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Soil mycoflora of Thi-Qar marshes and their enzymatic activities

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Abstract - A survey on soil mycoflora in Thi-Qar marshes have been studied. A total of 22 fungal species belonging to 13 genera were isolated and identified. The average of the total fungal colony count ranged between 1550-4150 colonies/gm of soil. *Aspergillus*, *Alternaria*, *Cladosporium*, *Stachybotrys* and *Phoma* were of the mostly occurred genera in soil samples constituting 98.67%, 82.67%, 69.33%, 57.0% and 52.0%, respectively, and the mostly occurred species were; *A. terreus, A. flavus, C. cladosporioides and S. atra*; constituting 74.67%, 69.33%, 69.33% and 57%, respectively. Nine fungal species: *Alternaria chlamydospora*, *Aspergillus flavus*, *A. fumigatus*, *A. terreus*, *Bipolaris spicifera*, *Fusarium oxysporum*, *Exserohilum rostratum*, *Trichoderma viride* and *Ulocladium botrytis* were identified to produce the four investigated enzymes: cellulase, amylase, lipase and protease. All the tested isolates were identified as cellulase and amylase producers, *A. candidus*, *A. flavus*, *B. spicifera* and *C. cladosporioides* were an excellent producer of cellulase, while *A. terreus*, *Stachybotrys atra* and *T. viride* were the most active amylase producers. *A. flavus*, *F. oxysporum* and *U. atrum* showed the highest lipase production. The higher activity of protease was produced by *A. candidus*, *A. flavus* and *T. viride*.

Keywords: Soil mycoflora, enzymatic activity, fungal occurrence, Thi-Qar marshes.

Introduction

The southern Iraqi marshes are considered as a complete ecosystem of long history and regarded as one of the largest wetland in the Middle East and of West Asia (Maltby, 1994). The southern Iraqi marshes are located at a triangular area which their heads are: Emara province at the north, Souq Al-Sheiukh from the west and Basrah province from the east and south (Hasek, 1979). Their area was estimated to be between 15000-20000 km^2, but this area is variable depending on season, it is larger during winter and smaller during summer (Al-Hilli, 1977). During 1991 onwards, the marshes were desiccated and their area was greatly reduced to about 3% of its original status (Partow, 2001). The importance of the marshes comes from the fact that it plays an important role in the environmental equilibrium, since it is regarded as a large sedimentation basin to precipitate sludge and through their different aquatic plants which act as filters together with their associated microorganisms may act as integrated system to remove organic matter, inorganic nutrients and metals from water (Partow, 2001). The dominant aquatic plants were *Phragmites australis* that covers large areas of these wetlands and *Typha domingensis* which grow at marshes

periphery (Alwan, 2006). Few studies were carried on the fungi of the southern Iraqi marshes; Abdul-Qadir (1985) studied the fungi associated with aquatic plant, *Typha dommingesis*, where eight ascomycetous species were isolated and identified from submerged plant debris at different wetlands in Basrah Province (Abdullah and Abdulkhadir, 1987). Also a new genus and new species belonging to fungi imperfecti were identified by Abdullah *et al.* (1989). In other study, new ascomycetous species associated with submerged plant debris were described, namely*: Zopfiella cephalothecoidae, Z. submers*e and *Preussia aqualirostrata* (Guarro *et al.*, 1996; 1997 a and b).

Because of the changes in the southern Mesopotamian marshlands ecosystem due to the desiccation of water bodies in the southern marshes through a massive hydro-engineering program by the government from 1991-1995, and rehabilitation of the marshes started at 2003-2004, the present study has been carried out to monitor the diversity, occurrence and frequency of mycoflora in marshland soils and to study the enzymatic activity of some fungal isolates.

Materials and Methods

Samples collection:

Seventy five soil and mud samples were collected at a depth of 10 cm below the soil surface from different localities in Thi-Qar marshlands including marshland boarders, near plant roots, and soil samples from agricultural areas in the marshes, during the period of March-2008 t0 March-2009, brought to the laboratory and processed at the same day of collection or the day after.

Isolaion of fungi:

Fungal species were isolated from soil samples by direct plat method as described by Warcup (1950), and by dilution method described by Jonhson *et al.* (1959), using three types of culture media: malt extract agar (MEA), Potato carrot agar (PCA), and potato dextrose agar (PDA). The antibacterial antibiotic, chloramphenicol (250 mg/l) was added to each medium then sterilized by autoclaving at 121 ºC and 15 Ib/ square inch for 20 minutes. The inoculated Petri dishes were incubated at room temperate for 3-5 days. Each medium was used in duplicates.

