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On the thermophilic and thermotolerant mycoflora of Iraqi soils

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Thirty-five species of thermophilic and thermotolerant fungi were isolated from 200 soil samples collected from different parts of Iraq. Eleven species are new records for this country. For five species thermotolerance has been demonstrated for the first time, whilst true thermophily was shown only by six species. The study revealed that thermophilic and thermotolerant fungi are widely represented in the mycoflora of Iraqi soils. The majority of the species isolated can produce one or more enzymes involved in the degradation of starch, carboxymethylcellulose, lipids and proteins incorporated in solid media.

Several papers have dealt with the ecology and taxonomy of fungi from Iraqi soils (ABDULLAH & al., 1986; ABDULLAH & AL-BADER, 1989; ISMAIL & ABDULLAH, 1977; AL-DOORY & al., 1959; TOLBA & al., 1957; EL-DOHLOB & AL-HELFI, 1982; UDAGAWA & al., 1985, 1986), but comparatively few are known that describe the incidence and activity of thermophilic fungi in this country (ABDULLAH & al., 1986; ABDULLAH & AL-BADER, 1989; UDAGAWA & al., 1986). Thermophilic soil mycoflora, by contrast, has been investigated by several workers in different parts of the world (APINIS, 1963, 1972; COONEY & EMERSON, 1964; EGGINS & MALIK, 1969; TABER & PETIT, 1975; KUTHUBUTHEEN, 1982; MOUSTAFA & al., 1976; ABDEL-HAFEZ, 1982; JACK & TANSEY, 1977; TUBAKI & al., 1974; WARD & COWLEY, 1972; EGGINS & al., 1972; GOCHENAUR, 1975).

In this paper we report on the occurrence of thermophilic and thermotolerant fungi in Iraqi soil and on the results of investigations on their extracellular enzymatic activity.

Material and methods

200 soil samples (150–200 g each) were collected in the August 1985 and 1987 in different localities in Iraq. Soil temperatures ranged from 37 to 44 °C at the time the collections were made. Soil samples were taken from a depth of 5 cm and stored in polythene bags at 5 °C. The samples were processed within 1 to 2 weeks after collection.

The soil plate (WARCUP, 1950), the dilution plate (JOHNSON & al., 1959), and the heat and alcohol treatment method (WARCUP, 1951) were used to survey the occurrence of termophilic and thermotolerant fungi. Potato carrot agar (PCA: potatoes 20 g, carrots 20 g, agar 15 g, distilled water 1000 ml), 2% malt extract agar (Maknur, Canada); and Emerson YpSs agar (Maknur, Canada) were utilized as plating substrates. To each medium 50 mg/l Chloramphenicol (SDI) was added after autoclaving to inhibit bacterial growth. Plates were incubated at 45 °C.

The effect of the temperature on the linear growth of 27 selected fungal species was studied. Plates of YpSs agar were inoculated with mycelial discs (2 mm diam.) removed with a sterile cork-borer from the margin of actively growing colonies. Triplicate cultures were prepared for each species. The inoculated plates were sealed and incubated at 25, 30, 35, 40, 45, 50, and 55 °C. Linear growth in each plate was measured after 4 days incubation.

Amylase, cellulase, protease, and lipase activities by 17 isolates were determined according to HANKIN & ANAGNOSTAKIS (1975). Cultures were incubated at 45 °C.

Results and discussion

Thirty-five thermotolerant and thermophilic fungal species were isolated. Their frequency of occurrence is presented in Tab. 1. The fungi isolated have been divided in four groups according to their percentage of occurrence: high frequency (>50%); moderate frequency (20–49%); low frequency (5–19%); and rare frequency (<5%).

Aspergillus terreus, A. fumigatus and A. niger were present with frequencies of occurrence of 70%, 68%, and 60% respectively. The two former species have been reported as the most frequent fungal isolates of the thermophilic and thermotolerant mycoflora of soils in Kuwait, Qatar, and Saudi Arabia (Moustafa & al., 1976; Moubasher & AL-SUBAI, 1987; ABDEL-HAFEZ, 1982). The moderate frequency group was represented by Emericella nidulans, A. candidus, and Corynascus sepedonium. Their frequencies of occurrence were 35%, 30%, and 20% respectively. Corynascus sepedonium is a new record for the Iraqi soil mycoflora. Emericella sp., Rhizomucor miehei, Chaetomium rectopilium, Acrophialophora levis, Myrioconium thermophilum, Gilmaniella macrospora, Cunninghamella echinulata and *Talaromyces* sp. were representatives of the low-frequency group. Chaetomium rectopilium, A. levis, and M. thermophilum are new records for Iraqi mycoflora and G. macrospora is newly reported as a thermotolerant species. The remaining 21 species were rare and their frequencies ranged between 0.5 and 4%. The majority of species are new records for the Iraqi soil mycoflora.

