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Aspergillus niger; Aspergillus terreus HDJZ-ZWM and Paecilomyces variotii as polyextremophilic fungi isolated from Egyptian soil

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Abstract

Three polyextremophilic fungal isolates were isolated from agriculture soil at Mitsalsil, Dakahlia Governorate, Egypt. They tolerated temperature till 45 ºC and pH range from (1-14). They fully identified morphologically and genetically as *Aspergillus niger; Aspergillus terreus* HDJZ-ZWM and *Paecilomyces variotii*. The three fungal isolates secreted cellulase enzyme at neutral and acidic conditions also at different temperature degrees (28 ºC and 45 ºC). Lipase enzyme not secreted by *Aspergillus terreus* while secreted in neutral condition at 28 ºC and 45ºC by *Aspergillus niger* and at 45 ºC in acidic pH by *Paecilomyces variotii*. All fungal isolates secreted amylase at acidic pH at 45 ºC. Protease was secreted at all pH and temperatures used by *Paecilomyces variotii*, while secreted in neutral condition at 45 ºC by *Aspergillus niger* and at 28 ºC and 45 ºC by *Aspergillus terreus.*

Keywords: Filamentous fungi; Acidophilic microorganisms; Thermotolerant microorganisms; Extracellular enzymes; polyextremophiles.

1. Introduction

Microorganisms that are extremophilic can survive and even thrive in hostile settings. Thermophiles, acidophiles, psychrophiles, alkaliphiles, halophiles, piezophiles, barophiles, metalophiles, and radiophiles are among the functionally diverse extremeophiles, which are taxonomically widespread [1]. Organisms classified as extremophiles have evolved to live in ecological niches that are hostile to other species. Inland saline systems, soda lakes, solar salterns, cold and hot deserts, deep-sea hydrothermal vents, hot springs, solfataric fields, rock or lithic settings, and deep-sea saline systems are a few examples of such ecosystems [2]**.** Two or more extreme situations nearly always have an impact on extreme settings. There are several extremes present in many terrestrial and interplanetary ecosystems. Research on polyextremophiles has the potential to establish the boundary of habitability by imposing both theoretical and empirical restrictions on biological processes because all organisms exist somewhere in a multidimensional niche space [3,4]. Acidophiles are often suited to situations with high temperatures, high salinity, or heavy metal concentrations in addition to adaptations for acidic conditions because these factors regularly co-occur, for example in areas of acid drainage [5,6]**.** Thermophiles and psychrophiles are those who are sensitive to heat. Thermophiles are further broken down into the moderate, extreme, and hyperthermophile categories based on the temperature ranges they enjoy. The moderate thermophiles have an optimal growth temperature of 40 to 60 °C. There are acidophiles and alkaliphiles in terms of pH [7]**.**

Extremophiles are being studied more and more; a number of them have been acquired in pure culture, had their genomes investigated, and had their enzymes defined in university or commercial labs [8,9]. Extremozymes, which are enzymes that have evolved molecular mechanisms of adaptability to extreme physicochemical circumstances, can be used in biotransformation processes as an industrial biocatalyst in addition to whole microbial cells

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[10]. Thermozymes are extremozymes made by microorganisms that are thermophilic and hyperthermophilic. Additionally, these enzymes are frequently able to withstand proteolysis, severe environments, the presence of denaturing agents, high salinity and organic solvents. The use of thermozymes has advantages such as lowered contamination risk, decreased viscosity, and increased substrate solubility [2]**.** The study was aimed to isolate and characterize some thermoacidophilic fungi. It is worth to mention that there is no much more work was done on thermoacidophilic fungi especially in Egypt.

2. Materials and methods

2.1. Soil Sample

A soil sample was collected from canal at Mitsalsil, Dakahlia Governorate, Egypt.

2.2. The physical and chemical characteristics of soil sample:

The physical and chemical characterization of soil sample were carried out at Desert Research Center, central laboratory (water and soil analysis unit).

2.3. Fungal isolation:

Fungal isolation was carried out by two methods:

2.3.1. Direct cultivation method

Direct inoculation method was made by spreading 0.5 g of soil sample on the surface of Dox peptone agar medium which adjusted at pH 1and pH3 and incubated at 45^{o} C.

2.4. Growth parameters of the isolated fungi:

2.4.1. Influence of different temperature degrees on growth of fungal isolates

Dox peptone agar medium was adjusted (Triplicate sets of Petri dishes) at pH 1 using 2N HCl after autoclaving at 1.5 atm. for 20 minutes. Petri dishes were inoculated at the center and incubated at different temperatures (20,28,40, 45ºC, 50 and 60 ºC) for one week. After incubation period the growth diameters were determined.

2.4.2. Influence of different pH values on growth of fungal isolates

Dox peptone agar medium was adjusted (Triplicate sets of Petri dishes) at different pH values (1, 2, 3, 4,5,7,9,11 and 14) using Na_2CO_3 (2.5%) and 2N HCl, after autoclaving at 1.5 atm. for 20 minutes. Petri dishes were inoculated at the center and incubated at 45ºC±2 for one week. After incubation period the growth diameters were determined.

2.5. Image analysis of fungal isolates:

Image analysis was made at Regional Center for Mycology and Biotechnology Cairo, Egypt by using Olympus microscope X40, and by using Olympus microscope at collage.

2.6. Identification of fungal isolates:

Identification of fungal isolates was carried out morphologically and genetically.

2.6.1. Colonial identification of fungal isolates:

Morphological identification of fungal isolates was made by using media of Czapek-Dox agar, Potato dextrose agar (PDA), Malt extract agar (MEA), Sabouraud dextrose agar(SDA), Rose Bengal Agar (RBA) ,Oatmeal agar(OA) and Czapek Yeast Autolysate (CYA) media according to [11-17] , respectively. All culture media made in triplicates, pH was adjusted at (2 and 7) by using 2.0 N HCl solution after sterilization of media in autoclave at 121 ºC for 20 minutes at 1.5 atm. Fungal cultures were incubated at 45ºC±2 for one week. Colony color, diameter, texture, sulcation, reverse of colony, pigmentation, and exudates were determined for each fungal isolate. This was done by observing the colonies by naked eye.

