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Identification of Four Thermophilic *Geobacillus* Isolates from Hammam Pharaon, Sinai, Egypt

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ABSTRACT

The present study was conducted to identify thermophilic bacteria from local habitats and to test their ability for producing thermostable α-Amylase, cellulase, lipase and protease in order to pave the way for their use in industrial processes. Out of 29 isolates initially obtained mainly from Hammam Pharaon, four potential isolates were selected based on thermo-tolerance ability and enzyme activity. The four isolates were characterized using phenotypic and 16S rRNA sequencing which recognized the isolates as *Geobacillus*. All four isolates were found to be variable for indole test, methyl red and rod shaped morphology. However, they were found to be variable for citrate utilization, catalase test starch hydrolysis, gelatin hydrolysis, casein hydrolysis, and Tween 80 hydrolysis. These bacteria grow at temperatures ranging from 50 to 90°C and the pH ranged from 6 to 9. This study added a lot to our knowledge about the thermophilic bacteria inhabiting Hammam Pharaon and their characters that would open the door for their use in biotechnological applications..

Keywords: α-Amylases, Cellulase, protease, lipase, *thermophilic Geobacillussp*., Hammam Pharaon

INTRODUCTION

Prokaryotes inhabit all possible environments including those with extreme conditions. Thermophilic microorganisms, for instance, are adapted to grow at temperatures from 55°C to 115°C and their cellular components, enzymes, proteins and nucleic acids are thermo-stable (Haki and Rakshit, 2003).Thermophiles fall in three categories; moderate, extreme and hyperthermophiles (Bertoldo and Antranikian, 2002). Geothermal vents could be the main source of such organisms (Gupta *et al.*, 2014).

Thermophiles have adapted to high temperature by several structural and functional features. Thermophiles have adjusted the composition of cell membrane including the increase in acyl chain length and the degree of saturation and branching of fatty acids and cyclization, thus maintaining optimal membrane structure and function in high temperature that could increase the fluidity and permeability of membranes (Rothschild and Mancinelli, 2001; Charlier and Droogmans, 2005).Thermophiles contain a higher content of saturated straight- and branched fatty acids, but rarely contain significant amounts of polyunsaturated acids (O'Leary, 1962; Kaneda, 1963; Moss and Cherry, 1968).The fatty acid composition of several thermophilic bacteria of the genus *Bacillus* was recently investigated (Daron, 1970).

Thermophiles are a potent source of thermo-stable enzymes characterized by stability and activity under conditions of high temperature. Thus, these enzymes are rapidly used in industrial applications (Haki and Rakshit, 2003).Among industrially important enzymes, amylases, cellulase, protease and lipases which become more attractive ,and have wide applications in many fields. For instance, amylases have a great significance in present-day biotechnology as it represent 25% of the global enzyme market (Van Der Maarel *et al.*, 2002).

Amylases are intensively used in several industrial applications such as detergent and baking industries, ethanol production and HFCS (high fructose corn syrup) according to their characteristics(Gupta *et al.*, 2003) and it is also broadly used in several fields including medicinal and analytical chemistry(Kandra, 2003).Textile industry, laundry detergents, animal feed as well as juice processing require cellulases (Singh *et al.*, 2007). Proteases also have many industrial application and they represent 60 % of the enzyme market(Rao *et al.*, 1998).Lipases catalyze the hydrolysis and the synthesis of esters formed from glycerol and long-chain fatty acids. Lipases occur widely in nature but only microbial lipases are commercially significant (Sharma *et al.*, 2001) that are employed in paper industry, obtaining lipid by-products such as mono- and di-glycerides (Markossian *et al.*, 2000).Antibiotics in addition to several bio-active molecules have been reported to be obtained from thermophiles (Gonzalez *et al.*, 2003; Aanniz *et al.*, 2015)..

In Egypt, There are many hot springs surrounding the coast of the Gulf of Suez accompanying to the tectonic action of the Red Sea area and Gulf of Suez rift (Lashin and Al Arifi, 2010; Lashin, 2013; Lashin and El Din, 2013).Hammam Pharaon is advised the hottest sulfuric hot spring in Egypt with temperature ability to 70ºC, (Morgan *et al.*, 1983; Morgan *et al.*, 1985).Environmental conditions and the nutritional status available in Hammam Pharaonrepresent a selective media toward a particular group of microbial population that are not sufficiently studied. This study aims to study and identify the potential thermophilic bacteria mainly isolated for Hammam Pharaon and screening their potentialities to be used as a source of several thermostable enzymes.

