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Full Length Article

Fatty acids and survival of bacteria in Hammam Pharaon springs, Egypt

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ABSTRACT

A great lack of knowledge of Hammam Pharaon's microbial community; the most famous hot spring in Sinai, Egypt, derived this work. Three different hyperthermophilic bacterial were isolated from vents in the area, where the temperature was above 80 °C. Response Surface Methodology algorithm such as Central Composite Design determined the optimum cultivation conditions for these isolates. Accordingly, the best growth conditions were at 70 °C and at neutral to slightly acidic pH values. The constructed phylogenetic tree built using the 16S rRNA gene sequences has shown that the isolated strains (HM101, HM102 and HM103) belong to *Geobacillus, Rhodothermus and Thermus* bacteria, respectively. The fatty acid profiles, an indicative of thermotolerance, dominated by the short chain Dodecanoic acid (Lauric acid; (12:0), which represented about 40% of the total fatty acid contents for each of the three isolates. The enzymatic capabilities of the three strains were determined and α-amylase was found to be the most prominent one. Our own data had led us to conclude that the length of the fatty acid chain and the degree of saturation could be species specific. Moreover, the biotechnological potentials of these local isolates could contribute to fighting viral diseases and/or improve their amylolytic activities for sugar industry; where thermotolerance is really an important factor.

1. Introduction

Extremophiles are members of the extreme environment-tolerant organisms, which belong to Archaea, eubacteria, and eukaryote. These group of organisms can live, survive and flourish at temperatures above 50 °C and may reach 80 °C and up [1]. The normal temperature sensitive macromolecules (enzymes, proteins, lipids and nucleic acids) have demonstrated tolerance/resistance to this denaturing high temperatures. This adaptability of the thermophiles and hyperthermophiles cellular components is simply described as thermostability. These thermophiles and hyperthermophiles bacteria have been isolated from different habitats including hydrothermal vents and deep ocean-basin cores. From amongst them Gram positive/negative, spore or non-spore forming bacteria were isolated which exhibited aerobic or anaerobic metabolism [2] (See Table 1).

Overall, Thermophilic bacteria are the least explored due to difficulties in isolation and maintenance of pure culture. Biotechnological potentials of thermophiles and extremophiles were justified by their pools of amylases, proteases, lipases, xylanases and DNA polymerases. Theses enzymes tolerate not only high temperature but also extreme pH and salinity [3]. Additionally, extremophiles were reported to produce several bioactive molecules such as antibiotics, sulfur-reducing enzymes and *exo*-polysaccharides [2].

Accurate identification of any bacteria is a must to proceed forward. Despite it has been established for over a century ago, cultural, phenotypic, biochemical techniques were not satisfying. Therefore, recent nucleic acid based techniques (e. g. 16S rRNA gene sequence) and fatty composition (microbial fatty acid methyl esters, FAME) of the cell membranes has gained popularity due to their undisputed reliability and reproducibility [4–6].

Amongst the many thermal vents localized in Egypt, Hammam Pharaon, that lies in South Sinai at latitude 29, 197112 and longitude 32, 956179 has gained popularity due to the tourist attraction. In the present study, an endeavor was made to explore the bacterial community of Hammam Pharaon. The bacterial isolates were characterized at the morphological and molecular and fatty acid levels. Moreover, the biotechnological potentials of the isolates were explored, especially their amylolytic, cellulolytic, lipolytic and proteolytic activities.

2. Materials and methods

2.1. Samples collection and isolation of bacteria

Water samples and soil deposits from Hammam Pharaon (South Sinai, Egypt, at latitude 29, 197112 and longitude 32, 956179) were

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Table 1
Hammam Faraun water Sample analysis showing the high amount of sulfur pointed by the black arrow.

Sample no/name	Hot vents inside Hammam Faraun	Max values
Sample type	Saline hot water	
Turbidity	4.25	1NTU
Residual chlorine	———	0.2–2 PPM
pH	6.66	6.5–8.5
TDS	12920 (calculated) 21854 (weighed at 120 °C)	1000 mg/L
EC	19,370	µs/Sec
T.alkalinity	1100	300 mg/L
chlorides	7742.3	250 mg/L
T.Hardness	4500	500 mg/L
Ca.Hardness	2700	350 mg/L
Mg.Hardness	1800	150 mg/L
Ca	1080	mg/L
Mg	432	mg/L
Sulfate	353.441	250 mg/L
Nitrate	0.875	45 mg/L
Iron	0.113	0.3 mg/L
Manganese	0.959	0.4 mg/L
Fluoride	1.797	0.8 mg/L

collected in 500 ml sterile thermal glass containers and immediately transferred to the laboratory. The mineral composition of the water samples was determined according to the standard protocols [7]. Native bacteria were isolated from one gram of wet soil deposits as described by [8]. Then a 1 ml of the sample was transferred into 100 ml of Zobell broth marine medium containing g/L: 1.29 yeast extract, 3.75 peptone, 9 NaCl, 2 $MgCl_2$ and 0.525 KCl dissolved in Hammam Faraun water and pH was adjusted to 7.5. Cultures were incubated at 70 °C for two weeks, then, a 500 µl of each growing culture was transferred into fresh Zobell agar plates and incubated for another two weeks at 70 °C. Morphological properties of colonies and cells were scored as size, colour, margin, elevation and Gram stain preference, too. Morphologically distinct colonies were purified and stored in 25% glycerol at −80 °C for further studies.