2.6.2. Genetic identification of fungal isolates:

Genetic identification of thermoacidophilic fungal isolates were carried by analyzing 18S rRNA full sequencing according to **[18]** at Macrogen Company for Humanic Genomics by using primers, Korya:

Target (18SrRNA), Primer Name NS1, Forward, 5' (GTA GTC ATA TGC TTG TCT C) 3' and Primer Name NS8, Reverse, 5' (TCC GCA GGT TCA CCT ACG GA) 3'.

Phylogenetic analysis

The UPGMA algorithm was employed to infer the evolutionary history [19]**.** The ideal tree was displayed with a branch length sum of 0.01341364 (next to the branches). The Maximum Composite Likelihood technique was used to calculate the evolutionary and are in the units of the number of base substitutions per site distances [20]. The analysis involved 6 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. Positions with gaps and missing data were all removed. The final dataset contained 386 locations altogether. In MEGA7, evolutionary analyses were carried out [21]**.**

2.7. **Screening the secretion of some extracellular enzymes by the polyextremophilic fungal isolates at different conditions.**

The fungal isolates were examined for their ability to produce extracellular enzymes, protease, amylase, lipase and cellulase. Enzymes production was determined on solid media. To visualize the enzymatic activity, each enzyme substrate was added to the culture medium as a carbon source or nitrogen source. After inoculation and incubation of culture for 4 days depending on the enzyme and growth rate of the isolate the appearance of a clear halo or precipitation around the fungal growth indicates enzyme production.

2.7.1. **Amylase activity** was determined using nutrient agar medium supplemented with 20.0 g/L of soluble starch. After incubation period, the cultures were submerged with iodine solution. The presence of amylase is shown by the formation of a clear zone around the fungus [22].

2.7.2. **Cellulase activity** was evaluated on medium supplemented with (g/L) of KH_2PO_4; 2.0, K_2HPO_4; 0.1, $MgSO_4$ 7 H_2O; 1.0, $(NH_4)_2SO_4$;0.6, yeast extract 0.6, 10 of microcrystalline cellulose and 15.0 agar. At the end of the incubation time, the cultures were incubated at 50 ºC for 16 h to accelerate the action of the enzyme [23]. The cultures were then submerged with 5 ml of iodine and rinsed with distilled water to visualize the hydrolysis zone [24].

2.7.3. **Protease activity** was determined on milk agar medium containing 30% skim milk and 2% agar. After incubation, the degradation of casein was determined by a clear zone around the thallus [25]**.**

2.7.4. **Lipase activity** was detected on culture medium containing (g/L), 10.0; peptone, 5.0; NaCl, 0.1; $CaCl_2$ $2H_2O$, 17 agar and 10 mL/L Tween 80). Tween 60 was first added to the sterile medium after being separately sterilised. The cultures were placed at 4 ºC for 12 hours after the incubation period to help observe the formation of an opaque precipitation around the thallus [22].

2.7.5. **Enzymatic Index:**

The enzyme activity was evaluated by an enzymatic index (EI). Fungal isolates with an EI equal to or greater than 2 are considered as good producers of the studied enzyme [22]**.**

$$(EI) = R/r$$

Where: R: being the diameter of the zone, r: the diameter of the fungal growth.

2.8. **Statistical analysis** All obtained data were calculated as means ± SD. Multiple comparisons was performed by the Tukey-Kramer multiple-comparisons test with GraphPad InStat 1992–1998.

3. Results

3.1. **Chemical characteristics of soil sample:**

Table (1) contains the physical and chemical analysis of soil used in the isolation of thermoacidophilic fungal isolates. The soil sample was collected from canal at Mitsalsil, Dakahlia Governorate, Egypt.

The hydrogen ion concentration of the sandy clay soil sample tends to be alkaline (pH 8.02). This soil contains high amounts of calcium, bicarbonate, and sulfate (3.214,2.240, 1.904) meq/L respectively, while magnesium, sodium, potassium and chlorine recorded (0.460 ,0.435 ,0.230 and 0.200 meq/L) respectively. The electrical conductivity was 0.401 ds/m. Nil amount of carbonate was detected in the soil sample. The soil contains total dissolved salts as (235 mg/L).

Table 1: Physical and chemical properties of soil sample used in the isolation of thermoacidophilic fungi.

Physical texture	pH	E.C. Ds/m	(TDS) mg/l	Soluble Cations meq/L				Soluble anion meq/L			
				Ca++	Mg++	Na+	K+	CO3--	HCO 3-	CL-	SO4
Sandy clay	8.02	0.401	235	3.214	0.460	0.435	0.230	0.000	2.24	0.20	1.904

E.C.: electrical conductivity, TDS: total dissolved salts, meq/L= ml equivalents/L

3.2. Growth parameters of the thermoacidophilic fungal isolate.

3.2.1. Influence of different temperature degrees on growth of fungal isolates.

Figure (1) revealed the effect of different temperature degrees on the growth of fungal isolates represented by colony diameter(mm) that grown at acidic medium (pH 1). From the results it was appeared that, all fungal isolates were able to grow at temperature degrees 20, 28, 40 and 45 ºC. The optimum temperature for isolate No. 3 was 45 ºC while that of isolate No.5 was at 40 ºC, and for isolate No. 9 was 28- 40 ºC, the fungal isolate No. 5 had good growth from 28-45 ºC. All fungal isolates couldn't grow at 50 and 60 ºC.