MATERIALS AND METHODS

Materials:

Soluble starch, Carboxy methyl cellulose (CMC), Azo casein and Gum acacia were purchased from sigma chemical company, otherwise all chemicals were analytically grade.

- Sampling sites and sample collection

The main source of the intended isolates in this study was the thermal water and soil obtained from Hammam Pharaon, Sinai, Egypt .The Water and soil samples were collected in sterilized falcon tubes and immediately transferred to the lab.The temperature of water ranged between 55 to 70°C according to the site of collection and the water pH was 6.5- 7.The chemical constituents of this site has already been reported(Selim *et al.*, 2014).

- Isolation of thermophilic bacteria and growth conditions

Both dilution plate method and enrichment method were used for isolation (Holt *et al.*, 1994). 0.45 µm membrane filters were used to filtrate water samples and the obtained filtrate was inoculated in Nutrient Agar (NA)medium(Prescott *et al.*, 2002). For soil deposits, one gram of soil was serially diluted in sterile distilled water and plated on nutrient agar plates and incubated at 60ºC for 24-48 hrs. Colonies with different shape, size and pigmentation were individually picked and sub-cultured on nutrient agar plates to obtain pure isolates (Mohapatra *et*

al., 2014).Further characteristics were obtained by Gram staining, Endospore staining and capsule staining. The purified bacterial isolates were maintained under 20% glycerol and kept at -20 ºC till use for further experiments.

- Screening of extracellular thermostable enzymes

1- Screening for α-Amylase Activity

The obtained isolates were inoculated in a solid medium containing soluble starch (Castenholz, 1969)to test the activity of α-Amylase. After incubation for 24-72h at 60ºC, 1% iodine solution was added to the pre-inoculated plates and the development of clear zones around colonies indicated positive amylolytic activity(Yanmis *et al.*, 2015). Quantitavely,the tested isolates were inoculated in the afro-mentioned broth medium at 60°C for 72h then; the culture broth was centrifuged at 5000 rpm for 20 min at 4°C. To evaluate the activity, an assay mixture (total volume 10 ml) containing 1% soluble starch, crude enzyme solution in 60 mM potassium phosphate buffer pH 7.0 and the mixture was incubated at 60°C. The increase in the reducing sugar was monitored over time intervals by using dinitrosalicylic acid reagent (Miller, 1959). Maltose was used as standard and one unit of α-amylase activity was defined as the amount of enzyme that releases one µmol reducing sugar equivalent to maltose per min.

2- Screening for cellulase Activity

CMC containing solid medium (Bragger *et al.*, 1989) was used to screen cellulase activity. The obtained isolates were inoculated and then incubated at 60ºC for 5-7 days. The formation of clear zone around the colonies after addition of Congo red solution indicated positive result(Yanmis *et al.*, 2015).Quantitavely,to determine cellulase activity, the tested isolates were inoculated in the afro-mentioned broth medium and incubated for5-7 days at 60ºC then; the culture broth was centrifuged at 5000 rpm for 20 min at 4°C. The quantification of the resulted reducing sugars was performed by using the same assay mixture and dinitrosalicylic acid reagent was used to estimate released sugars. Glucose was used as standard and the unit of cellulase activity was defined as the amount of enzyme that releases one µmol reducing sugar equivalent to maltose per min.

3- Screening for Protease Activity

Minimal Synthetic Medium (MSM) containing skimmed milk were used as a source of protein (Priest and Alexander, 1988).After inoculation, the cultures were incubated for 24-72h at 60ºC and the formation of clear zones around colonies indicated positive proteolytic activity(Panda *et al.*, 2013). For the purpose of quantifying the protease enzymes; the tested isolates were inoculated in the afro-mentionedbroth medium and the cultures were incubated for5-7 days at 60ºC then; the culture broth was centrifuged at 5000 rpm for 20 min at 4°C. To evaluate the activity, an assay mixture (total volume 10 ml) containing 0.5% azocasein and the crude enzyme solution in 60 mM potassium phosphate buffer pH 7.0 and the mixture was incubated at 60°C.The reaction was started by adding 1 ml of enzyme source to the assay mixture andthe reaction was stopped by 1 ml trichloroacetic acid. The unit of protease activity was defined as the amount of enzyme release of one µmol of tyrosine per minute per µg protein.