Tab. 1. Percentage frequency of occurrence of thermophilic and thermotolerant fungi isolated from Iraqi soils.

Fungi	% fre-	frequency
	quency	group
Aspergillus terreus Thom	70	H
A. fumigatus Fres.	68	H
A. niger van Tieghem	60	H
Emericella nidulans (EIDAM) VUILLEMIN	35	M
Aspergillus candidus LINK: FR.	30	M
Corynascus sepedonium (Emmons) von Arx	20	M
Emericella sp.	16	L
Rhizomucor miehei (Cooney & Emerson) Schipper	16	L
Chaetomium rectopilium Fergus & Amelung	15	L
Acrophialophora levis Samson & Tariq	12	L
Myrioconium thermophilum (Fergus) van der Aa	9	L
Gilmaniella macrospora Moustafa	8	L
Cunninghamella echinulata (THAXTER) THAXTER	6	L
Talaromyces sp.	6	L
Trichoderma sp.	4	R
sterile mycelium (black)	4	\mathbf{R}
Torula terrestris MISRA	3	\mathbf{R}
sterile mycelium (white)	3	\mathbf{R}
Paecilomyces variotii BAIN	3	\mathbf{R}
Rhizopus sp.	3	\mathbf{R}
Scytalidium thermophilum (COONEY & EMERSON) AUSTWICK	1.5	R
Penicillium sp.	1.5	R
Byssochlamys vertucosa Samson & Tansey	1.5	R
Thermomyces lanuginosus Tsiklinksy	1.5	R
Cladosporium sp.	1.5	R
Absidia corymbifera (Cohn) Sacc. & Frott.	1	R
Myceliophtora sp.	1	R
Sporotrichum thermophilum Apinis	1	R
Malbranchea sulphurea (MIEHEI) SIGLER & CARMICHAEL	1	R
Thermoascus aurantiacus Miehei	1	R
T. aegyptiacus Ueda & Udagawa	0.5	R
Thielavia sp.	0.5	R
Chaetomium subcurvisporum Abdullah & Al-Bader	0.5	R
Thermophymatospora fibuligera Udagawa, Ueda & Abdullah	0.5	R
Mycotypha africana Novak & BACKUS	0.5	R

The effect of the temperature on the linear growth of 27 fungal isolates is presented in Tab. 2. According to COONEY & EMERSON'S (1964) definition of thermophilic fungi, only Malbranchea sulphurea, Rhizomucor miehei, Scytalidium thermophilum, Sporotrichum thermophilum, Thermomyces lanuginosus, and Trichoderma sp. are true thermophiles, having a maximum temperature for growth at or above 50 °C (Tab. 2). The majority of the remaining species are considered thermotolerant (CRISAN, 1964; MOUSTAFA & al., 1976; MILLNER, 1977; ABDEL-HAFEZ, 1982), since they grow at temperatures up to 45–50 °C, with the optimum between 30–40 °C. Five fungal isolates, Cun-

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ninghamella echinulata, Torula terrestris, Gilmaniella macrospora, Emericella sp., and Cladosporium sp. are reported for the first time as thermotolerant species.

Tab. 2. Effect of temperature on the linear growth of thermophilic and thermotolerant fungi isolated from Iraqi soils. Measurements were taken after 4 days incubation; each value is the mean of three readings. ND: not determined.