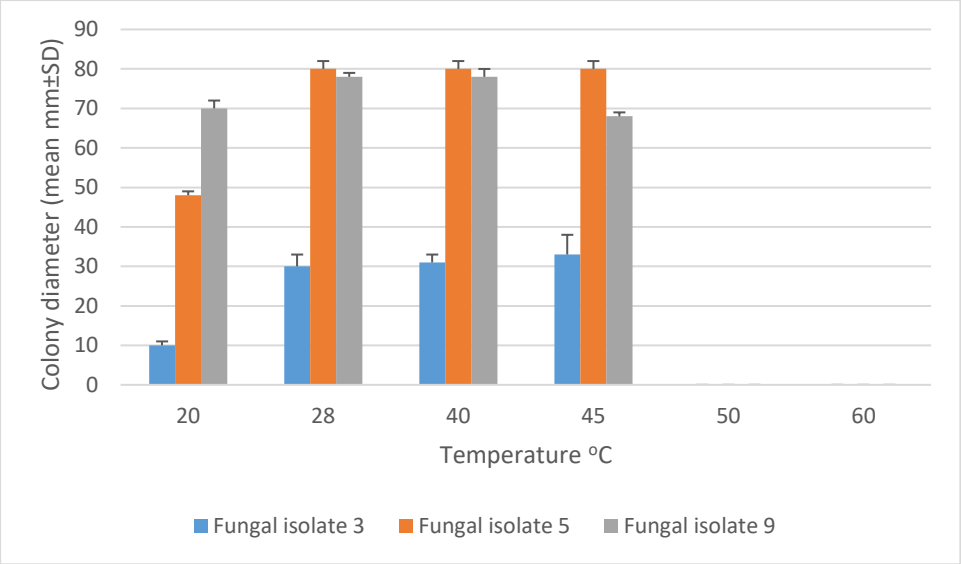

Fig. 1. Influence of different temperature degrees on growth of thermoacidophilic fungal isolates.

3.2.2. Influence of different pH values on growth of fungal isolates.

The effect of different pH values on the growth of fungal isolates (that were cultivated at 45 ºC) was determined (Fig. 2). It was appeared that all fungal isolates were able to grow at wide range of pH values from 1 to 14 except the fungal isolate No. 9` that grown at pH from 1 to 11. The optimum growth of fungal isolates was obtained at pH 7, 5 and 4 for isolate No. 5 (51 mm), isolate No.5 (88mm) and isolate No.9 (90 mm), respectively.

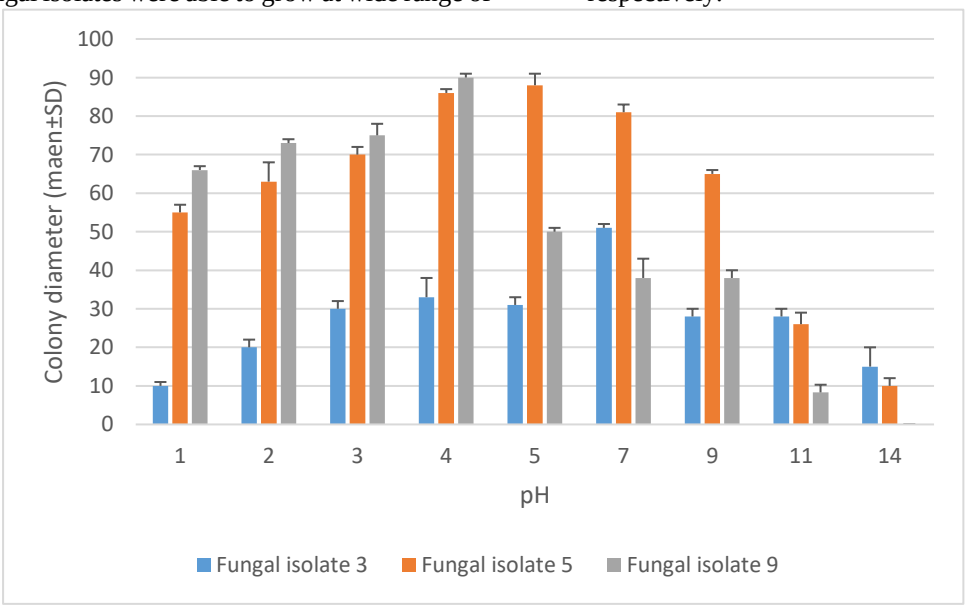

Fig. 2. Influence of Different pH on growth of isolated thermoacidophilic fungal isolates.

***3.3.* Image analysis of fungal isolates:**

Table (2) demonstrated the statistics of image analysis of the fungus and fig. (3) appears the fungal image at pH 1 and 45°C. Hyphae were septate and hyaline. Conidial heads are biseriate, containing metula that support phialides and columnar vesicle. Smooth-walled and hyaline conidiophores typically end in globose vesicles. Conidia are globose, spherical, and tiny. On submerged hyphae, globose, hyaline, sessile accessory conidia were frequently generated. All these characteristics resemble the *Aspergillus terreus*.

Table 2: The statistics of image analysis of the fungal isolate No. 3 at pH 1 and 45°C.

Statistical Function	Conidiophore diameter	vesicle length	Vesicle width	1st sterigmata length	1st sterigmata width	2nd sterigmata length	2nd sterigmata width	conidia diameter
Mean± S D	6.19±0.96a	14.14± 1.39b	14.63± 0.74b	6.50± 1.15a	1.37± 0.26c	6.27± 0.70a	1.23± 0.27c	1.91± 0.55c

Mean±SD followed with the same letter was not significant P > 0.05, mean±SD followed with the different letter was significant P < 0.05

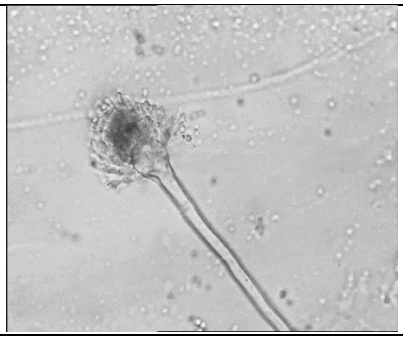

Fig. 3. Image (X40) of fungal isolate No.3 at pH 1 and temperatures degree 45 ºC.