4- Screening for Lipase Activity

Tween 20/tween 80 containing solid medium were used to test lipase activity (Kumar *et al.*, 2012). After incubation for 2 days at 60ºC, the observation of white precipitation around the colonies indicated positive lipase activity(Ertuğrul *et al.*, 2007).Quantitavely, to determine lipase activity, the tested isolates were inoculated in the afro-mentioned broth medium 5-7 days at 60ºC then, the culture broth was centrifuged at 5000 rpm for 20 min at 4°C. The lipase activitywas measured in an assay mixture containing homogenized olive oil in 10 % (w/v) gum acacia as a substrate and the crude enzyme source in 60 mM potassium phosphate buffer pH 7.0 and the mixture was incubated at 60°C(Jensen *et al.*, 1983). The reaction was stopped by the addition of 1 ml of acetone: alcohol (1:1) mixture and titration was performed against 0.1MNaOH.The unit of lipase activity was defined as the amount of enzyme that liberated one µmol fatty acid per minute per µg protein.

- Identification and characterization of the potential thermophilic isolates

1. Morphological, biochemical and physiological identification:

Morphological and biochemical identification was done by applying the methods described in Bergey's Manual of Determinative Bacteriology (Vos *et al.*, 2011).

Optimum pH and temperature of the most potential producers were determined using the Central Composite Design (CCD) algorithm in which a factorial CCD was proposed with two factors. 13 sets per experiment was formulated to obtain the optimum pH and temperature for growth(Box *et al.*, 1978).The CCD matrix included 5 levels for each variable, 6 center points and star points to estimate the curvature. The CCD provided an indication of the main effect of each factor in addition to the interaction between them. All the experiments were carried out at least in triplicate (Fossi *et al.*, 2011).

All isolates were inoculated in 50 ml LB medium with particular pH and then incubated in the temperature indicated in table 4for 10 days. Final biomass were collected into a 50 ml falcon centrifuge tube and centrifuged at 5,000 rpm for 15 minutes. The cell pellets were transferred into a 15 ml screw tube and freeze-dried and the dry weights were determined (Tamilarasan *et al.*, 2012) . In the RSM, the interactive effects of pH, and temperature were studied to obtain optimum growth. Both factors in were studied at five different levels (-2, -1, 0,+1, +2) in which the minimum and maximum ranges of variables were tested with respect to their values in actual and coded for mass displayed in table 1.Minitab 15 software (StatEase, Inc, Minneapolis, MN, USA) was used in this experiment.

Table 1. Different pH and temperature levels in terms actual and coded factors were tested during optimization using Central Composite Design (CCD) experiment

Variables	Range of levels				
	-2	**-1**	**0**	**+1**	**+2**
pH	5	6	7	8	9
Temperature(°C)	50	60	70	80	90

2- The molecular identification:

The genomic DNA of the isolates was obtained by using Insta-Gene Matrix Genomic kit (Bio-Rad, USA) and then it was used as a template for amplification of the 16S rRNA gene. PCR was performed using518F as forward primer (5'CCAGCAGCCGCGGTAATACG) and 800R as a reverse primer (5'-TACCAGGGTATCTAA TCC). Montage PCR Clean up kit (Millipore) was used to obtain PCR pure product. Sequencing was performed by using ABI PRISM®BigDyeTM Terminator Cycle Sequencing Kits. The sequence obtained was checked and manipulated by

Finch TV version and the phylogenetic tree was generated via Sea view software using the closest published type strains sequences.

RESULTS

- Isolation of thermophilic bacteria and growth conditions

In the present study, Hammam Pharaon was the main source of thermophilic bacteria. At the time of sampling, the thermal water temperature ranged from 55 to 70°C, and pH 6.5-7. The obtained 29 isolates were further screened for the desired hydrolytic activities.

