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Full Length Research Paper

Thermo-aerobic bacteria from geothermal springs in Saudi Arabia

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Biodiversity in geothermal springs in Saudi Arabia appears scanty and has not been thoroughly investigated. Geothermal springs are scattered in several areas in Saudi Arabia. Water samples were collected from ten hot springs and analyzed for different physical and chemical parameters. Fifteen isolates of thermo-aerobic bacteria were found. *Bacillus cereus, B. licheniformis, B. thermoamylovorans, Pseudomonas* sp., *Pseudomonas aeruginosa* and *Enterobacter* sp. were dominant in hot springs. Genetic relatedness indicated that eleven *Bacillus* spp. grouped together formed several clusters within one main group with high similarity. Plastic composite support tubes (PCS) were employed to stimulate the bacterial growth at high temperatures. The optimum temperature of isolates was 60°C. Thus, different blend of PCS were used to enhance the growth at higher temperatures. PCS blended with 20 and 15% (w/w) of dates pits and 5% (w/w) of yeast extract stimulated the growth of *B. thermoamylovorans* at 90°C.

Key words: Thermophilic bacteria, geothermal springs, genetic relatedness, extreme environments, 16S rDNA sequencing.

INTRODUCTION

Until recently, most extreme environments were considered too hostile to support any life form, but in the last few decades, it has become evident that they actually provide a natural habitat for certain microorganisms (Islas et al., 2007). Extreme environments are scattered in most parts of the world. In Saudi Arabia, there are ten geothermal springs. Six of these springs are located in Gizan. They include Ain Khulab, Ain Khulab Quwa, Ain Mijara Quwa, Ain ad Damad, Ain al Wagrah and Ain al Wagrah Dam. The remaining four are located in Al-Lith area and includes Ain al Harram, Ain Jumah, Ain Markub, and Ain ad Darakah (Rehman and Shash, 2005). Aquatic prokaryotic communities harbor a significant fraction of global genetic diversity in the biosphere. Microbial diversity and abundance are influenced both by environmental factors, such as the nature of the organic substrates, nutrients and water chemistry, and by biological factors (Liu et al., 2009). Geothermal systems are populated by diverse thermophilic bacteria and archaea (Kaur et al., 2008). Many thermophilic and more than 20 different genera of hyperthermophilic archaea have been isolated from geothermal and hydrothermal environments (Arab et al., 2000). Thermophilic bacilli grow best at temperatures between 45 and 70°C. The first research about the characterization of thermophilic bacteria, which were forming aerobic spores and able to grow at 70°C was done by Miquel (1888). Since then several reports on thermophilic *Bacillus* strains have been published. Particularly, many strains of the thermophilic bacteria have been characterized by the spore forming bacteria belonging to *Bacillus* and *Clostridium* genera (Maugeri et al., 2001; Belduz et al., 2003). Regardless of varying environmental conditions, the ability of thermophiles to thrive in extremely hot environments lies in extremozymes, enzymes geared to work in extremely high temperatures. It is possible to isolate them from different environments, such as permanently cold habitats, deep ocean-basin cores, shallow marine hot springs, petroleum reservoirs, deep-sea hydrothermal

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vents and the leachate of a waste pile from a canning factory (Bae et al., 2005).

For several decades, thermophilic bacteria have attracted the interest of many scientists due to their biotechnological potential (Adiguzel et al., 2009). In particular, phenotypic and genotypic characterization of thermophilic bacteria has been done for many geothermal areas in different parts of the world, including Turkey (Gul-Guven et al., 2008; Adiguzel et al., 2009), Italy (Maugeri et al., 2001), Bulgaria (Derekova et al., 2008), Greece (Sievert et al., 2000), China (Lau et al., 2009), India (Sharma et al., 2008) and Iceland (Takacs et al., 2001).

Advances in molecular biology techniques, such as rep-PCR profilings, and 16S rRNA sequencing have provided excellent opportunity for identification and characterization purposes of microorganism at species and subspecies levels (Adiguzel, 2006; Zaliha et al., 2007). These methods have been used also for studying the diversity in ecosystem, presenting the phylogenetic relation between strains, and discriminating the microorganism, which are genetically close to each other (Adiguzel, 2006).

The nutritional requirements of many thermophilic and hyperthermophilic bacteria have been investigated and frequently found to be complex (Beffa et al., 1996; Tomova et al., 2010). The major problem with enumeration of hyperthermophiles has been their inability to obtain sufficient cell mass. The microbial communities in these habitats have attracted the attention of microbial ecologists because of the unique adaptations to these harsh environments and valuable source for the exploitation of novel biotechnological processes. Culture dependent methods with some modification have been employed for studying the diversity of microbes in geothermal springs in Saudi Arabian studies (Zakaria, 2008). Recently, three thermophilic bacteria were isolated from Al-khoba and Al-Arida hot springs in Saudi Arabia (Khalil, 2011)

In this study, Plastic composite support tubes (PCS), which can be customized to support specific microbial requirements, support long term fermentation and stimulate microbial attachment (Pometto et al., 1997) were employed to isolate thermophilic and hyperthermophilic bacteria from water samples collected from geothermal springs in Saudi Arabia. These isolates were identified and characterized using molecular genotypic methods.

MATERIALS AND METHODS

Study sites

Gizan area of Saudi Arabia is a part of south Tihama with a steppe type of coastal plain, which stretches some 300 km along the Red sea coast and is 30 to 40 km wide. It extends between 16° and 18°N latitude and between 41° and 45° E longitude. Al-Lith site is a coastal town in the western area of Saudi Arabia. It extends between 20° and 39°N latitude and between 40° and 42°E longitude.

