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A Study on Soil Thermophilic and Thermotolerant Fungi in Thamar-Yemen

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الملخص: جمعت 38 عينة تربه من مواقع مختلفه في محافظة ذمار/ اليمن وذلك للفترة من 2012/4 -2013/2. اتبعت طريقتي الزرع المباشر والتخافيف لعزل الفطريات وحساب الوحدات المكونة للفطريات وذلك باستعمال وسطي آكر البطاطا والجزر وأميرسون. عُزل 23 فطارً تعود لـ14 جنساً تشمل 11 فطرا ناقصاً و 10 فطريات كيسيه وفطرين لاقحيين فضلاً عن الخيوط الفطرية العقيمة السوداء والبيضاء. اربعة عشرة عزلة فطريه منه عُدت تسجيلا جديدا للمجتمع الفطري اليمني هي **Acremoniummurorum, Acrophialophorafusispora, Chaetomiumatrobrunneum, C.gracile,C.strumarium ,C.therophilevar.coprophpile ,Corynascussepedonium ,Emericellarugulosa, Melanocarpusalbomyces , paecelomycesinflatus Papulosporathermophila, Rhizomucormiehi, Scytalidiumthermophilum, and Talromycesthermophilus.** و ظهرت الوحدات المكونة للفطريات عددا اعلى اثناء الاشهر المعتدلة الحرارة والرطبه (36667) مما في الاشهر الجافه (20400)وكانت الفروق معنويه عند مستوى اقل من 0,05 بينما كانت النسبة المؤية للتماثل الكلي بين الاجناس الفطريه عاليا بين الفترتين (78,57%) . اظهرت نتائج اختبار النشاط الانزيمي للفطريات المعزولة قابلية متفاوته في لانتاج السليوليز، الاميليز، اللاييز، البروتيز، الزايلينيز، والفينوال اوكسيديز فيما اعطت عزلات **Corynascus sepedonium, Eemericellane dulansand Malbranchea cinnamomea .** نتيجة موجبه لجميع الانزيمات .

الكلمات المفتاحية: الفطريات ، محبة للحرارة ، التربة، التذبذب الفصلي ، الانزيمات ، اليمن.

Abstract: Thirty-eight soil samples were collected from different sites in Thamar province during the period from 4/2012 to 2/2013.The direct plate, and dilution plates methods were followed to isolation of counting colony forming units, the potato carrot agar (PCA) and Emerson's media were used A total of 23 fungi belong to (14) genera were isolated, they include (11) Hyphomycetes, (10) Ascomycetes, and (2) Zygomycetes besides black and white sterile mycelium. A fourteen isolates were regarded asnewly record for Yemeni mycobiota,they include Acremonium murorum, Acrophialophorafusispora, Chaetomiumatrobrunneum, C.gracile,C.strumarium, C.therophilevar.coprophpile, Corynascussepedonium ,Emericellarugulosa, Melanocarpusalbomyces, paecelomycesinflatus Papulosporathermophila, Rhizomucormiehi, Scytalidiumthermophilum, and Talromycesthermophilus Soil dilution method showed that the colony forming units (CFUs)in soil had seasonal fluctuation , they were significantly higher (36667) during moderate/humid month than cold/dry months (20400) ($p<0.05$), while the total similarity% is high between fungal genera which were isolated during the two periods (78.57%) .A test of 15 isolates to produce cellulase, amylase, lipase, protease, xylanase and phenoloxidase showed a different activities in number of enzymes and levels of production. Corynascussepedonium, Eemericellanedulans and Malbrancheacinnamomea gave a positive result for all enzymes

Keywords Fungi, Thermophilic, Soil, Enzyme, Seasonal fluctuation, Yemen

1. Introduction

Thermophilic and thermotolerant fungi(TTF) are a group of mycobiota which prefer to grow at high level of temperature more than the other fungi. Thermophilic fungi are grow at or above 20C° as a minimum temp. up to 60-62° C [1]. In 1964 [2] mentioned that the thermotolerant fungi are grow between (20°C to about 55°C). Several studies were attributed about (TTF), In 2007[3] listed (88) of thermophilic fungi belong to ascomycetes, zygomycetes, and deutromycetes and in the same time[4] listed (86) heat tolerant fungi. The ecology of (TTF) were a goal of several studies, they were isolated from soil, composts, piles of hays, stored grains, wood chip piles, nesting material of birds and animals, snuff, and municipal refuse [3,5,6]. Others have been dealt with enzyme activity of (TTF)[1] .