Table (3) and Fig. (4) represented the statistics and image analysis of the fungal No. 5 at pH2 and temperature 45 ºC. Hyphae are septate and hyaline. Conidial heads were radiate initially, splitting into columns at maturity. The biseriate vesicle creates phialides. Conidiophores were long, smooth, hyaline, and get darker at the tip until ending in a globose vesicle (36.462 µm in diameter). Metulae and phialides (width 2.29 ± 0.47 and length 11.63 ± 1.35 µm) cover the entire vesicle. Conidia were brown to black, globose and very rough. The data of this fungus revealed that it resembles *Aspergillus niger*.

Table 3: The statistics of image analysis of the fungal isolate No. 5 at pH 2 and 45°C.

Statistical Function	Conidiophore width µm	Vesical diameter µm	Phialide length µm	Phialide width µm	Conidia diameter µm
Mean ±SD	8.63 ± 0.77a	36.46 ±2.00b	11.63 ± 1.35a	2.29 ± 0.47c	3.44 ± 0.48c

Mean±SD followed with the same letter was not significant P > 0.05, mean±SD followed with the different letter was significant P < 0.05

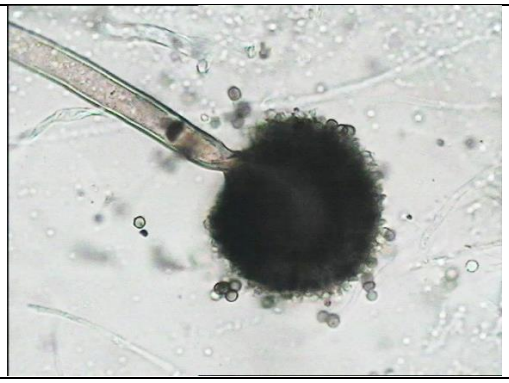

Fig. 4. Image (X40) of fungal isolate No.5 at pH2 and temperature of 45°C.

In fungal isolate No. 9, the conidiophores, which resemble irregularly branching brushes and bear the conidia, have tapering phialides with pointy tips. Conidia are single-celled, hyaline, lemon-shaped and positioned terminally in chains. Table (4) and Fig (5) represented the statistics analysis and the image of fungal isolate No. 9. This feature resembling *Paecilomyces variotii*.

Table 4: The statistics analysis of the images of fungus No. 9 at pH 2 and 45°C.

Statistical Function	Conidia length	Conidia width	Phialide length	Phialide width	Conidiophore length
Mean±SD	5.13±0.80a	2.29±0.56b	13.26±1.25c	2.28±0.58b	5.50±0.74a

Mean±SD followed with the same letter was not significant P > 0.05, mean±SD followed with the different letter was significant P < 0.05

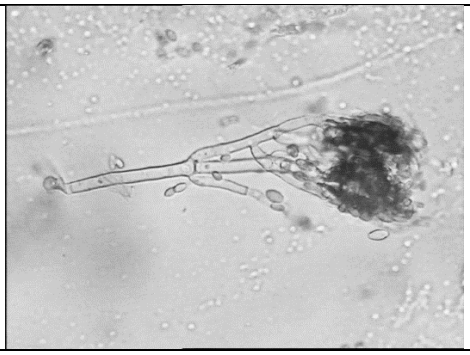

Fig. 5. Image (X40) of fungal isolate No. 9 at pH2 and temperature degree 45 ºC.

3.4. Colonial characterization of the fungal isolates:

The colonies of fungal isolate No. 3 on SDA were funiculose yellow in center and buff creamy at margin at pH 7 while they were funiculose white at pH 2. On DAM, PDA and OMA at pH 7 the colony was leathery to cottony creamy in center (tend to be cinnamon color) and with white margin. On MA and OMA at pH 7, it has flat powdery to leathery colony with yellowish buff brownish color. The colony of the fungus at pH 2 has leathery funiculose white creamy colony with grove sulcations on all media used (Plate 1).