Sample collection

A total of ten water samples were collected from geothermal springs in Saudi Arabia. Six samples were collected from Gizan, these were 1) Ain Khulab, 2) Ain Khulab Quwa, 3) Ain Mijara Quwa, 4) Ain ad Damad, 5) Ain al Wagrah and 6) Ain al Wagrah Dam. Four water samples were collected from Al-Lith geothermal springs, which included 7) Ain al Harra, 8) Ain Jumah, 9) Ain Markub, and 10) Ain ad Darakah. The samples were collected between July and August 2008. These samples were collected in 500 ml glass screw cap bottles and brought to the laboratory in an ice box. The physical and chemicals properties of the geothermal springs were studied.

pH and conductivity of water samples were measured using Conductivity Benchtop Meters (OAKTON). Chemical analyses of geothermal spring waters, including elements, alkalinity, ammonia, nitrate, phosphate, and sulfate, were performed in the laboratory using Standard Methods (APHA, 1995).

Microbial enrichment

Plastic composite support tubes (PCS)

The PCS was developed using a blend of polypropylene (PP) and agricultural products as described by Ho et al. (1997). Different blend of PCS were used to enhance thermophilic and hyperthermophilic growth and to stimulate the bacterial biofilm (Table 2).

Initial PCS repeated-batch fermentations

Customized 500-ml reactors using wide-mouthed glass bottles fitted with silicone stoppers were designed for small-scale repeated-batch fermentations. 50 gm of plastic composite support (PCS) blends as described by Ping-Shing (2003) with some modification: 50% (wt/wt) poly-poropylene, 5% (wt/wt) yeast extract, 5% (wt/wt) bovine albumin, 5% (wt/wt) mineral salts, and 40% (wt/wt) cellulose, were placed in each reactor to stimulate bacteria growth and to provide slow release of nutrients. The reactor with PCS was autoclaved with 10 ml of deionized water at 121°C, 15 psi for 30 min. Then the deionized water was pumped out. Each reactor was supplied with 250 ml of sterilized LB media containing 10 g/L of tryptone peptone, 5 g/L of yeast extract, 5 g/L of sodium chloride, and 0.5 g/L of maltose, 3.0 g/L of $(NH_4)_2SO_4$, 5.03 g/L of Na_2HPO_4, 1.98 g/L of KH_2PO_4, 0.20 g/L of $MgSO_4 \cdot 7H_2O$, 0.20 g/L of NaCl, 0.05 g/L of $CaCl_2 \cdot 2H_2O$, and 1 ml/L of trace element solution (Pridham and Gottlieb, 1948). The pH was adjusted to 7.0 with 1 N NaOH prior to sterilize. The reactor was inoculated with 50 ml of non-sterilized geothermal spring water and incubated at 60°C for 7 days. Ten sets of experiments were performed each with one of geothermal spring water.

Microbial isolation

Bacterial cultures were recovered from PCS by stripping sand using a method developed by Ho et al. (1997). Five pieces of PCS were shaken vigorously for five seconds in 0.1% peptone, and then transferred to a culture tube containing 9 ml of 0.1% (wt/vol) peptone and 5 gm sand. The tube was vortexed in intervals of 30 s for 1 min (Ping-Shing, 2003) and the mixture was centrifuged at 2000 × *g* for 5 min at 4°C. The precipitate was then resuspended in 0.1% (wt/vol) peptone and subjected to Gram staining (Figure 1).