A taxonomic studies on (soil TTF) and their enzyme activity were down in our region[7,8,9,10,11] .

There are a little attention to Yemen mycoflora[12],and the present study is aimed to distinguish the thermophilic and thermotolerant fungi in soil of Thamar-Yemen, and to test their extracellular enzyme activities on solid media.

2. Materials and Methods

Site characterization/ Thamar province lies in middle west of Yemen, it is 1600-3200 meter above sea level. Most of the rains comes down at summer (May-August). Because the sampling area is a flat high plateau, the temperature during the year was not reach (30°C). Data about annual temperature and rains fall were obtain from civil aviation and metrological services/Sanaa.

Total colonies count/Three locations were selected as constant stations to record the fluctuation of colony forming unites of (TTF) in the soil. They include Rasaba (R), university campus (U), and Ellesy (E). From each station and during 4/2012 to 2/2013, soil samples (triplicates) were collected bimonthly in polyethylene bags, then were transferred to the lab, and were kept at 4°C. Each sample was collected from the upper (5 cm) of soil, it was about (100 gm). Dilution plating method was followed to record CFUs [13]. Soil suspension 10^{-2} was prepared,1 ml of the suspension was transferred to 9 cm sterile plates then 30 ml of sterile Emerson's culture medium (before hardness) were poured, the plates were rotated to homogenize the sample. A replicate was used for each sample, and the mean of CFUs numbers were calculated. All plates were incubated at 45°C. A standard method was followed to test soil texture of constant stations (R, U, E), also C% and N% were analysis at (Public Authority of Agriculture Research and Extension/ Ministry of Agriculture-Thamar)

The survey samples/Thirty-eight soil samples were collected from different sites of Thamar in nylon bags, the upper 5 cm of soil were collected. Each sample was 100-150 gm. They were brought to the lab and were kept at 4°C for the farther study.

Isolation and identification of fungi/For isolation fungi, the direct plating method was followed [14], potato carrot agar and Emerson's culture media supplemented by rose Bengal stain were used. About 1gm

of soil sample was spread on the surface of culture medium, and were examined after a week up to 4 weeks to record the developing fungi. To all culture media, a chloramphenicol(250mg/L) was added and were incubated at 45°C.

Pure cultures of observed fungi were prepared and the identification was done after [3,4,10,15].

Total similarity% (TS)/To represent the total similarity% genera isolated during the (humid/warm) season and (cold/ dry) season, the following formula was used:

T.S.%= (no. of isolated genera from the two periods/no. of total genera) x 100

3. Results and Discussion

The soil analysis that have been done for soil samples according to Agricultural Researches Center /Thamar-Yeman represent in table (1)

Table (1) soil texture (S.T.), pH, carbon (C%), and nitrogen(N%)of (R,U,and E)stations

Station	S.T.	pH	C%	N%
Rasaba (R)	Sandy loam	9	1.06	0.8
Uni.campus (U)	Sandy loam	9	0.37	0.1
Ellesy (E)	Sandy	9	0.37	0.01

Twenty four thermophilic and thermotolerant fungi were isolated during the study (Table-1) they belong to(14) genera, and include ascomycetes(10), hyphomycetes (10),and zygomycetes (2) beside the black and white sterile mycelia. These fungi represented 28.9% of the species and 48% of the genera previously mentioned by [4].This indicate to a high diversity of TTF in Thamar soil ,although Thamar province represented the lowest yearly temperature in Yemen (<30°C).The high diversity of TTF which appear in the present study may be due to the effects of microenvironment factors rather than to the climatic factors (macro), and also it may be caused by transfer of fungal propagules by air from the warm region surrounding Thamar(table-2)

Table (2): The isolatedthrmophilic and thermotolerant fungi, their taxonomic group(TG) ,(H=hyphomycetes) (A=ascomycetes) (Z=zygomycetes) and sample locations (R=Rasaba), (U=university campus), and (E=Ellesy).