As indicated in table 2,of the 29 isolates it was found that; 17 isolates are capable of degrading starch due to their amylase activity. In addition; 9 isolates were found to be able to produce cellulase enzyme, 15 isolates were able to produce lipases enzyme and 14 isolates were able to produce proteases enzymes. In order to select the best in each group, enzyme activity was measured for all enzymes in each group. The isolate (HF_84) gave the highest assay for amylases activity compared with the other isolates with total activity 24.16 U/ml. The isolate (HF_85) gave the highest activity of cellulase with a value of 11 U/ml. The isolate (HF_86) gave the highest protease activity among other isolates with total activity 49.7 U/ml. Finally, the isolate (HF_87) gave the highest value of lipase activity with total activity 1.72 U/ml.

- Morphological and biochemical identification of thermophilic bacteria

The obtained four isolates (HF_84, HF_85, HF_86 and HF_87) that gave the highest activity in the quantitative assays were further subjected to morphological and biochemical tests as represented in table 3. The colony shape of the four strains was different and all of them showed the ability to survive at 50-90°C, pH 5-9 and 1-5% NaCl. These strains were further selected for molecular identification.

Table 2. The obtained isolates and their corresponding enzyme assays. (+) represent positive activity while (–) represent absence of the enzyme activity.

Isolate code	Alpha amylase	Cellulase	Protease	Lipase
HF_84	+	+	+	+
HF_85	+	+	+	+
HF_86	+	+	+	+
HF_87	+	+	+	+
HF_88	+	-	-	-
HF_89	-	-	-	-
HF_90	+	-	+	-
HF_91	-	+	-	-
HF_92	+	-	+	+
HF_93	+	+	+	+
HF_94	+	-	-	+
HF_95	+	-	+	+
HF_96	+	-	-	-
HF_97	+	-	-	-
HF_98	+	-	+	+
HF_99	+	-	+	-
HF_100	+		-	+
HF_101	-	-	-	-
HF_102	+	-	-	-
HF_103	-	-	-	-
HF_104	-	+	-	+
HF_105	-	+	+	+
HF_106	-	-	-	-
HF_107	-	-	-	+
HF_108	-	-	-	-
HF_109	+	-	+	-
HF_110	-	+	-	+
HF_111	-	-	+	-
HF_112	-	-	+	+

Table 3. Morphological, biochemical and physiological characterization of the four isolates

Morphological characterization	HF_84	HF_85	HF_86	HF_87
Colony size	small	moderate	moderate	large
Colony shape	circular	circular	circular	circular
Margin	Entire	Entire	Entire	Entire
Form	regular	regular	irregular	regular
Elevation	Slightly raised	Slightly raised	Slightly raised	Slightly raised
Color	white creamy	white-yellow	white	white creamy
Surface texture	Smooth	Smooth	Smooth	Smooth
Light transmission	Translucent	Translucent	Opaque	Transparent
Consistency	moist	moist	dry	moist
Nutrient broth culture	Sediment	Turbid	Turbid	Turbid
Growth form on slant	Arbores cent	Effuse	Effuse	Arbores cent
Gram's nature	G+ve rods	G+ve rods	G+ve rods	G+ve rods
Spore position	Terminal	Terminal	Terminal	Terminal
Spore shape	Oval	Ellipsoidal	Ellipsoidal	Oval
Sporangium shape	Swollen	swollen	Swollen	Swollen
Motility	motile	motile	motile	motile
Acid fast staining	-ve	-ve	-ve	-ve
Capsule staining	+ve	+ve	+ve	+ve
Amylase activity	+ve	+ve	+ve	+ve
Cellulase activity	+ve	+ve	+ve	+ve
Protease activity	+ve	+ve	+ve	+ve
Lipase activity	+ve	-ve	+ve	+ve
Gelatinase activity	-ve	-ve	-ve	-ve
Catalase	+ve	+ve	+ve	-ve
Oxidase	+ve	+ve	+ve	-ve
Urease	-ve	+ve	+ve	-ve
Macconkey agar	-ve	-ve	-ve	-ve
Eosin methylene blue agar	-ve	-ve	-ve	-ve
Indole test	-ve	-ve	-ve	-ve
Methyl red test	-ve	-ve	-ve	-ve
Citrate utilization test	+ve	+ve	+ve	-ve
H_2S test	+ve	+ve	+ve	-ve
Voges-proskauer test	-ve	-ve	-ve	+ve
Sugar fermentation test	+ve	+ve	+ve	+ve
Triple Sugar Iron test	+ve	+ve	+ve	+ve
Physiological characterization				
Growth at 50-90ºC	+ve	+ve	+ve	+ve
Growth at pH 5-9	+ve	+ve	+ve	+ve
Growth at 1-5 % NaCl	+ve	+ve	+ve	+ve

Table 4. Matrix and responses of CCD applied for optimum pH and temperature for growth of the four isolates and the higher dry weight were displayed as Bold.