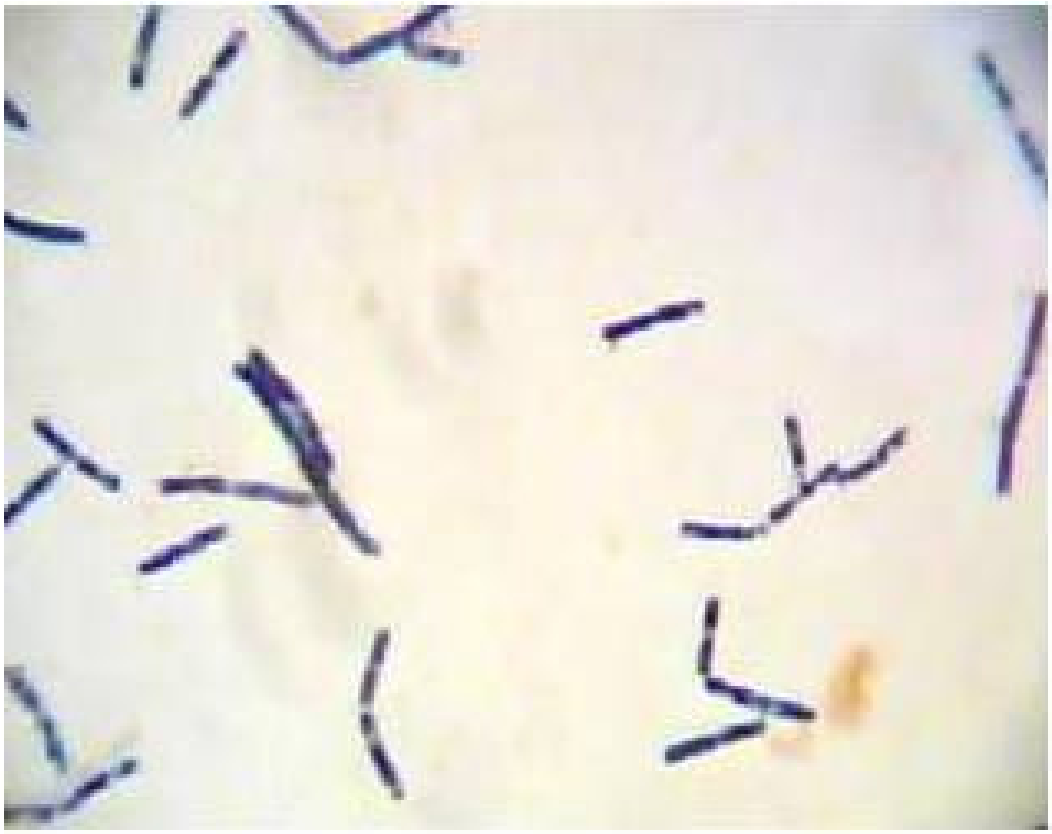

Figure 1. *B. thermoamylovorans* was collected from PCS culture grew at 60°C after centrifugation the mixture and staining by Gram stain.

The Total Bacteria Count (CFU) was recorded using plate count technique with GELRITE- basal medium. Each plate received 0.5 ml of resuspended mixture (Lin and Casida, 1984). The plates were incubated at 30, 40, 50 and 60°C and the CFU were recorded (Figure 2).

The ability of growth at high temperatures of the two dominant isolates grown on plates at 60°C was evaluated using the previous customized 500-ml reactors with 50 g of PCS and liquid media described by Sako et al. (2001). Each reactor was supplied with different kinds of PCS1, PCS 2, PCS 3, PCS 4, PCS 5, and PCS 6 (Table 2). The reactor was continuously aerated with filter-sterilized CO_2-free humidified air. The reactors were incubated on sand bath to maintain temperatures at 60, 70, 80, and 90°C. The CO_2 in the exit gas was trapped by 10 ml of 2 N NaOH. The trapped CO_2 was measured daily for 7 days (Figure 3a to d) by pH titration using a Mettler T70 automatic titrator (Metler-Toledo, Hightown, NJ) (Pometto III et al., 1998).

Isolates identification

DNA extraction and touchdown PCR

Bacterial DNA was isolated from bacterial samples by silica-based selective adsorption as per manufacturer's instructions (QIAamp DNA Mini kit, Valencia, CA), followed by 1% agarose gel electrophoresis to evaluate the extraction. Extracted DNAs were quantified by using spectrophotometer (GeneQuant, Ge Healthcare). The yield was between 100-200 ng/µl with purity ratio of 1.8. Touchdown PCR was performed to minimize non-specific amplification. In touchdown PCR, the DNA polymerase was added to the reaction mixture at 80°C (after the denaturing step). The reaction was then ramped down to 65°C for primer annealing. Each subsequent annealing step decreased temperature by 1°C until 55°C was reached (10 cycles), then the remaining cycles were performed at this annealing temperature. Touchdown PCR reactions included 10 x ExTaq buffer with $MgCl_2$ 2 mM /reaction, dNTP mix 2.5 mM each /reaction, ExTaq (Fisher Scientific, Pittsburgh, PA) DNA polymerase 1.25 U/reaction and 100 ng/µl of DNA, and total volume was brought up to 50 µl by ddH_2O. The PCR reactions were used to amplify regions within 16 S rDNA by using universal oligonucleotide primers targeting highly conserved regions (Not in Table 2). The reaction tubes were placed in GenAmp 9700 thermal cycler (Applied Biosystems) at 94°C initial denaturation for 5 min, at 80°C ExTaq with proof reading activity added, at 94°C for 30 s and at 65°C for 1 min. The annealing temperature declined 1°C per cycle for 10 cycles until it reached 55°C, 3 min, 72°C for 10 min for 35 cycles. All PCR products were gelpurified by QIAquick Gel extraction kit (Qiagen, Valencia, CA).

Cloning and sequencing 16S rDNA

To identify bacteria present in the samples, the regions of the 16S rDNA gene were amplified by PCR. Amplicons were gel-purified using GFX PCR DNA and gel band purification kit (GE Healthcare) and sense and anti-sense strands of 16S rDNA were sequenced in an ABI 3130 Genetic Analyzer (Applied Biosystems, Foster City,

