growth optimum, 50–60ºC), extreme thermophiles (growth optimum, 60–80ºC), and hyperthermophiles (growth optimum, 80–110ºC) [13]. Thermophiles have been isolated from different ecological zones (e.g., hot springs and deep sea) of the earth. The organisms with the highest growth temperatures (103–110ºC) are members of the genera Pyrobaculum, Pyrodictium, Pyrococcus, and Melanopyrus belonging to Archaea; within Fungi, the Ascomycetes and Zygomycetes classes have high growth temperatures [14], while, in case of bacteria, Thermotoga maritime and Aquifex pyrophilus exhibit the highest growth temperatures of 90 and 95ºC, respectively [15]. Thermophilic microorganisms can be classified as Gram-positive or Gram-negative, they can exist under aerobic or anaerobic conditions, and some of them can form spores. Due to their increased importance, potential applications, and roles in different fields, scientists have concentrated their studies to discover new genus and species across the world [16–18].

The city of Almaty, situated in south region of Kazakhstan, is rich in geothermal hot springs, with temperature ranging between 30 and 98 ºC. Their detailed distribution and characteristics have been described in the literature [19]. One of such hot springs is the Zharkent. The microbial diversity if this hot spring has not yet been fully studied. Therefore, the aims of this study were to isolate thermophilic bacteria from sample of Zharkent geothermal hot spring, determine the thermostability of the isolates, screen for industrial enzymes and study the phylogenetic affiliation of the thermophilic
bacterium in comparisons with other bacterial isolates occurring as mesophiles, thermophiles and hyperthermophiles.

2. Materials and methods

2.1. Study site and collection of samples. The Zharkent geothermal hot spring is located at 43 °97’14.93 »N, 79 ° 66’ 12.09 »E, 273 km. from Almaty city, not reaching Zharkent town, at 80 km from China border. Several wells are located within the Zharkent town, depression in the Zharkunak tract. The Zharkunak underground water field is understood as the central part of the Zharkent geothermal spring, including wells No. 5539, 1-RT. All wells are located within the land use area of LLP «Baiserek-Agro» (Kazakhstan company) (Figure 1). Due to its remoteness it is less influenced by human interferences and believed to have rich microbial wealth. The temperature of the hot spring during the sampling period was 87°C. The pH was recorded to be in the range of 7 – 8 indicating alkaline environment. Sampling was performed in January 19, 2019. At 8.30 a.m. Water sample was collected from a part of conduit in the outlet of the spring in 50 ml sterile tubes. The total number of taken samples 100 ml. Tubes were brought to the laboratory and kept at 4°C in refrigerator till further processing.

The chemical composition of well No. 1-PT is characterized as sulfate-sodium chloride, slightly mineralized, alkaline, very slightly radonic, siliceous hydroterm with a content of natural iodine ion and high fluorine content [19].

(A) Map of Kazakhstan showing the location of Zharkent. (B) Close-up photograph of the source of the Zharkent hot spring with sampling site indicated by an arrow. (C) Sampling of the water.

Figure 1 – Location of study site
2.2. Isolation of bacteria. Geothermal hot spring sample 100 ml is get into the tubes samples and then labeled. Water sample was used for enrichment in nutrient broth (HiMedia, Mumbai) at 81°C during 6 days enrichment culture was streaked on nutrient agar (HiMedia, Mumbai) to obtain separate colonies. All bacterial isolates obtained on plates were selected and purified by streaking onto the same medium at least three times. The isolates were considered pure after microscopic observation of a single morphological per culture. Plastic freezing bags were used to avoid drying of the samples during incubation. The isolates’ purity cell morphology, sporulation ability, and motility were determined by microscopy (Micros, Austria) of freshly prepared wet mounts.