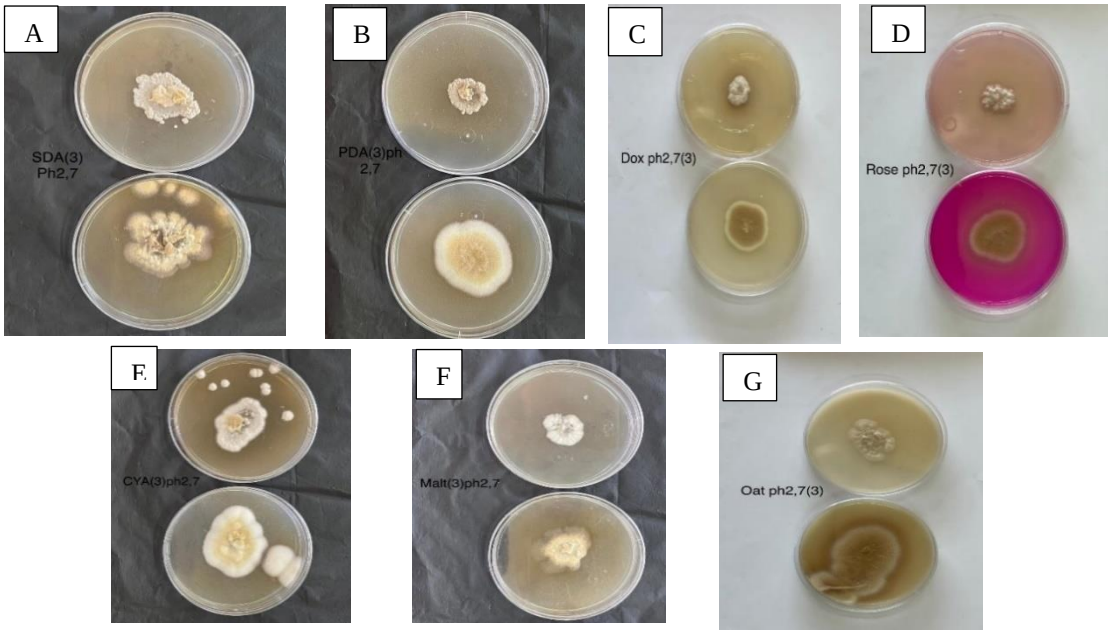

Plate 1. The colonial growth of the fungal isolate No. 3 at different pH values(2&7) and at 45 ˚C on different media. where A: SDA medium, B :PDA medium ,C: DOX medium, D: Rose Bengal Agar medium E: CYA medium, F: Malt extract and G : OAT medium.

The colony of the fungal isolate No. 5` was large black color at pH7 on SDA and Dox peptone while at pH 2 the colonies were spetmaller and delay the sporulation to some time and then became dark after sporulation. On PDA ,CYA ,OMA and MEA at pH 7 the fungus had dark grenish colonies , dark greenish black large colonies at pH 7 while at pH 2 on OMA it had white creamy fluccose small colony and on MEA it had dark green fluccose small colony. At pH 2 on CYA and on PDA the colonies were powdery dark grenish.The fungus had small green color colonies at all pH used (Plate 2).

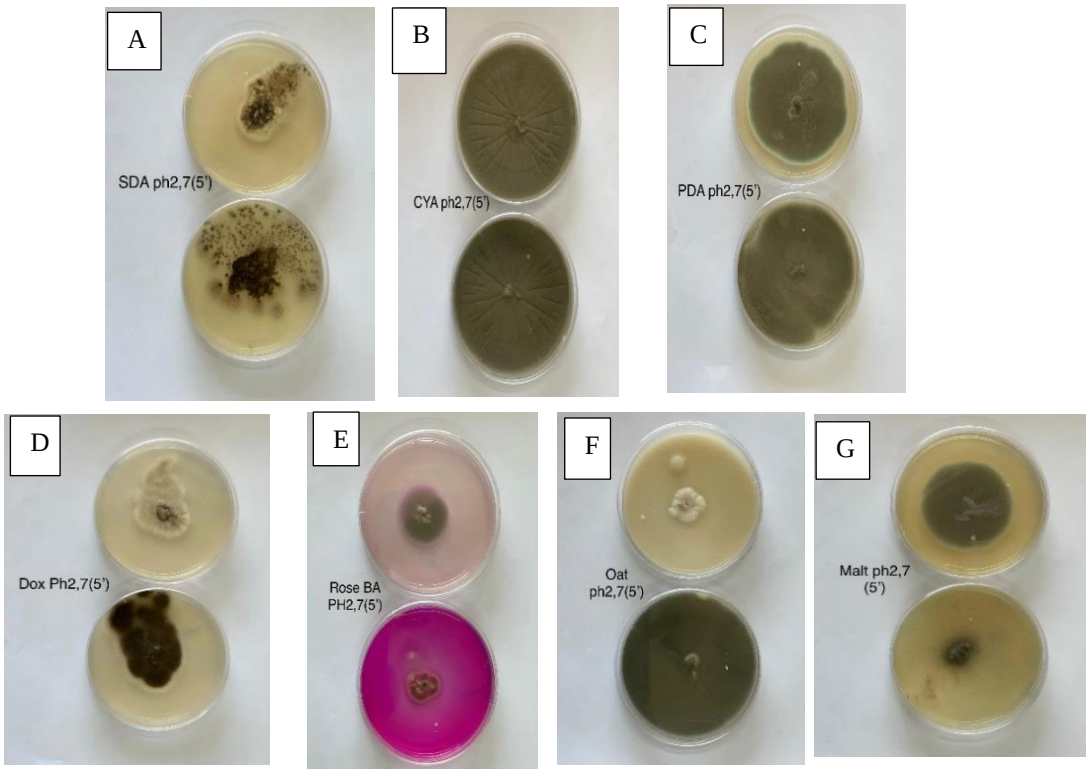

Plate 2. The colonial growth of the fungal isolate No. 5` at different pH values(2&7) and at 45 °C on different media . where A: SDA medium, B : CYA medium ,C: PDA, D: DOX medium E: Rose Bengal Agar medium, F: OAT and G : Malt extract medium.

The fungal isolate No. 9 was characterized by faster growing yellowish greenish colonies from pH 2 and pH 7 at 45 °C on Dox peptone medium. On PDA; MAM and on SDA media it has white floccose sulcation colonies at pH 2 while it had yellow ochraceous colonies at pH 7 on these media. On RAM and OMA media it had greenish powdery colony at pH 7 whereas it had yellow greenish colony at pH 2 on RAM it had yellowish white colony at pH 2 on OMA (Plate 3).