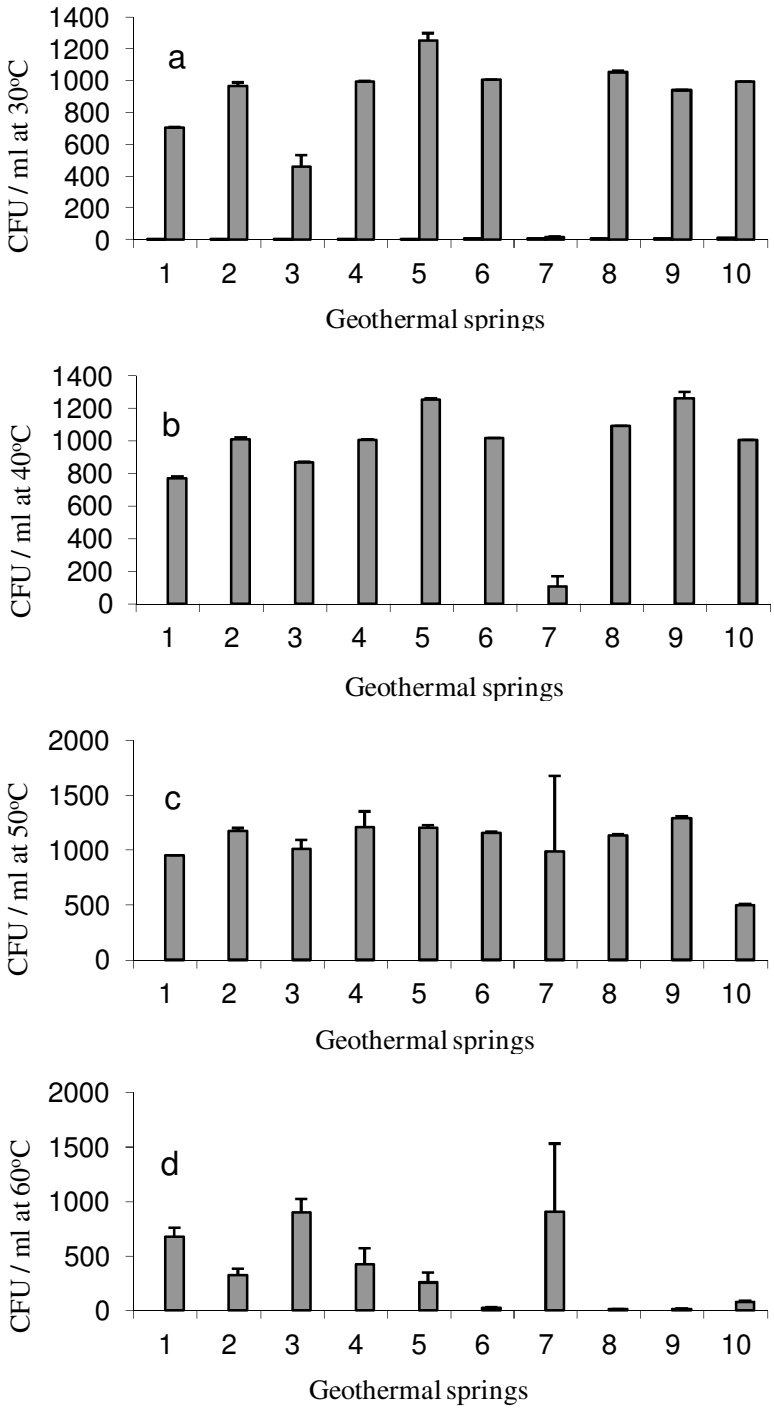

Figure 2. The total bacterial count in the geothermal spring samples incubated at 30, 40, 50 and 60°C after 7 days.

CA.) using ABI BigDye terminator cycle sequencing ready reaction kit chemistry according to manufacturer's recommendations. Following sequencing, each sequencing data was identified using basic local alignment search tool (BLAST) (www.ncbi.nlm.nih.gov/BLAST) and ribosomal databases of Michigan State University (http://rdp.cme.msu.edu/index.jsp). Sequencing data were aligned and the evolutionary relationship of the sequencing information was studied by phylogenetic analyses (Figure 4).

RESULTS AND DISCUSSION

All investigated water samples were collected and analyzed in triplicates. The results of physico-chemical characteristics of the samples are shown in Table 1. All sample showed that they have similar physical and chemical characteristics. The lowest temperature in

Table 1. Physical and chemical properties of the geothermal springs in Gazan and Al-Lith areas. The concentrations are represented in mgl^{-1} except temperature, pH and conductivity.

Variable	Geothermal spring									
	Ain Khulab	Ain Khulab Quwa	Ain Mijara Quwa	Ain ad Damad	Ain al Wagrah	Ain al Wagrah Dam	Ain al Harra	Ain Jumah	Ain Markub	Ain ad Darakah
Temperature (^oC)	75.3	60.5	59.0	58.7	60.4	60.0	83.0	50.0	47.0	40.0
pH	8.1	7.1	7.7	7.3	8.0	7.3	8.0	7.3	6.8	7.4
Conductivity ($\mu S\ cm^{-1}$)	3300	1831	2800	2022	1940	1210	3532	2494	2605	2881
Sodium	300 ±0.9	227±2.3	361±1.6	184±0.1	279±6.9	280±3.6	350±1.3	283±1.7	301±2.7	294±5.1
Calcium	150 ±3.1	77.9±6.6	150±0.8	160±2.6	95±4.1	120±1.1	153±4.4	89±2.1	94.3±2.7	99.4±1.5
Potassium	350 ±5.3	115±3.7	288±1.7	245±2.2	204±0.5	179±3.4	205±2.4	199±4.8	197±3.6	273±3.3
Magnesium	8.3 ±1.1	9.2±2.4	6.4±0.8	3.1±0.4	4.1±0.6	10.0±2.1	12.3±3.1	8.3±2.5	7.2±1.5	5.2±0.3
Manganese	1.0 ±0.01	0.6±0.1	0.8±0.01	0.3±0.02	0.6±0.01	0.8±0.01	0.5±0.1	1.0±0.02	0.4±0.01	0.9±0.01
Iron	0.1 ±0.02	0.1±0.01	0.08±0.01	0.05±0.001	0.1±0.001	0.05±0.02	0.1±0.01	0.04±0.01	0.1±0.001	0.1±0.001
Zinc	0.01± 0.001	0.001±0.0001	0.01± 0.001	0.03±0.01	0.01±0.001	0.01± 0.001	0.01±0.001	0.01±0.001	0.01±0.001	0.01±0.001
Fluoride	2.1 ±0.3	1.3±0.01	2.7±0.5	1.7±0.04	0.9±0.03	0.4±0.01	3.1±0.02	1.7±0.06	0.8±0.03	1.7±0.2
Chloride	400± 9.3	119±6.3	350±9.7	94.6±4.6	166±10.3	470±10.3	503±11.4	331±5.3	306±5.3	332±7.8
NO_3^-	7.5 ±0.3	3.8±0.01	6.7±0.3	4.1±0.02	6.2±0.04	7.4±0.3	8.3±1.0	5.9±0.8	2.7±0.05	4.2±1.09
SO_4^{2-}	2380± 20.1	884±13.5	993±7.3	736±16.3	874±5.5	988±10.8	2445±23.0	873±3.5	814±7.2	653±10.2
NH_4^+	45.8± 1.7	33.8±0.5	45.0±1.3	33.2±0.6	40.2±3.3	48.0±0.8	60.7±5.2	39.7±3.3	40.3±5.7	51.1±2.7
PO_4^{3-}	6.8± 0.3	4.1±0.01	6.6±0.5	5.1±0.1	3.9±0.4	8.4±0.5	3.4±0.3	2.7±0.07	5.7±0.7	3.02±0.02