2.3. Determination for thermos-tolerance. Pure cultures of the bacterial isolates were determined for their thermophilic characteristics. Each bacterial isolates were inoculated into 10 ml of nutrient broth medium (HiMedia, Mumbai) in test tube at 81°C during 6 days. After specified incubation period each broth culture of bacteria were streaked onto freshly prepared nutrient agar (HiMedia, Mumbai) medium, they were cultivated at 65°C 24 h. Bacterial isolates growing in the plates were selected and again tested for their thermos-tolerance at higher temperature in test tube at 81°C during 6 days. Finally isolates that could tolerate at temperature of 81°C was selected for further study.

2.4. Metabolic and Biochemical Characterization of the Isolates. Thermophilic bacteria isolates were studied for various morphological characteristics viz., color, gram reaction, shape, spore formation and motility. Various biochemical tests were carried out for the biochemical characterization of selected bacteria isolates such like fermentation of sugars (glucose, sucrose, lactose) (Case and Jonson), H₂S production, oxidation of Mn and Fe, obligate aerobes, facultative anaerobes [20]; the presence of catalase and oxidase enzymes was investigated according to the methods described by Prescott et al [21].

2.5. Optimization of growth conditions. To determine the effect of temperature on the growth of the isolates, microorganisms were grown at varied incubation temperature in the range of 50-90°C with regular 5°C increment for 24 hours. Besides, the effect of pH on the growth of isolated microorganism were studied by growing the organism for 24 hours at 82°C in nutrient broth medium adjusted to different pH ranging from 4.0 – 9.0 separately.

2.6. Assessment of Enzymatic Production

2.6.1. Screening and Identification of Cellulase Producers. Ten microliters of overnight grown culture was spot plated on CMC agar (NaNO₃ – 2.0 g, K₂HPO₄ – 1.0 g, MgSO₄ – 0.3 g, KCl – 0.3 g, carboxymethylcellose (CMC) – 0.2 g, NaCl -0,1 g, peptone 1.0 g (HIMEDIA, India), bacto agar – 20,0 g. (g/l)). Plates incubated at 65°C for 24 hours. From cellulase-producing microorganisms, four potential bacterial strains AW2 (Gram -ve), AW4 (Gram -ve), AW5 (Gram -ve), AW6 (Gram -ve), AW7 (Gram -ve), AW8 (Gram -ve), were selected for identification and further studies. Screening of cellulose producers were done on CMC agar. After incubation the plates were flooded with Gram’s iodine (2.0 g KI and 1.0 g iodine in 300 ml distilled water HiMedia, Mumbai) for 3 to 5 minutes by Ramesh et.al. [22]. The formation of clear zone of hydrolysis indicated cellulose degradation as adopted from Shaikh et al. [23].

2.6.2. Screening and Identification of Amylase Producers. Screening of amylase producers were done on using starch hydrolysis method. The starch agar plates (Tripton – 10.0 g, yeast extract – 5.0 g, NaCl – 5.0 g, starch solution (potato starch ) – 1.0 g, bacto agar – 20.0 g (France), (g/l)) were streaked by microbial isolates followed by their incubation at 65 °C for 24 hours. After incubation, 1 % iodine solution (HIMEDIA, India) (freshly prepared) was flooded on the starch agar plate. The presence of blue color around the growth indicated negative results [24].

2.6.3. Screening and Identification of Lipase Producers. Lipase activity was observed by the appearance of a turbid halo around the inocula on solid medium containing next elements per liter: yeast extract – 5.0 g, peptone – 10.0 g (HIMEDIA, India), NaCl – 5.0 g, CaCl₂·H₂O – 0.1 g, MgSO₄·H₂O – 0.3 g, KH₂PO₄ – 0.3 g. K₂HPO₄ – 0.3 g, agar – 20,0 g, g/l. The medium supplemented with 1% Tween 80 (AppliChem, GmbH) at 65 °C for 24 hours as explained by Rollof et al. [25].