	Fungi	TG	R	U	E
1	*Acremonium murorum (*Corda)W.Gams	H	+	0	0
2	*Acrophialophorafusispora*(S.B.Saksena) Samson	H	+	0	0
3	*Aspergillus fumigates* Fresinius	H	+	+	0
4	*A.terreus* Thom	H	0	+	+
5	*Aspergillus* sp.	H	+	0	0
6	*Chaetomium atrobrunneum*Ames	A	+	+	+
7	*C.gracile*(Udagawa)von Arx*et al*	A	+	+	+

8	*C.strumarium* Rai,Tewari and Mukerji	A	0	+	+
9	*C.therophilevar.coprophpile*Cooney and Emerson	A	0	+	+
10	*Corynascus sepedonium*(Emons)von Arx	A	+	+	+
11	*Emericella nidulans*(Eidam)Vuillemin	A	+	0	+
12	*E.regulosa*(Thom and Raper) Bengamin	A	+	0	+
13	*Emericella* sp.	A	+	0	0
14	*Malbranchea cinnmomea*(Libert)van Oorschot and deHoog	H	+	0	+
15	*Melanocarpus albomyces*(Cooney and Emerson) von Arx	A	0	+	0
16	*Mycelia sterilia*(black)	H	0	+	+
17	*Mycelia sterilia* (white)	H	+	+	0
18	*Paecilomyces inflatus*(Burnside)J.W.Carmech	H	+	0	0
19	*Papulospora thermophila*Fergus	H	+	+	0
20	*Rhizomucor miehi*(Cooney and Emerson) Schipper	Z	+	0	0
21	*Rhizopus oligosporus* Saito	Z	+	+	0
22	*Scytalidium thermophilum* (Cooney and Emerson)Austwick	H	+	+	+
23	*Talromyces thermophilus* Stolck	A	0	+	0
24	*Thermomyces lanuginosus*Tisklinskya	H	+	+	+

It is worth noting that (14) fungi were identified as new record for themycobiota of Yemen. They include *Acremonium murorum, Acrophialophora fusispora C,.atrobrunneum, C.gracile, C.strumarium, C.therophilevar.coprophpile ,Corynascussepedonium ,E.rugulosa, Melanocarpusalbomyces , paecelomyces inflatus Papulospora thermophila, Rhizomucormiehi, Scytalidiumthermophilum*, and *Talromyces thermophilus*. Most of these fungi (table-1) were previously isolated from soil and self-heated materials in middle east countries [7,9,11].

Four species only wer erepresented in the three stations *Chaetomium atrobruneum, Corynaascus sepedonium,Scytalidium thermophilum, Thermomyce slanuginosus*). *C.atrobrunneum* was listed as thermotolerant fungus while the rest are thermophilic [3,11].

Aspergillus and Scytalediumonly represented high occurrence%(60<) (table-3). , while Malbranchea,Emericilla, Chaetomium , Corynascus, and Melanocarpus , had moderate occurrence% (20-59) , and the genera Talaromyces, Paecilomyces , Rhizomucor, Papulospora , Acremonium, Acrophialophora beside Mycelia sterilia with low occurrence% (20>).(Table-3)

Table(3): Occurrence % of isolated genera of (TTF) /(H=high),(M=moderate),(L=low)