Run	Variables		Responses (growth in term of Dry Weight Biomass)			
	pH	Temp.	HF_84 (g/L)	HF_85 (g/L)	HF_86 (g/L)	HF_87 (g/L)
1	7	70	4.31	4.53	3.52	5.97
2	8.4	70	3.80	5.11	3.90	2.69
3	7	70	4.33	4.51	3.53	5.96
4	7	70	4.31	4.53	3.52	5.97
5	7	55.8	2.11	3.64	2.89	5.40
6	8	60	3.48	2.17	3.33	5.55
7	5.5	70	1.02	1.26	1.89	1.06
8	6	80	2.35	5.91	1.74	3.60
9	7	70	4.33	4.51	3.53	5.96
10	7	70	4.31	4.53	3.52	5.97
11	7	84	1.08	1.04	1.00	1.31
12	8	80	2.07	2.33	346	5.89
13	6	60	3.95	2.79	4.15	5.15

Statistical analysis of the effect of the tested factors (pH and temperature) tested for growth of *Geobacillus*_HF84 showed the significant effect of pH,$pH^2$and $temp^2$ on the dry weight as their P values were less than 0.1 (α value),P value was used as a cut off to test the significant at 90% level of confidence ,their P values were 0.059 and 0.009 respectively. Statistical analysis of the effect of the tested factors (pH and temperature) tested for growth of *Geobacillus*_HF85showed the non- significant effect of pH,pH^2 and $temp^2$ on the dry weight as their P values were more than 0.1 (α value),P value was used as a cut off to test the significant at 90% level of confidence , their P values were 0.414 and 0.163 respectively. Statistical analysis of the effect of the tested factors (pH and temperature) tested for growth of *Geobacillus*_HF86 showed the significant effect of pH, temp, pH- Temp and $temp^2$ on the dry weight as their P values were more than 0.1 (α value),P value was used as a cut off to test the significant at 90% level of confidence, their P values were 0.028,0.008, 0.013 and 0.033respectively. Finally, Statistical analysis of the effect of the tested factors (pH and temperature) tested for growth of *Geobacillus*_HF87 showed the significant effect of pH^2 on the dry weight as their P values were more than 0.1 (α value),P value was used as a cut off to test the significant at 90% level of confidence, its P value was 0.034.Statistical analysis of the CCD experiment for the growth of the four isolates were summarized in Table 5 at 90% level of confidence. Significant P value(<0.1) displayed as Bold. The interaction among the tested variables could be summarized using contour plot (Fig.1).

Table 5. Statistical analysis of the CCD experiment for the growth of the four isolates at 90% level of confidence. Significant P value(<0.1) displayed as Bold

	Geobacillus HF84		
Variables	**Summation of Squares**	***F Value***	***P Value***
pH	0.012653	1.81	0.220
Temp	0.024939	3.57	0.101
pH^2	0.022809	5.08	0.059
$temp^2$	0.087497	12.54	0.009
pH- Temp	0.000090	0.01	0.913
*Geobacillus*_HF85			
pH	0.017509	7.58	0.028
Temp	0.030664	13.28	0.008
pH^2	0.000166	0.49	0.508
$temp^2$	0.025211	10.92	0.013
pH- Temp	0.016129	6.99	0.033
*Geobacillus*_HF86			
pH	0.001937	0.07	0.793
Temp	0.000197	0.01	0.933
pH^2	0.011758	0.76	0.414
$temp^2$	0.063395	2.43	0.163
pH- Temp	0.021904	0.84	0.390
*Geobacillus*_HF87			
pH	0.031190	1.51	0.259
Temp	0.061147	2.96	0.129
pH2	0.128292	6.95	0.034
temp2	0.033832	1.64	0.242
pH- Temp	0.008930	0.43	0.532

According to Fig.1, it was found that; the optimum conditions for the growth of *Geobacillus*_HF84 at pH 7 and temperature 70°C., *Geobacillus*_HF85 at pH 6 and temperature 80°C, *Geobacillus*_HF86 at pH 6 and temperature 60°C, and finally *Geobacillus*_HF86pH 7 and temperature 70°C