2.6.4. Screening and Identification of Protease Producers. Protease activity was detected on solid medium containing next elements per liter: glucose – 1.0 g, peptone – 10.0 g (HIMEDIA, India), yeast extracts – 0.2 g, casein – 10 g (HIMEDIA, India), CaCl₂ – 0.1 g, K₂HPO₄ – 0.5 g, MgSO₄·0,1 g, agar 20,0 g. Plates were streaked with test isolates followed by incubation at 65 °C for 24 h. The presence of a transparent zone around the colonies indicated caseinase activity as explained by Daniel [26].
3. Results and Discussion

3.1. Characterization of the Sample. Out of all sample collected from Zharkent geothermal hot spring eight different bacterial isolates were isolated. The isolates were given codes viz., AW1, AW2, AW3, AW4, AW5, AW6, AW7, AW8. Altogether eight bacterial isolates AW1 (Gram – ve), AW2 (Gram – ve), AW3 (Gram + ve), AW4 (Gram – ve), AW5 (Gram – ve), AW6 (Gram – ve), AW7 (Gram – ve), AW8 (Gram – ve) were obtained from water sample collected from geothermal hot spring of Zharkent, Almaty region, Kazakhstan.

3.2. Morphological and Biochemical Examination of the Isolates. Various identification test like endospore formation, motility, catalase, and oxidase, fermentation of sugars (glucose, sucrose, lactose), obligate aerobe, facultative anaerobe were performed (Table 1). Based on Gram staining, the isolates were found mostly to be Gram-negative and microscopic observation revealed seven non spore forming and one spore forming bacteria arranged in chain. Growth occurred on solid and liquid media.

Table 1 – Biochemical characterization of the bacterial isolates

<table>
<thead>
<tr>
<th>Biochemical tests</th>
<th>AW1</th>
<th>AW2</th>
<th>AW3</th>
<th>AW4</th>
<th>AW5</th>
<th>AW6</th>
<th>AW7</th>
<th>AW8</th>
</tr>
</thead>
<tbody>
<tr>
<td>H2S production</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
</tr>
<tr>
<td>Catalase</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>Oxidase</td>
<td>+ve</td>
<td>+ve</td>
<td>-ve</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Starch hydrolysis</td>
<td>+ve</td>
<td>+ve</td>
<td>-ve</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>Fermentation of sugars</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
</tr>
<tr>
<td>Sucrose</td>
<td>-ve</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
<td>-ve</td>
<td>+ve</td>
<td>-ve</td>
<td>-ve</td>
</tr>
<tr>
<td>Lactose</td>
<td>-ve</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
<td>-ve</td>
<td>+ve</td>
<td>-ve</td>
<td>-ve</td>
</tr>
<tr>
<td>Oxidation of Mn</td>
<td>-ve</td>
<td>+ve</td>
<td>-ve</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
<td>-ve</td>
<td>-ve</td>
</tr>
<tr>
<td>Oxidation of Fe</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
</tr>
<tr>
<td>Obligate aerobe</td>
<td>+ve</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Facultative anaerobe</td>
<td>-ve</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
<td>ND</td>
<td>+ve</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

+ indicates positive and – indicates negative test; ND – not determined

The isolates were negative H2S production test, strict aerobes, oxidation of Fe. Catalase, starch hydrolysis, oxidation of Mn, fermentation of sugars, oxidase, facultative anaerobe were found positive.

Morphologically, the isolates showed some variation in the color, margin, shape, and texture of the colonies (Table 2). The selected bacterial isolates were observed and growth characteristics were studied (Figure 2).

They color were; four creamy, one grey, one white and two white-creamy. The light transmission were; four opaque and four translucent. The consistency were; one rough, one smooth four smooth or mucous and two mucous. The shape were; two regular, two irregular and four circular. The elevation were; one convex, four flat and three raised. The margin were; one filiform, three entire and four undulate. Colonies might appear finely wrinkled and adherent to the agar surface.

3.3. The effect of different temperature and pH on the growth. The bacterial isolates were screened for their thermos-tolerance property in different temperatures starting from 50 ºC to 90 ºC. With isolates in solid medium the optimum temperature was 65°C, and maximum and the minimum temperature were 70° and 60°C, respectively. With isolates in liquid medium the optimum temperature was 81°C, and maximum and the minimum temperature were 70° and 85°C, respectively.