	The genera	Occurrence%
1	*Aspergillus spp.*	84 (H)
2	*Scytaledium sp.*	68 (H)
3	*Malbranchea sp.*	50 (M)
4	*Emericella sp.*	36 (M)
5	*Chaetomium sp.*	34 (M)
6	*Corynascussp.*	23 (M)
7	*Melanocarpus sp.*	23 (M)
8	*Talaromyces sp.*	13 (L)
9	*Paecilomyces sp.*	7 (L)
10	*Rhizomucor sp.*	7 (L)
11	*Papulospora sp.*	5 (L)
12	*Mycelia sterilia*	5 (L)
13	*Acremonium sp.*	2 (L)
14	*Acrophialophora sp.*	2 (L)

The results showed that Rasaba's soil with the highest C% and N% (table-1) gave the highest no. of total CFUs of TTF (table-4) followed by (U) and (E) soil, they were 28234, 16200, and 11733 respectively. Both(C) and (N) are two of the most important elements that effect the soil's productivity [(14)]. No doubted that the sandy loam texture of (R) and (U) soil had better water holding capacity than sandy soil of (E) and so enhanced fungal development.

Table(4) :The total colony forming unites (CFUs) during the sampling period in Rasabaa, university campus, and Ellesy.

Station	Rasaba	University campus	Ellesy	Meantemp.C°
Month				
April	4200	2500	2700	15.8°
June	8767	4367	2233	19.8°
August	6500	3467	1933	18.2°
October	3500	2733	1900	16.0°
December	3267	1700	1567	13.9°
February	2900	1433	1400	13.7°
Total no.	28234	16200	11733	

The total no. which have been counted at moderate/humid period -April,June, and August- were 36667, it was higher than that of cold/dry period -October, December, and February-(204000). The Mann-Whitney test between warm and cold periods exhibit significantly higher number of TTF in the first period compare with the second period ($p<0.05$). Such result was supported by [16] who suggested that the fungal thermophily is an adaptation to transient seasonal and diurnal high temperatures, rather than simply an adaptation to specialized high-temperature environments. Total similarity% of genera are (78.57%) between the two periods, the survival spores and other live propagules may lead to high similarity in tested soil samples, especially the warm months are coinciding ith rain fall.

The enzyme activity tests for 15 isolates showed a different levels for the same enzyme among different isolates, and alsoa different activity levels for the same isolate according to different enzymes(table-5).

Table(5):The enzyme activity of (TTF) The enzyme activity of (TTF) .(Cell.=cellulase), (Amy. = amylase), (Lip.= lipase), (Prot.= protease), (Phen. = phenoloxidase), (Xyla. = and xylanase)// (+++ high) (++ moderate) (+ low) (- none) (x not tested).

	Enzyme Fungi	Cell.	Amy.	Lipa.	Prot.	Phen.	Xyla.
1	*A.fumigatus*	++	X	++	+++	-	++
2	*A.terreus*	-	X	++	++	-	X
3	*C.olivaceum*	+++	-	++	++	+	X
4	*C.strumarium*	±	-	++	+++	+	±
5	*C.thermophilum*	-	++	++	++	+	±
6	*C.sepedonium*	+++	++	++	±	+	±
7	*E.nidulans*	±	±	++	++	+	±
8	*E.rugulosa*	+++	-	±	+++	-	-
9	*S.thermophilum*	±	+++	++	++	-	+++
10	*M.cinnamomea*	++	++	++	+++	+	±
11	*M.albomyces*	±	-	+++	+++	-	-
12	*P.inflaus*	±	-	±	++	+	-
13	*R.oligosporus*	++	±	+++	+++	-	+++
14	*T.thermophilus*	+++	+++	+++	-	-	-
15	*T.lanuginosus*	++	±	++	++	-	++

The isolates (6,7,10) in(table5)showed positive results for all tested enzymes, while (1,4,5,9,13,15) isolates showed a positive results for five from six testes. A several researches were mentioned the high activity of TTF in producing enzymes ,and using selected isolates in biotechnology to produce them (11,18,19, 20) .

4. Conclusion:

Soil of the tested area (Thamar-Yemen) has a high diversity of thermophilic and thermotolerant fungi although the mean of yearly temperature about thirty . From only (38) soil samples collected during this study ,we recorded 28.9% species and 48%genus from the list reviewed by[4] in 2007.

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