geothermal springs was 40°C in Ain ad Darakah and the highest was 83.0°C in Ain al Harra. The water at all sambling points had a neutral or slightly alkaline pH. The lowest pH value was 6.8 in Ain Markub and the highest was 8.1 in Ain Khulab. The lowest conductivity was 1831 $\mu S\ cm^{-1}$ in Ain Khulab Quwa and the highest was 3300 $\mu S\ cm^{-1}$ in Ain Khulab. The temperature, pH and conductivity were determined in Ain Al-harra and Ain Al-khoba in previous study (Basahy, 1994). Compared to this study's measurements, the pH and conductivity of water samples varied insignificantly. However, the temperatures changed significantly. Interestingly, according to the dwellers around Ain Al-khoba, the temperature of Ain Al-khoba has increased significantly in recent years. In 1994, Basahy recorded the temperature in Ain Al-khoba to be 57°C, while recently, the temperature ranges between 70 to 75.5°C (Rehman and Shasha, 2005; Mohamed, 2008). The change in temperature might be explained by the change in tectonic features of the areas and the change in crust thickness in those areas. In particular, those geothermal springs are located along the west region coast, which had an earthquake recently. In contrast, variations in water chemistry were not significant among the geothermal springs in this study and the previous study (Basahy, 1994). Knowledge on biodiversity in geothermal springs in Saudi Arabia is still scanty and has not been compared to other hot springs located at different geographic areas.

The microorganisms in geothermal springs are fastidious and exist in limited number. Therefore, the total counts of bacteria in samples ranged from undetectable to approximately 10^5 CFU ml^{-1}. Relatively, higher bacterial counts were observed within samples collected from geothermal spring in Gazan. The total counts of the majority of the samples were in the range of 170 - 1320 CFU ml^{-1} (Figure 2). Low bacterial counts were observed in all Al-Lith water samples and most of the reporteddata mentioned that hot springs possess low density bacterial populations. Culture-dependent methods consisted of isolating and culturing microorganisms prior to their identification. This approach identifies only a small fraction (0.1–10%) of the microorganisms in natural environments (Ranjard et al., 2000). To overcome the limitation associated with the cultivation approaches, plastic composite support (PCS) were

