

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 thermophilic 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	% frequency	frequency group
<i>Aspergillus terreus</i> THOM	70	H
<i>A. fumigatus</i> FRES.	68	H
<i>A. niger</i> VAN TIEGHEM	60	H
<i>Emericella nidulans</i> (EIDAM) VUILLEMIN	35	M
<i>Aspergillus candidus</i> LINK: FR.	30	M
<i>Corynascus sepedonium</i> (EMMONS) VON ARX	20	M
<i>Emericella</i> sp.	16	L
<i>Rhizomucor miehei</i> (COONEY & EMERSON) SCHIPPER	16	L
<i>Chaetomium rectopilium</i> FERGUS & AMELUNG	15	L
<i>Acrophialophora levis</i> SAMSON & TARIQ	12	L
<i>Myrioconium thermophilum</i> (FERGUS) VAN DER AA	9	L
<i>Gilmaniella macrospora</i> MOUSTAFA	8	L
<i>Cunninghamella echinulata</i> (THAXTER) THAXTER	6	L
<i>Talaromyces</i> sp.	6	L
<i>Trichoderma</i> sp.	4	R
sterile mycelium (black)	4	R
<i>Torula terrestris</i> MISRA	3	R
sterile mycelium (white)	3	R
<i>Paecilomyces variotii</i> BAIN	3	R
<i>Rhizopus</i> sp.	3	R
<i>Scytalidium thermophilum</i> (COONEY & EMERSON) AUSTWICK	1.5	R
<i>Penicillium</i> sp.	1.5	R
<i>Byssoschlamys verrucosa</i> SAMSON & TANSEY	1.5	R
<i>Thermomyces lanuginosus</i> TSIKLINSKY	1.5	R
<i>Cladosporium</i> sp.	1.5	R
<i>Absidia corymbifera</i> (COHN) SACC. & FROTT.	1	R
<i>Myceliophthora</i> sp.	1	R
<i>Sporotrichum thermophilum</i> APINIS	1	R
<i>Malbranchea sulphurea</i> (MIEHEI) SIGLER & CARMICHAEL	1	R
<i>Thermoascus aurantiacus</i> MIEHEI	1	R
<i>T. aegyptiacus</i> UEDA & UDAGAWA	0.5	R
<i>Thielavia</i> sp.	0.5	R
<i>Chaetomium subcurvisporum</i> ABDULLAH & AL-BADER	0.5	R
<i>Thermophymatospora fibuligera</i> UDAGAWA, UEDA & ABDULLAH	0.5	R
<i>Mycotypha africana</i> 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-*

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 °C	30 °C	35 °C	40 °C	45 °C	50 °C	55 °C
<i>Acrophialophora levis</i>	13	18	20	21	14	0	0
<i>Aspergillus candidus</i>	14	19	31	18	12	0	0
<i>A. fumigatus</i>	17	26	28	29	25	0	0
<i>A. niger</i>	23	30	34	21	8	0	0
<i>A. terreus</i>	13	22	25	28	9	0	0
<i>Byssosclamyces verrucosa</i>	10	15	16	12	11	0	0
<i>Chaetomium rectopilum</i>	12	23	26	26	20	0	0
<i>C. subcurvisporum</i>	15	20	35	37	19	0	0
<i>Cladosporium</i> sp.	12	16	14	11	5	0	0
<i>Corynascus sepedonium</i>	18	23	31	38	30	0	0
<i>Cunninghamella echinulata</i>	52	80	82	84	50	0	0
<i>Emericella nidulans</i>	14	25	33	33	22	0	0
<i>Emericella</i> sp.	13	26	28	30	12	0	0
<i>Gilmaniella macrospora</i>	12	16	38	52	22	0	0
<i>Malbranchea sulphurea</i>	0	11	19	25	26	15	0
<i>Mycotypha africana</i>	11	12	ND	16	8	0	0
<i>Myrioconium thermophilum</i>	81	84	84	90	38	0	0
<i>Paecilomyces variotii</i>	13	25	40	30	10	0	0
<i>Penicillium</i> sp.	11	17	18	18	11	0	0
<i>Rhizopus</i> sp.	18	29	41	50	23	0	0
<i>Rhizomucor miehei</i>	14	28	40	53	45	21	0
<i>Scytalidium thermophilum</i>	0	15	30	48	44	22	0
<i>Sporotrichum thermophilum</i>	10	30	39	65	52	15	12
<i>Talaromyces</i> sp.	13	17	18	18	9	0	0
<i>Thermomyces lanuginosus</i>	19	23	30	55	60	42	40
<i>Torula terrestris</i>	15	17	13	10	10	0	0
<i>Trichoderma</i> 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; KUTHUBUTHEEN, 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 activity, *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
<i>Acrophialophora levis</i>	+	+	+++	+
<i>Aspergillus candidus</i>	-	++	-	+
<i>A. fumigatus</i>	++	+	+	+
<i>A. niger</i>	++	ND	+	+
<i>A. terreus</i>	+	+	ND	+
<i>Chaetomium rectopilum</i>	++	+	++	-
<i>Cladosporium</i> sp.	++	+++	ND	-
<i>Corynascus sepedonium</i>	++	+	++	++
<i>Gilmaniella macrospora</i>	-	+	-	-
<i>Malbranchea sulphurea</i>	+++	++	+++	+
<i>Myrioconium thermophilum</i>	++	+	++	++
<i>Paecilomyces variotii</i>	+	+	-	++
<i>Scytalidium thermophilum</i>	+	+	+	+
<i>Sporotrichum thermophilum</i>	+	ND	+	+++
<i>Talaromyces</i> sp.	++	++	+	+
<i>Thermomyces lanuginosus</i>	++	++	+	+
<i>Torula terrestris</i>	+	+	++	-

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; KUTHUBUTHEEN, 1982; UDAGAWA, 1985). The present investigation has shown that thermophilic and thermotolerant fungi are widely represented also in Iraqi soils.

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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