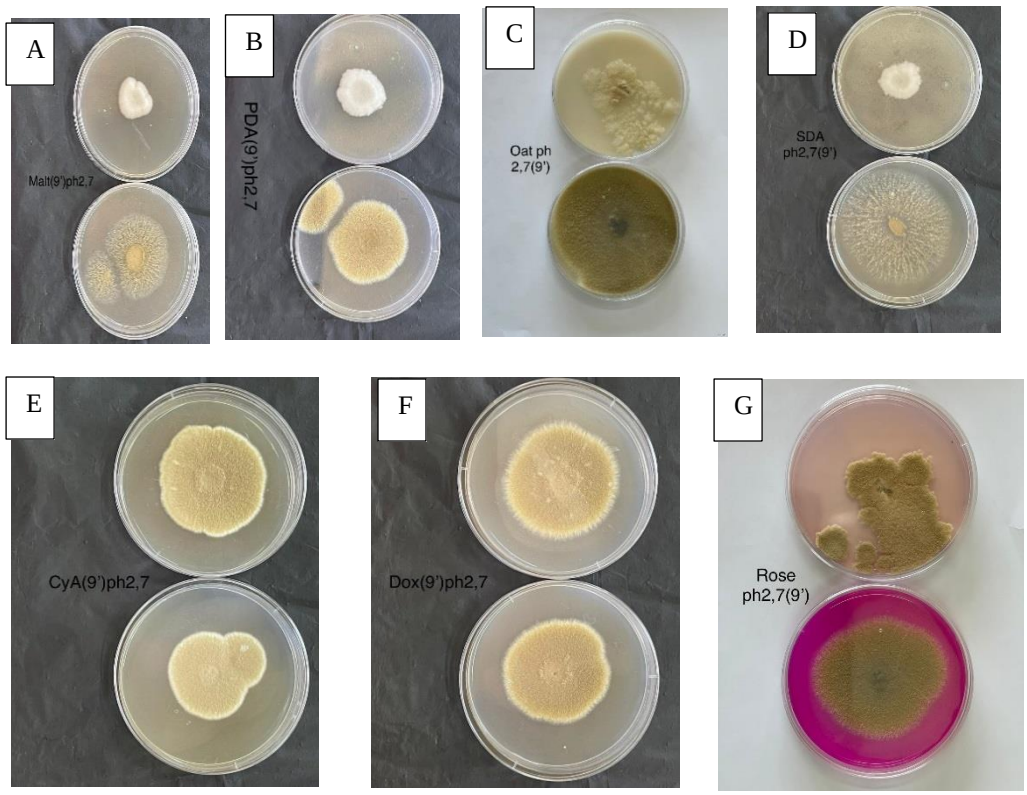

Plate 3. The colonial growth of the fungal isolate No. 9` at different pH(2&7) and at 45 °C on different media . where A: Malt extract medium, B : PDA medium ,C: OAT medium, D: SDA medium E: CYA medium, F: DOX medium and G : Rose Bengal Agar.

3.5. Genetic identification of the fungal isolates.

Table (5) showed the BLAST results of the three fungal isolates which reveals that, the black isolate (5`) was related to *Aspergillus niger* by identity 99 %, the yellow ochraceous one (3) was related to *Aspergillus terreus* HDJZ-ZWM by identity 99 % and the yellowish green fungus(9`) was related to *Paecilomyces variotii* with 99 % identity. So, the morphological identification agreed with the phylogenetic identification. The isolates were blasted, and based on % similarity, the closest neighbors in the data bank were identified. The isolates were all dispersed across three taxa and grouped together within the phylum Ascomycota: two *Aspergillus* and one *Paecilomyces.* The constructed phylogenetic tree indicated the phylogenetic position of the isolates (Fig. 6). The bootstrap (evolutionary distances) values suggested that the various isolate strains were connected to known strains from Genebank in a close manner. To validate the strong resemblance between the isolates, the bootstrap values were connected to morphological traits.

Table 5: Results obtained from BLAST showing the close relative to each fungal isolate.

Query	Subject					Score		Identities		Accession No	Name of fungal isolate
Name	AC	Gene	Length	Start	End	Bit	Raw	Match	Pct.(%)		
H210908-020_A05_A_NS1	KF562841.1	*Aspergillus sp.* F13 18S ribosomal RNA gene, partial sequence	1093	16	1067	1925	1042	1049	99	ON705118	*Aspergillus terreus* HDJZ-ZWM ribosomal RNA gene, partial sequence
H210908-020_C05_A_NS24	KM462826.1	*Aspergillus terreus* isolate ATE1 small subunit ribosomal RNA gene, partial sequence	1708	1658	586	1969	1066	1071	99		
H210909-035_A01_B_NS1	KP036601.1	*Aspergillus niger* strain AN0512 18S ribosomal RNA gene, partial sequence	1669	8	997	1821	986	989	99	ON705126	*Paecilomyces variotii* ATHUM:8891 ribosomal RNA gene, partial sequence
H210909-035_C01_B_NS24	KY825168.1	*Aspergillus niger* strain ANTS 18S ribosomal RNA gene, partial sequence	1713	1696	696	1823	987	997	99		
H210908-020_E05_C_NS1	AY526473.2	*Talaromyces spectabilis* strain CBS 101075 18S small subunit ribosomal RNA gene, partial sequence	1729	12	1065	1929	1044	1051	99	ON705120	*Aspergillus niger* LA small subunit ribosomal RNA gene, partial sequence
H210908-020_G05_C_NS24	KU376436.1	*Paecilomyces variotii* culture-collection ATHUM:8891 18S ribosomal RNA gene, partial sequence	1670	1665	577	2001	1083	1088	99		