Species	Linear growth (mm)						
	$25 \ ^{\circ}\mathrm{C}$	$30 \ ^{\circ}C$	35 °C	40 °C	45 °C	$50 \ ^{\circ}\mathrm{C}$	55 °C
Acrophialophora levis	13	18	20	21	14	0	0
Aspergillus candidus	14	19	31	18	12	0	0
A. fumigatus	17	26	28	29	25	0	0
A. niger	23	30	34	21	8	0	0
A. terreus	13	22	25	28	9	0	0
Byssochlamys verrucosa	10	15	16	12	11	0	0
Chaetomium rectopilium	12	23	26	26	20	0	0
C. subcurvisporum	15	20	35	37	19	0	0
Cladosporium sp.	12	16	14	11	5	0	0
Corynascus sepedonium	18	23	31	38	30	0	0
Cunninghamella echinulata	52	80	82	84	50	0	0
Emericella nidulans	14	25	33	33	22	0	0
Emericella sp.	13	26	28	30	12	0	0
Gilmaniella macrospora	12	16	38	52	22	0	0
Malbranchea sulphurea	0	11	19	25	26	15	0
Mycotypha africana	11	12	ND	16	8	0	0
Myrioconium thermophilum	81	84	84	90	38	0	0
Paecilomyces variotii	13	25	40	30	10	0	0
Penicillium sp.	11	17	18	18	11	0	0
Rhizopus sp.	18	29	41	50	23	0	0
Rhizomucor miehei	14	28	40	53	45	21	0
Scytalidium thermophilum	0	15	30	48	44	22	0
Sporotrichum thermophilum	10	30	39	65	52	15	12
Talaromyces sp.	13	17	18	18	9	0	0
Thermomyces lanuginosus	19	23	30	55	60	42	40
Torula terrestris	15	17	13	10	10	0	0
Trichoderma sp.	85	90	84	82	33	6	0

Humicola insolens and H. grisea var. thermoidea, two otherwise frequent thermophilic species commonly found in tropical and subtropical soil (Abdel-Hafez, 1982; Moustafa & al., 1976; Kuthubu-THEEN, 1982) were not detected in this study.

Species of *Acrophialophora* are considered thermotolerant and are mostly present in soil in tropical countries (SAMSON & MAHMOOD, 1970). *Acrophialophora fusispora*, the most common thermotolerant species reported to inhabit tropical and subtropical soils (SAMSON & MAHMOOD, 1970; Abdel-Hafez, 1982), was not isolated. The results of the enzyme studies are summarized in Tab. 3. Fifteen isolates were able to degrade starch incorporated in solid medium. *Malbranchea sulphurea* showed the highest amylolytic activity. All species tested produced cellulase. Twelve species showed proteolytic avtivity, *A. levis* and *M. sulphurea* being the most active. Of the 17 species tested in this work *Sporotrichum thermophilum* showed the highest enzymatic activity for lipase.

Tab. 3. Production of amylase, cellulase, protease and lipase by thermophilic and thermotolerant fungi. Activity zone in mm: no activity: -; 1-2 mm: + (slightly active); 3-6 mm + + (intermediate activity); > 6 mm: + + + (high activity); ND: not determined.

Species	Amylase	Cellulase	Protease	Lipase
Acrophialophora levis	+	+	+++	+
Aspergillus candidus	-	++	-	+
A. fumigatus	++	+	+	+
A. niger	++	ND	+	+
A. terreus	+	+	ND	+
Chaetomium rectopilium	++	+	++	_
Cladosporium sp.	++	+++	ND	_
Corynascus sepedonium	++	+	++	++
Gilmaniella macrospora	-	+	-	-
Malbranchea sulphurea	+++	++	+++	+
Myrioconium thermophilum	++	+	++	++
Paecylomyces variotii	+	+	-	++
Scytalidium thermophilum	+	+	+	+
Sporotrichum thermophilum	+	ND	+	+++
Talaromyces sp.	++	++	+	+
Thermomyces lanuginosus	++	++	+	+
Torula terrestris	+	+	++	-

Acrophialophora levis, Aspergillus fumigatus, Corynascus sepedonium, Malbranchea sulphurea, Myrioconium thermophilum and Scytalidium thermophilum were found to secrete cellulase, amylase, protease, and lipase. The present study showed that all the fungal isolates tested have the ability to secrete one or more of the enzymes involved in the degradation of starch, cellulose, lipids, and proteins when these substrates are incorporated in solid media. These substances represent the major constituents of the organic matter in soil and this suggests that these fungi may have a similar ability to degrade these substances in soil.

At the present time approximately 70 thermophilic and thermotolerant fungal species have been reported from different substrates in the world (SAMSON & TANSEY, 1977; TANSEY & BROCK, 1978; KUTHU-BUTHEEN, 1982; UDAGAWA, 1985). The present investigation has shown that thermophilic and thermotolerant fungi are widely represented also in Iraqi soils.

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Thermophilic and thermotolerant fungi are detected more frequently from sun-heated soil (TANSEY & JACK, 1976). JACK & TANSEY (1977) pointed out that temperatures in sun-heated soil were suitable for the germination and production of spores by thermophilic and thermotolerant fungi. Therefore, the frequent occurrence of this group of fungi in Iraqi soil had to be expected, since Iraq is considered one of the areas receiving the highest incidence of solar radiation in the Northern hemisphere (THALEN, 1979).

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