The growth of isolates was tested at different pH. The pH of media to 9.5 and 10.0 was adjusted with Na2HPO4 and NaOH. After the addition of 0.2 ml of seed culture to each 10 ml of a test media, the culture was incubated without shaking for 96 h. at 81 ºC with two tubes run at each pH. The optimum pH range for the growth was observed to be pH 7.0 to 7.5. No growth was observed below pH 6 and pH 9.
Isolation and Characterization of Thermophilic Bacteria from Zharkent geothermal hot spring

Figure 2 – Variation in colonial morphology of bacterial isolates from the hot spring using nutrient agar

Table 2 – Colony morphology of the eight isolates isolated from Zharkent geothermal hot spring

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Light transmission</th>
<th>Consistency</th>
<th>Shape</th>
<th>Margin</th>
<th>Elevation</th>
<th>Color</th>
</tr>
</thead>
<tbody>
<tr>
<td>AW1</td>
<td>Translucent</td>
<td>Smooth</td>
<td>Regular</td>
<td>Undulate</td>
<td>Flat</td>
<td>White-creamy</td>
</tr>
<tr>
<td>AW2</td>
<td>Opaque</td>
<td>Mucous, Smooth</td>
<td>Circular</td>
<td>Entire</td>
<td>Raised</td>
<td>Creamy</td>
</tr>
<tr>
<td>AW3</td>
<td>Opaque</td>
<td>Mucous, Smooth</td>
<td>Circular</td>
<td>Entire</td>
<td>Raised</td>
<td>Creamy</td>
</tr>
<tr>
<td>AW4</td>
<td>Opaque</td>
<td>Mucous</td>
<td>Circular</td>
<td>Undulate</td>
<td>Raised</td>
<td>Creamy</td>
</tr>
<tr>
<td>AW5</td>
<td>Translucent</td>
<td>Mucous, Smooth</td>
<td>Regular</td>
<td>Undulate</td>
<td>Flat</td>
<td>White-creamy</td>
</tr>
<tr>
<td>AW6</td>
<td>Opaque</td>
<td>Mucous</td>
<td>Circular</td>
<td>Entire</td>
<td>Flat</td>
<td>Creamy</td>
</tr>
<tr>
<td>AW7</td>
<td>Translucent</td>
<td>Rough</td>
<td>Irregular</td>
<td>Filiform</td>
<td>Flat</td>
<td>White</td>
</tr>
<tr>
<td>AW8</td>
<td>Translucent</td>
<td>Mucous, Smooth</td>
<td>Irregular</td>
<td>Undulate</td>
<td>Convex</td>
<td>Grey</td>
</tr>
</tbody>
</table>
3.4. Production of Extracellular Enzymes. Bacterial isolates collected from hot spring were screened for amylase, protease, lipase and cellulose activities (Figure 3). Among the eight identified isolates, at least two extracellular hydrolyzed enzymes was produced by each isolates (Table 3).

**Figure 3** – Enzyme activity of isolates isolated from Zharkent geothermal hot spring. (a) amylase activity, (b) cellulase activity, (c) protease activity, (d) lipase activity
Table 3 – Variation in enzymatic activity produced by isolates isolated from Zharkent geothermal hot spring

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Amylase</th>
<th>Cellulase</th>
<th>Protease</th>
<th>Lipase</th>
</tr>
</thead>
<tbody>
<tr>
<td>AW1</td>
<td>+ve</td>
<td>+ve</td>
<td>-ve</td>
<td>-ve</td>
</tr>
<tr>
<td>AW2</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
<td>-ve</td>
</tr>
<tr>
<td>AW3</td>
<td>+ve</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
</tr>
<tr>
<td>AW4</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>AW5</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>AW6</td>
<td>-ve</td>
<td>+ve</td>
<td>+ve</td>
<td>-ve</td>
</tr>
<tr>
<td>AW7</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>AW8</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
</tbody>
</table>

+ indicates positive and – indicates negative test

Seven isolates (90%) produced amylase, six isolates (80%) produced protease, seven isolates (90%) produced cellulose, and four isolates (50%) produced lipase. In addition, four isolates (50%) combined four of the tested enzymes, one isolate (15%) produced three extracellular enzymes, one isolate (15%) produced two extracellular enzymes, and only one isolate (15%) produced one enzyme screened.