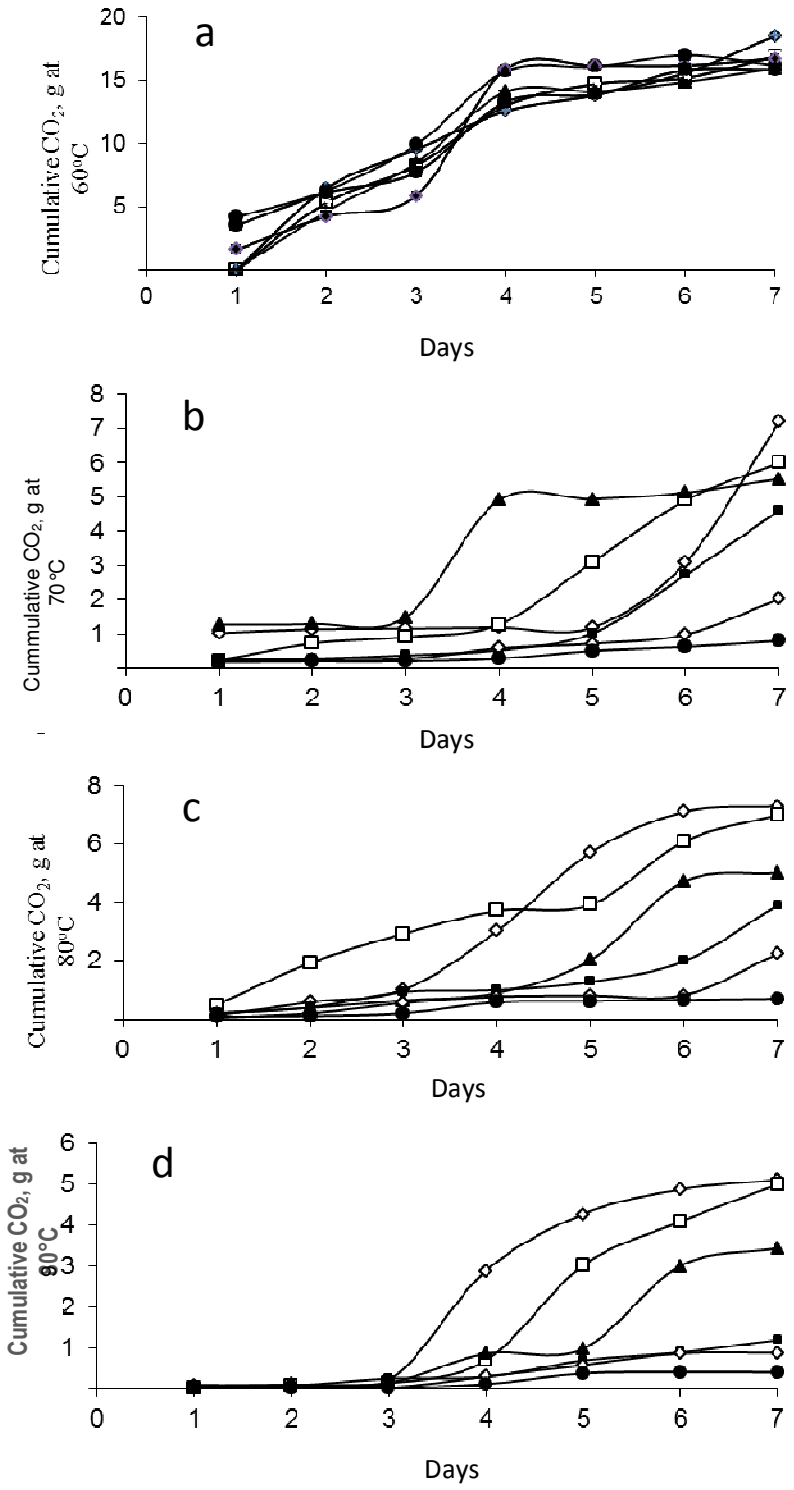

Figure 3. The figure shows the ability of *B. thermoamylovorans* to grow on different PCS blends, (◊) PCS 1, (□) PCS 2, (▲) PCS 3, (■) PCS 4, (○) PCS 5 and (●) PCS 6 at different temperatures (60 – 90°C).

used. The PCS was employed for isolation and characterization of thermophilic and hyperthermophilic microorganisms from food processing facilities (Ping-Shing, 2003). Furthermore, several studies have shown that plastic composite support (PCS) stimulate microbial growth, and biofilm formation, increase the final product