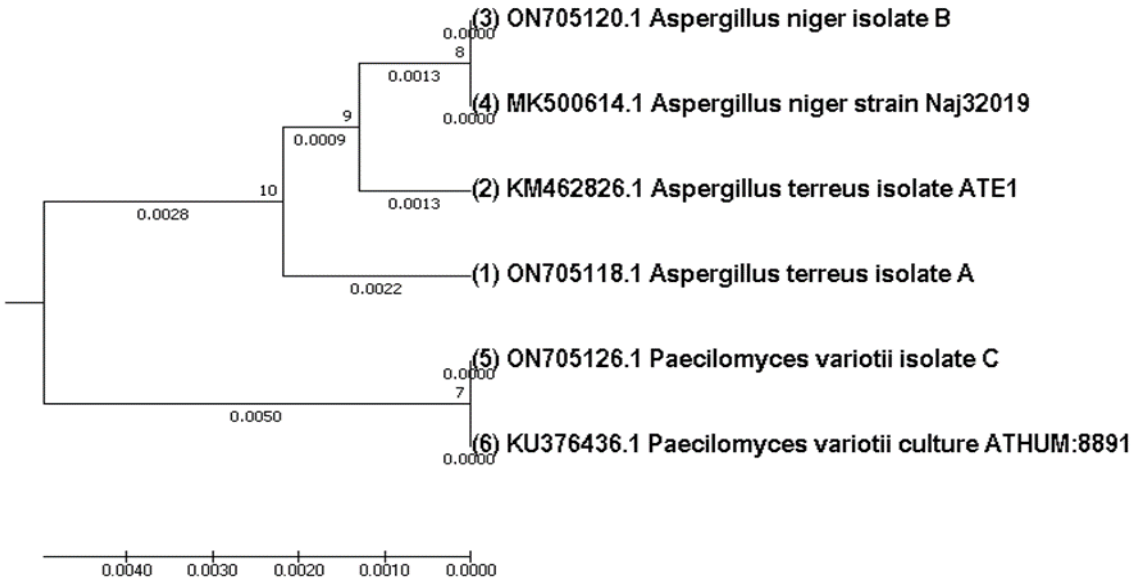

Fig. 6. Rooted Phylogenetic tree created using Neighbor-joining method and is based on a comparison of the 18S ribosomal DNA sequences of three fungal isolates and their closest phylogenetic relatives.

3.6. Screening the secretion of some extracellular enzymes by the polyextremophilic fungal isolates at different conditions.

The three fungal isolates secreted cellulase enzyme at neutral and acidic conditions also at 28 ºC and at 45 ºC. Lipase enzyme was secreted in neutral pH at 28 ºC and at 45 ºC by *Aspergillus niger* and at pH 7 and 45 ºC by *Paecilomyces variotii*. *Aspergillus niger* secreted amylase on acidic conditions at 28 and 45 °C, while it was secreted by *Aspergillus terreus* at 45 °C in acidic and neutral pH, whereas that of *Paecilomyces variotii* was secreted at 45 ºC at acidic pH. *Paecilomyces variotii* secreted protease enzyme in all conditions of pH and temperatures used (Fig. 7). *Aspergillus terreus* secreted protease at 28 and 45 °C in neutral pH only while that of *Aspergillus niger* was secreted at 45 °C in neutral pH.