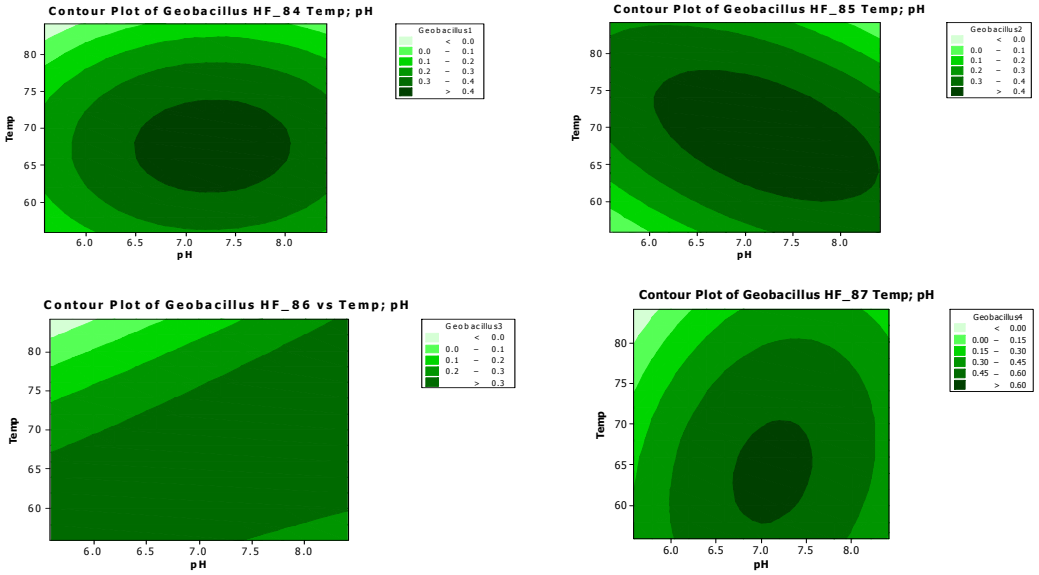

Fig. 1. Counter plot showing the interaction among the tested variables on the dry weight of the four isolates.

- Molecular identification of the selected isolates

The 16S rRNA sequence analysis showed that all the obtained isolates are members of the genus *Geobacillus*. The isolate HF_84 that is phylogenetically relevant to *Geobacillus caldoxylolyticus* (96.7 % identity),HF_85 and HF_86 showed very high sequence identity to *Geobacillus thermoglucosidasius*(97.9-96.3 % identity) respectively, and HF_87 showed high sequence similarity to *Geobacillus vulcani*(94.4 % identity). The obtained GenBank accession numbers for the four isolates HF_84,HF_85, HF_86 andHF_87 were KY084244, MG564474 , MF155645 and MF155646respectively. The phylogenetic tree of four local isolates (Fig. 2) has been obtained by Sea view software in which the common type strains were selected.

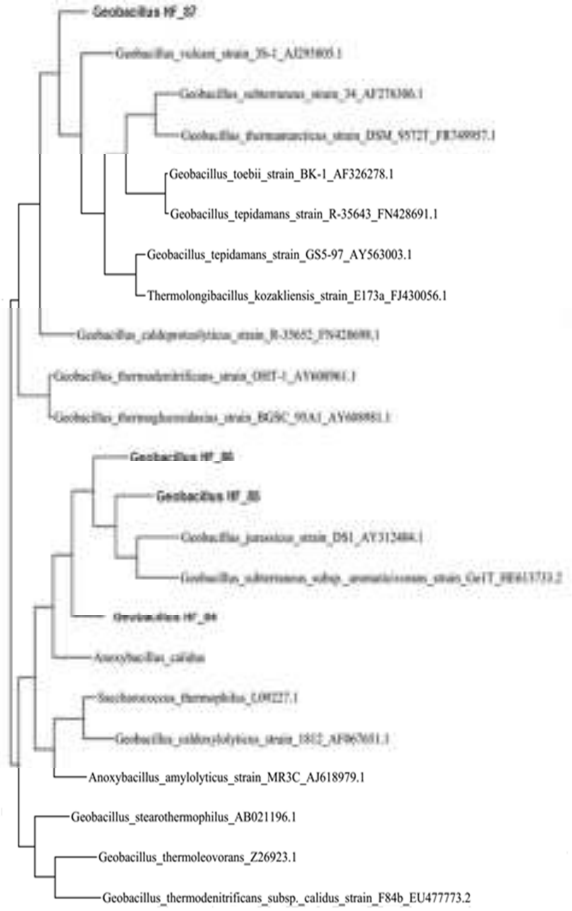

Fig. 2. Phylogenetic tree of the four isolates showing the relationship with other members of the genus *Geobacillus* upon the analysis of the 16S rRNA gene sequence and analysis.