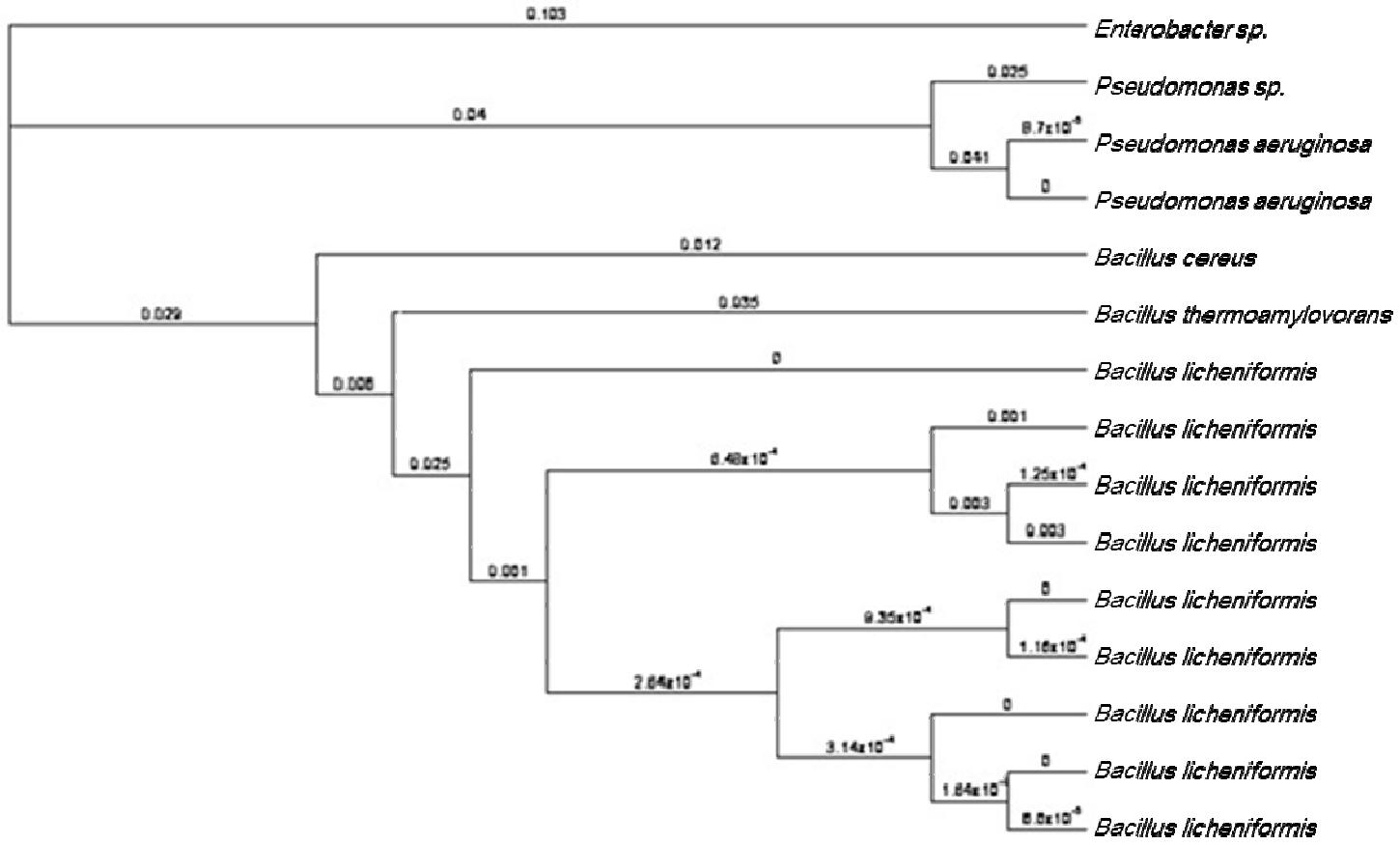

Figure 4. Neighbour-joining phylogenetic tree based on the nearly full length of the 16S rDNA gene of the different thermophilic and hyperthermophilic bacteria from geothermal springs in Saudi Arabia.

Table 2. Plastic composite support (PCS) blends (wt/wt).

Support[a]	Poly-poropylene	Yeast extract	Bovine albumin	Mineral salts[b]	Cellulose	Dates pits[c]
PCS	50	5	5	+	40	-
PCS 1	50	5	5	+	20	20
PCS 2	50	10	5	+	20	15
PCS 3	60	5	5	+	20	10
PCS 4	60	10	5	+	10	15
PCS 5	60	15	5	+	10	10
PCS 6	60	-	5	+	10	25

[a]: PCS tubes composed of % (wt/wt); [b]: mineral salts consist of: 2% (wt/wt) sodium acetate, 1.2% (wt/wt) magnesium sulfate, 0.06% (wt/wt) manganese sulfate; [c]: dried ground Khalas date pits (10-mesh).

and detoxify toxic compounds (Demirci and Pometto III, 1995; Demirci et al, 1997; Velazquez et al., 2001; Khiyami et al., 2005, 2006).

The isolated strains could be categorized as thermophilic since they thrive at a temperature of 60°C. Based on the morphological, biochemical, physiological and molecular characteristics, the optimal growth temperatures of these strains were found to be around 60 to 65°C after 48 h. Cluster analysis of fifteen bacterial isolates revealed that the dominant isolates in geothermal springs were assigned to *Bacillus licheniformis* (nine isolates), *B. cereus* (one isolate), *B. thermoamylovorans* (one isolate), *Pseudomonas aeruginosa,* (two isolates), *Pseudomonas* sp. (one isolate) and *Enterobacter* sp. (one isolate), (Figure 4). Genetic relatedness indicated that eleven *Bacillus* spp. grouped together formed several clusters within one main group with high similarity, *Enterobacter* sp. *Pseudomonas* sp. and *P. aeruginosa*

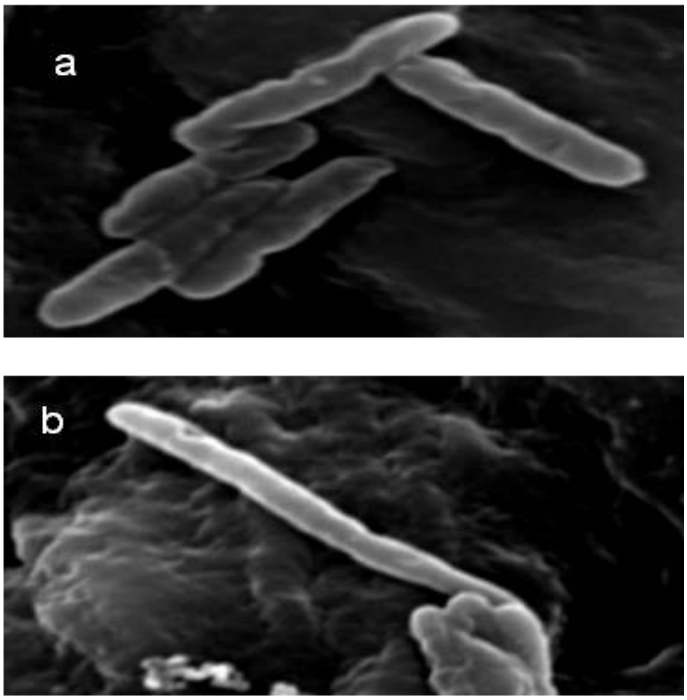

Figure 5. Scanning electron micrograph for *B. thermoamylovorans,* (a) at 60°C and (b) at 90°C.

are out of *Bacillus* cluster (Figure 4). Phylogenetic analyses of 16S rDNA sequences of nine isolates indicated that the nine isolates of *B. licheniformis* and the isolate of *B. thermoamylovorans* form one main subgroup, while *B. cereus* form a separate subgroup. The two isolates of *P. aeruginosa* and the isolate of *Pseudomonas* sp. form one group. Our results are in line with those obtained by Maugeri et al. (2001), who isolated 87 thermophilic, aerobic, spore-forming bacteria from shallow, marine, thermal vents of the Eolian Islands (Italy) using 16S rDNA sequences and found that most thermophilic species were members of *Bacillus*. They tested these bacteria for a broad spectrum of phenotypic characteristics. Later studies for thermophilic bacteria revealed significant evidence supporting reclassification of thermophilic members of the genus *Bacillus* as *Amphibacillus*, *Halobacillus*, *Thermobacillus* and *Marinibacillus* (Bae et al., 2005) based on the data of 16S rRNA sequence analysis. Recently, Adiguzel et al. (2009) identified and characterized thermophilic bacteria isolated from various hot springs in Turkey by using phenotypic and genotypic methods (PCR profilings, and 16S rRNA sequencing). The bacterial strains were classified into three phenotypic groups based on fatty acid profiles, which were confirmed by genotypic methods such as 16S rRNA sequence analysis and rep-PCR genomic fingerprint profiles. They concluded that rep-PCR fingerprinting using the (GTG)5 and BOXA1R primers could be considered a promising genotypic tool for the identification and characterization of thermophilic bacteria from species to strain level.