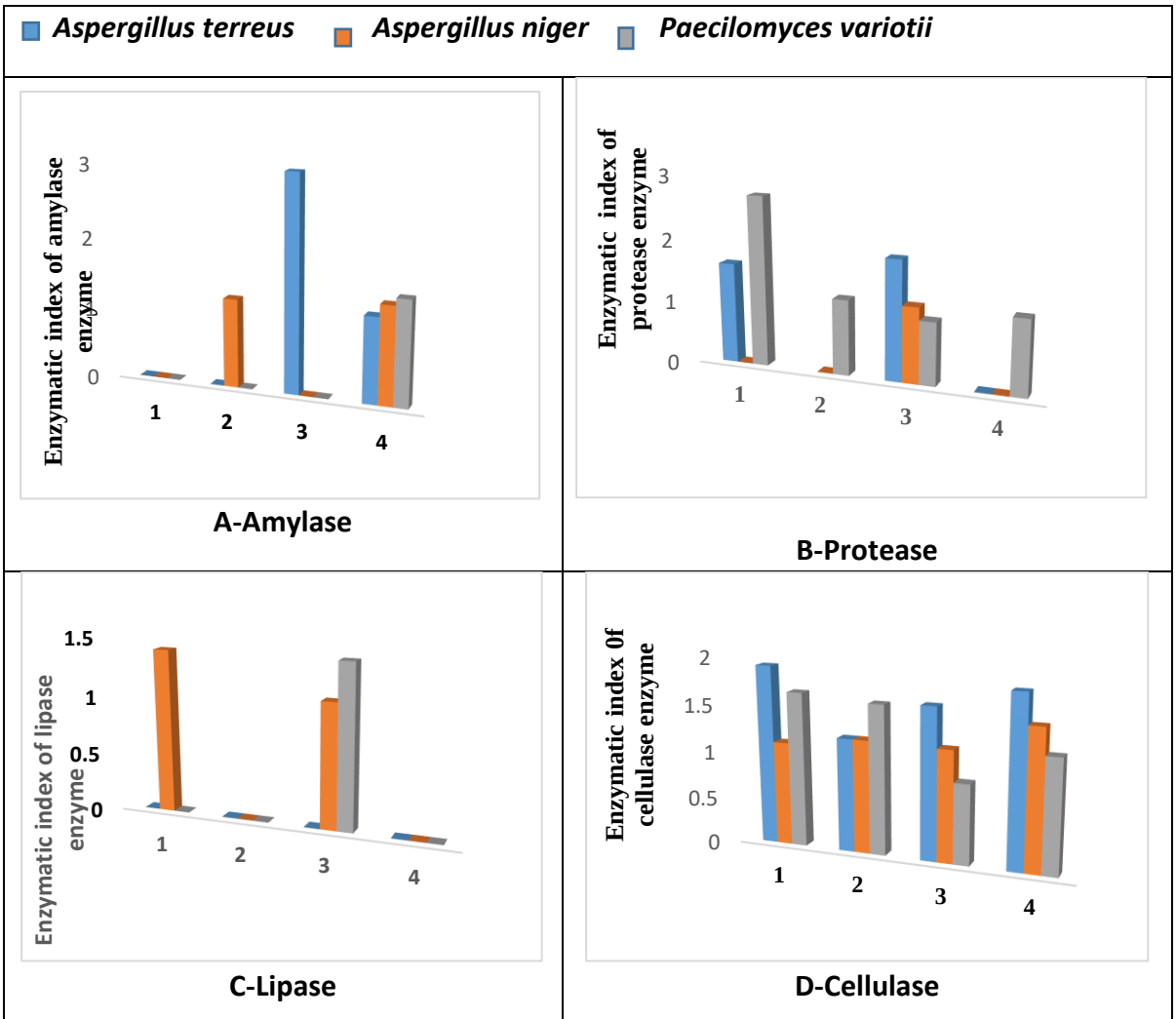

Fig. 7. Enzymatic index of extracellular enzymes of thermoacidophilic fungal isolates at different pH values and temperature degrees. Where: 1= At 28 ^{O}C and pH 7, 2 = At 28 ^{O}C and pH 2, 3 = At 45 ^{O}C and pH 7, and 4 = At 45 ^{O}C and pH 2

4. Discussion

In the current study, all fungal isolates couldn't grow at 50 and 60 ºC. [26]_isolated thermophilic species grew more quickly at 45 °C than at 34 °C, while thermotolerant species grew better or equally well at 34 °C than at 45 °C., so fungal isolates No.(3 and 5`) were considered as thermotolerant while fungal isolate No. 9` was considered as thermophilic. It has been observed that thermophilic fungus may thrive at temperatures as low as or higher than 20 °C and as high as 60 to 62 °C [27]. **[28]**

isolated 15 thermophilic fungi from soil and investigated their growth at 50 °C.

According to [29], who showed that microorganisms that live in continuously acidic conditions are referred to as "acidophiles," some are extreme acidophiles that normally grow at pH 3, and others are moderate acidophiles that grow best in the pH 3-5 range. All fungal isolates are considered as moderate acidophiles.

All the isolates clustered within the phylum Ascomycota and were distributed in two genera: *Aspergillus* and *Paecilomyces.* **[26]**, the only orders known to contain Candida thermophilic fungi are the Sordariales, Eurotiales, and Onygenales in the Ascomycota and the Mucorales. However, there may be another order that does. The Basidiomycota's thermophilic species were not supported by any evidence. *Aspergillus, Trichoderma*, and *Penicillium* are the genera that are most frequently used to produce hydrolytic enzymes by microorganisms [30].

Extracellular enzymes (amylase, protease, lipase and cellulase) of thermoacidophilic fungal isolates were produced in the present study depending on temperature and pH values. A novel *Aspergillus terreus* strain M11 was identified by [31] from compost that included cellulose**.** The isolate grows best at 45℃ and pH2.0. It was found that the activity of the CMC ase was up to 3.680IU/mL with high heat stability and the optimal reaction conditions of the CMCase were at 60℃ and pH2.0.

It may be especially beneficial to use enzymes from microorganisms that can live in extremely acidic environments. The ability of acidophilic microbes to maintain a neutral pH internally, however, is one of its distinguishing characteristics. As a result, the intracellular enzymes from microorganisms do not need to be adapted to harsh growing environments [32]**.**

A few thermophilic fungus extracellular enzymes are made commercially, and a few others may follow. Pure crystalline proteins have been generated by cloning and overexpressing the genes for the lipase, protease, xylanase, and cellulase from thermophilic fungi in heterologous fungi in order to better understand the mechanisms underlying their inherent thermostability and catalysis [27]**.**

Thermostable enzymes are required for industrial use because commercial procedures need for high temperatures. Consequently, the need for thermophilic and thermostable producers of cellulolytic and hemicellulolytic enzymes is growing, especially for those that are extremely stable and resistant to product inhibition [33,34].

Several starch-hydrolysis enzymes, such as amylases, glucoamylases, pullulanases and glucosidases, that are active at acidic pH were isolated by [35]**. [36]** used ragi husk, an agricultural waste product, as a substrate for the microbial fermentation to produce cellulase by thermo-acidophilic filamentous fungi *Aspergillus fumigatus* JCM 10253., The thermo-acidophilic fungus *Aspergillus terreus* M11 demonstrated high-level cellulase activity at pH 3, and the ideal temperature range for cellulase synthesis under SSF was achieved at 35-45 °C. The optimal cellulase activities were obtained at 50 °C at pH 2-4 [37]**.** *Comparing different microbes, Aspergillus terreus has been found to produce the most cellulase* [38]. At 45 °C, cellulase production was at its highest [27]**.** Numerous *Aspergillus* species have been reported to produce cellulase [39,40]**.** Based on hollow zones around colonies, **[28]** screened fifteen thermophilic fungi for their activity of hemicellulase, cellulase and lignin degradation. They also investigated *Aspergillus fumigatus* JCM 10253 as a potential producer of extracellular enzymes. According to [41], an *Aspergillus* sp. soil isolate had the highest lipase activity, and the lipase production peaked at pH 5.5 and 30 ℃. While [42] reported that *Aspergillus* strain lipases are active at pH values between 4 and 7 and between 40 and 50°C.*Thermomyces lanuginosus* produces extracellular amylase in solid state fermentation (SSF), according to research by [43]. They discovered that the enzyme activity was highest at 50 °C and pH 6.0. Protease synthesis by nine different thermophilic fungi was examined by [44] in solid-state fermentation (SSF) and submerged fermentation (SmF)**.**

5. Conclusion

The following work exhibited that fungal isolates, *Aspergillus niger; Aspergillus terreus* HDJZ-ZWM and *Paecilomyces variotii* was thermoacidophilic fungi. Also, extracellular enzymes (amylase, protease, lipase and cellulase) of these fungal isolates were produced depending on temperature and pH values. Some of the uses of these fungal isolates or their enzymes as the involvement of acidophiles in biomining of metals from low-grade ores as a result of their enzyme synthesis has various applications in the food sectors.

6. References

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