DISCUSSION

Out of 29 isolates obtained from Hammam Pharaon in Egypt. Among them, four isolates (HF_84, HF_85, HF_86 and HF_87) have been further characterized and identified on basis of their higher ability to hydrolyze starch, CMC, protein and lipids. In terms of phenotypic, physiological and biochemical characters that have been confirmed by 16s rRNA analysis, these isolates have been identified as *Geobacillus*(Logan *et al.*, 2009). Strains HF_84 The isolate HF_84 that is evolutionary relevant to *Geobacillus caldoxylolyticus* (96.7 % identity)(Fortina *et al.*, 2001), HF_85, HF_86 showed very high sequence identity to *Geobacillus thermoglucosidasius* (97.9 -96.3 % identity) respectively(Suzuki *et al.*, 1983; Nazina *et al.*, 2001), and HF_87 showed high sequence similarity to *Geobacillus vulcani*(94.4 % identity)(Nazina *et al.*, 2004).

Since 2001, the thermophilic *Bacillus* of group 5 were reclassified to be members of Geobacillus that is characterized by optimal growth at 45-70°C(McMullan *et al.*, 2004). For this group, Geobacillus (Bacillus) stearothermophilus was regarded as the type strain (Nazina *et al.*, 2001).At present, this genus includes several species such as *Geobacillus stearothermophilus*(Nazina *et al.*, 2001), *G. thermocatenulatus*(Golovacheva *et al.*, 1975) and *G. thermoleovorans*(Zarilla and Perry, 1987).

The obtained data for optimum temperature and pH required for growth of the obtained Geobacillus strains indicated that RSM could not only be used for optimization of medium components in the fermentation process (Puri *et al.*, 2002) but also for studying the combined effects of culture parameters(Zambare, 2011). The data obtained for the optimum temperature (60 - 70°C) and pH (6-7) agrees well with the data collected about the temperature and pH of the site of collection supporting that the obtained isolates are thermophilic bacillus (Geobacillus) adapted to live under the conditions of Hammam Pharaon.

From the results represented in this study, it could be concluded that the most attractive attributes of thermopiles is their production of thermoactive and thermostable enzymes which are required for several industrial processes such as α-amylase, cellulase, protease and lipase. Such enzymes have been employed in several industrial processes. Several enzymes which are promising enzymes regarding their characteristics particularly thermal stability and optimum pH. Further studies are of our consideration now to optimize its production from the parent strain to pave the way for its industrial commercialization.

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عزل و تعريف اربع سلالات من جنس *Geobacillus* من حمام فرعون - مصر

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استهدفت هذه الدراسة عزل وتعريف سلالات بكتيرية محبة لدرجات الحرارة العالية من البيئات المحلية و لها القدرة علي إنتاج انزيمات متحملة لدرجات الحارة العالية مثل الالفا اميليز و السيليوليز و البوتيز و الليبيز. تم عزل 29 سلالة من حمامات فرعون وقد تم إختيار اربعة منها لقدرتها علي انتاج الانزيمات سالفة الذكر بنشاط عالي. تم تعريف هذه السلالات علي المستوي الجزيئى حيث وجد انهم جميعا من جنس *Geobacillus* وكانت هذه السلالات مختلفة من حيث الشكل و الخواص البيوكيميائية مثل تكسير النشا و تحليل الجيلاتين و غيرها و كذلك كانت مختلفة فسيولوجيا فهي تنموا في درجات حرارة من 50 الي 90 ودرجة حموضة من 5-9. وختما فان هذه الدراسة تقدم معلومات هامة عن البكتريا ال محبة لدرجات الحرارة العالية من حمامات فرعون لما لها من خصائص تعطيها اهمية في العديد من التطبيقات البيوتكنولوجية.