The geothermal springs might also be contaminated with *Pseudomonas* and *Enterobacter*. These isolates were found only in Al-Lith geothermal springs that posses low temperatures (40, 47, and 50°C). In spite of the low bacterial counts observed in all Al-Lith water samples, the *B. thermoamylovorans* was dominant in Ain al Harra samples. Also, *B. thermoamylovorans* was isolated from Ain Khuulah in Gizan.

Members of the *Bacillus* genus and the obligate thermophilic bacilli generally have simple nutritional needs; therefore, they do not require specific amino acids for growth and are able to grow on simple media such as tryptone soya aga (TSA). The optimum growth temperature of the thermophilic bacilli is usually between 50 and 65°C, but varies between species and strains (Burgess et al., 2010). Thus, they are easily cultured and subcultured in the lab and used for extraction of beneficial compounds.

B. thermoamylovorans grew poorly on PCS (Figure 3), but with PCS1, PCS 2 and PCS 3 it grew significantly at high temperatures (Figure 3a to d). The PCS have the capability of releasing nutrients slowly in long-term fermentations. PCS blends can be customized for individual bacterial strains, and have been proven to increase lactic acid production rates, reduce lag phase of microorganisms, reduce requirements of micronutrients, increase cell density and could provide a platform for attachment of thermopiles and hyperthermophiles (Cotton et al., 2001; Urbance et al., 2003; Ping-Shing, 2003). The

B. thermoamylovorans growth was varied depending on the nutrient of PCS. The blend of high concentration of date pits and low concentration of yeast extract (PCS 1 and PCS2) showed significant effect on growth at 90°C. Dates pits contain 5-10% (w/w) moisture, 5-7% (w/w) crude protein, 7-10% (w/w) oil, 10-20% (w/w) crude fiber, 55-56% (w/w) carbohydrates and 1-2% (w/w) ash (Khiyami et al., 2008). On the other hand, the high concentration of dates pits and 0% concentration of yeast extract (PCS 6) slow the growth significantly at 70, 80 and 90°C. The yeast extract obligately required supraoptimal temperatures (De Souza and Leal Martins, 2001). The growth was slow during the first four days with all PCS blends, but increased significantly after day four especially with PCS 1, PCS 2, and PCS 3 (Figure 3c). These results might be due to the effect of nutrients releasing slowly in long-term fermentations. The isolate was moderately thermophilic, motile, spore-forming rods, aerobic or facultatively anaerobic and posses enzymatic activity (Nazina et al., 2001; Belkova et al., 2007). Interestingly, the PCS 1 and PCS 2 helped the *B. thermoamylovorans* to become hyperthermophilic tolerant. The growth at 90°C affects the cells shape and becomes long rod compared to growth at 60°C (Figure 5). Ability of *B. thermoamylovorans* to grow at high temperature is an indication of the presence of hyperthermophilic bacteria in geothermal springs in Saudi Arabia. These species are unable to grow easily *in vitro* due to the culture conditions and its low numbers. It is believed that the culture collection is not representative of the actual microbial diversity in geothermal springs. We are in the process of developing new technique to use PCS *in situ* to provide a shelter for hyperthermophilic bacteria.

The majority of industrial enzymes known to date have been derived from bacteria and fungi (Antranikian and Egorova, 2007). In biotechnology research, thermophiles have become increasingly important and the number of bioprospecting groups searching for useful organic compounds in nature have dramatically increased as well. Currently, thermophilic bacteria are used as a source of thermostable enzymes and industrial products (Maugeri et al., 2001). Bhardwaj et al. (2010) isolated and screened thermophilic bacteria from Tapoban geothermal field (Uttarakhand Himalaya, India) for the production of antibacterial compounds. With the constant developments of new technologies, such as genomics, developments in the cultivation and production of thermophiles, and the success in the cloning and expression of their genes in mesophilic hosts, their potential industrial application will only but increase. Genomic investigation of bacterial species is important in medicine, biotechnology and the environment. The information obtained has begun to increase not only our understanding of microbiology, but also general theory in cell and evolutionary biology. Comparative genomic analysis will help to understand adaptations made within the thermophilic bacilli compared with related mesophilic strains (McMullan et al., 2004).

Conclusion

The ecosystem in Saudi Arabia is still poorly investigated ecological zones of the Earth. The geothermal springs represent a challenge for the search of non-described biotechnological resources. This study shows the possibility of isolating and identifying and characterizing, and estimating genetic relatedness of thermophilic and hyperthermophilic bacteria from geothermal springs in Saudi Arabia using 16S rDNA primes. The dominant bacteria in geothermal springs were somewhat similar to the bacteria isolated from other habitats worldwide. In addition, the blend of PCS with date pits will help to isolate hyperthermophilic bacteria. There is a vast potential for investigating these thermophiles for their ability to produce products of great biotechnological importance that will play a big role in delivering clean, sustainable, novel and exciting industrial processes in the future. Thermophiles and their enzymes have become a hot research topic, which has opened a new era in biotechnology.

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