Thermal springs represent extreme niches whose pristine quality is maintained over a period of time. The terrestrial hot springs that exist on earth [27] represent hot spots for unusual forms of life, genes, and metabolites. Ever since Thomas Brock discovered the presence of Thermus aquaticus in the thermal vents of Yellowstone National Park, a number of researchers have investigated similar environments all over the world. The earth we are existing on is filled with variety of microorganisms that researchers are still far away from being able to complete their identification and isolation, this lead to intensive and extended researches to be carried out in order to fully investigate such promising microorganisms. Worldwide, geothermal areas which are favorable habitats for thermophilic organisms are limited to a restrict number of sites. In Zharkent, there are several hot springs renowned for their rejuvenating and medicinal qualities. The temperatures are often higher than 40°C. In these conditions living organisms have to cope with extremes temperature, low humidity, and low availability of nutritional compounds. These conditions reduce biodiversity but some bacteria developed survival strategies in order to adapt to such stress.

In this study, a total of eight isolates were isolated from hot spring were encoded in the form of AW1–AW8 and then subjected to various morphological, biochemical tests. Seven isolates were Gram – negative and non-endospore forming bacilli and filamentous (AW1, AW2, AW4, AW5, AW6, AW7, AW8) and one isolate was Gram – positive and endospore forming bacilli (AW3). One isolate should be classified into the genus Bacillus according to their characteristics (Gram-positive roads, endospore forming, aerobes, catalase positive, optimum growth temperature of 60-65°C and growth pH range). Therefore, they could be classified as thermophilic bacteria according to Brock [28], Perry and Staley [29], and Souza and Martins [30].

On the other hand isolates AW1, AW2, AW4, AW5, AW6, AW7, AW8 were tentatively identified as belonging to the genus Thermus, as shown by their biochemical and physiological tests, and in a report by Hudson et al. [31]. They grow in aerobic conditions at a pH range of 7-8 and temperature range of 40-78°C (optimum growth temperature was 65°C). They are oxidase and catalase positive. The strains of the genus Thermus form yellow, orange or reddish colonies (description in Bargey’s Manual, vol. 1 [32]), but the colonies of the 7 isolated isolates showed no pigmentation. Ramely and Hixson [33] reported a bacterium that resembles Thermus aquaticus morphologically but appears to lack the carotenoid pigment. On the basis of the features observed here and the literature, isolated isolates AW1, AW2, AW4, AW5, AW6, AW7, AW8 were tentatively classified as Thermus.

4. Conclusions

One isolate classified into the genus Bacillus and seven of isolates tentatively identified Thermus according to their characteristics. The thermophilic bacteria were isolated and from Zharkent geothermal
hot spring and their preliminary enzymatic potential was characterized. Four isolates (AW4, AW5, AW7, AW8) from eight showed good enzymatic characterization according to the results of their cultivations on solid medium. Isolation procedures were first carried out under various combinations of culture conditions; temperature (50, 60, 70, 80 and 90°C), different media (for amylase, cellulase, lipase producer), various pH (5, 6, 7, 8 and 10). Only bacteria reappeared steadily upon incubation at 60-80°C.

The identification procedures of the bacterial isolates were carried out on the basis of their morphology and biochemical tests. Bacterial isolates then identified, mainly based on descriptions in the references. However, the accepted description of Thermus seemed incomplete, thus preventing us from completely identifying such species. This is the first report on isolation of bacteria from Zharkent geothermal hot spring, Almaty region, Kazakhstan. These promising results can be exploited further for production of biotechnological important and industrial thermostable enzymes. This study widens the opportunities for further research to be conducted to explore more the immense significance of these isolates, where there is lack of intensive studies regarding this organisms.

Conflicts of Interest
The authors declare that they have no conflicts of interest.

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