2.2. Molecular identification of the bacterial isolates

This depended on the DNA sequencing of the gene encodes for the 16S rRNA by PCR using the universal primer pair of 518F: (5′CCAGCAGCCGCGGTAATACG3′) and 800R: (5′TACCAGGGTATCTAATCC3′) [9]. Subsequently; the PCR products were purified using the QIAquick PCR purification Kit protocol (Qiagen, Germany) and auto-sequenced by ABI PRISM using cycle sequencing kit (Macrogen, Korea). The sequences were analyzed and managed by the software CLUSTAL W 2.0, while the Phylogenetic trees were constructed by using Seaview software [10].

2.3. Scanning electron microscopy (SEM)

Bacterial isolates were further characterized by scanning electron microscopy (JSM 6501LV, Joel Japan) [11] In short, bacteria were primarily fixed in a mixture of formaldehyde and glutaraldehyde (1:1) for 24 h, followed by three washes (10 min each) with potassium phosphate buffer (pH 7.2). A post-fixation by 1% osmium tetraoxide was carried for two hours; samples washed with potassium phosphate buffer and dehydrated with different concentration of ethanol (50, 70, 80, 90, 95 and 100%) for 15 min each, in an Autosamdry-815 (USA) model. Finally, samples were coated with gold using SPI module sputter coater before being examined by SEM.

2.4. Analysis of the fatty acid methyl ester (FAME)

Fatty acids were extracted from each isolate as described by Gattinger (2002) [12]. Where 20 mg of each freeze-dried isolate was suspended in 2 ml of 5% Methanolic HCl, incubated at 70 °C water bath for 2 h, The mixture cooled at room temperature for 45 min, then 1 ml deionized water was added and vortexed. To remove the unsaturated fat methyl esters (FAME) were obtained by adding 2 ml hexane to each and the tubes were kept at ambient temperature for layers separation. The upper layer was moved into a clean glass tube and dried under nitrogen and was analyzed by the Gas Chromatography/Mass Spectroscopy. This Agilent GC was provided with splitter injector at 280 °C connected to Agilent MSD with the electron voltage 70 eV, source temperature 230 °C, quad temperature 150 °C, multiplier voltage 1800 V and interface temperature 310 °C, controlled by HP Compaq PC. The specimen (1 µl) in hexane was infused utilizing autosampler with the split open. After the fundamental dissolvable crest had passed the GC temperature system and the information obtaining initiated, partition was performed on an Agilent-combined silica fine section (30 m × 0.25 mm i.d) covered with 0.25 µm dimethyl poly-siloxane (HP-5) stage. The GC was temperature modified from 30 to 130 °C at 5 °C/min then to 300 °C at 20 °C/min and held at a last temperature for 5 min with helium as the bearer gas (stream rate of 1 ml/min, beginning weight of 50 kPa, split at 10 ml/min). Peaks were distinguished and named after correlation of their retention time and mass spectra [13].

2.5. Optimum pH and temperature for growth

The Central Composite Design (CCD) was used to determine the main effects and interaction between pH and temperature on bacterial growth in order to obtain the optimum condition for each isolate. All strains were grown in 50 ml of Zobell medium [14] at the optimum temperature and pH values for each isolate for 7 days. Final biomasses were collected by centrifuging at 4000 rpm for 15 min. The cell pellets were transferred into a 1.5 ml screw tube and freeze-dried and the dry weights were determined.

2.6. Enzymes assays

The amylolytic, cellulolytic, lipolytic and proteolytic activities of the three isolates were qualitatively assayed according to [15], [16] and [17], respectively. The α-amylase activity was tested by starch hydrolysis was monitored by Iodine reagent [15]. Lipase activity was evaluated using Tween 80 medium and measuring the clearing zones around the bacterial colonies. The monitoring of cellulose degradation activity depended upon the diameter of the clear zones around the bacterial colonies growing onto carboxymethylcellulose (CMC) agar media.

3. Results

3.1. Water samples analysis

There was a heavy smell of sulfur gas around the water sources of Hammam Pharaon and the water analysis confirmed the presence of high percentage of sulfur. The in-situ measurement of temperature and pH indicated that during the sampling period the temperature was in the range of 70–90 °C and the pH was recorded to be in the range of 6–7.5.