The isolated fungal colonies were identified according to the criteria described by: Ellis (1971, 1976), Domsch *et al.* (1980), Arx *et al.* (1986 and 1988) Sivanesan (1987), Klich and Pitt (1988), Hoog and Guarro (1995), Pitt and Hocking (1997), Klich (2002).

The percentage occurrence of genera and species of the isolated fungi were calculated as follows:

$$\textbf{Occurrence \%} = \frac{\text{Number of samples from which fungal species were isolated}}{\text{Total number of samples}} \times 100$$

Enzymatic Activity:

The ability of some fungal isolates to produce the enzymes; amylase, cellulase, lipase, and protease in solid media were carried out as follows:

1. *Amylase*: The ability of some of the isolated species to hydrolyse starch and to produce the enzyme, amylase has been done using the culture media described by Gessner (1980) and by using KI solution as reagent.
2. *Cellulase*: The culture medium described by Mandeles *et al.* (1975) was used to study the ability of the fungal isolate to produce cellulase using hydrochloric acid- iodine solution as reagent.
3. *Lipase*: Lipase production by the isolated fungi was detected, using the medium described by Sierra (1957) through the formation of clear hallo around the colonies, or by the formation of white precipitate under the colonies or the appearance of white crystals of calcium salt surrounding the colonies.
4. *Protease*: To detect protease, the culture media described by Hankin and Angnostakis (1975) which contain gelatin as protein constituent was used together with Frazier's reagent ($HgCl_2$, 15 g; concentrated Hcl, 20 ml and 100 ml DW). The specific culture media used for enzymes production were inoculated with disks of fungal growth (5 mm diameter), incubated at room temperature for 3-7 days and the percentage of the enzymatic activity of the tested strains were measured as follows:

$$\textbf{Enzymatic activity \%} = \frac{\text{Diameter of the clear zone-Fungal colony diameter}}{\text{Fungal colony diameter}} \times 100$$

Results and Discussion

In the present study, 22 fungal species belonging to 13 genera were isolated from mud and soil samples from Thi-Qar marshlands. All the isolated species were Deuteromycota (Table 1). The result coincides with that of Abdullah *et al.* (2000) who found that the fungi isolated from Shatt-Al-Arab and North East Arabian Gulf sediments were of imperfecti represented by 47 species belonging to 21 genera, while ascomycetous and zygomycetous species were represented by 23 and 21 species respectively, but the differences were in that there is a decrease in the diversity of fungal genera and species outlined in the present results, and this may be due to the desiccation program in 1991-1995. However, in salt marshes of Kuwait, 79% of the fungi were imperfecti (Mustafa, 1975). Also most of the isolated fungi from salt lakes in Egypt were imperfecti (Abdel-Hafez *et al.*, 1977). The wide characteristic distribution of fungi imperfecti in the present soil samples, were in accordance with the results of other studies (Muhsin and Al-Helfy, 1982; El-Dohlob *et al.*, 1982; Abdullah *et al.*, 2010a) and related to the ability of these microorganisms to grow in different media, either natural on artificial, and to grow on plant debris and remains (submerged or unsubmerged), and their ability to produce propagules in large numbers (Domsch *et al.*, 1980).

Table 1. Percentage occurrence of genera and species of the isolated fungi from soil and mud of marshlands in southern Iraq.

Fungal genera and species	Soil and mud samples	
	Occurrence (%)	No. of sample
Alternaria	82.67	62
A. alternate (Fr.:Fr.) Keissler	57.33	43
A. chlamydospora Mouchacca	28	21
A. phragmospora van Emden	20	15
A. radicina Meier, Drechsler & Eddy	8	6
Aspergillus	98.67	74
A. candidus Link : Fr.	20	15
A. flavus Link : Fr.	69.33	52
A. fumigatus Fresen	29.33	22
A. niger van Tiegham	62.67	47
A. terreus Thom	74.67	56
Bipolaris	45.33	34
B. spicifera (Bainier) Subram	45.33	34
Cladosporium	69.33	52
C. cladosporioides (Fresen) de Vries	69.33	52
Curvularia	20	15
C. lunata (Wakker) Boedijn	20	15
Exserohilum	36	27
E. rostratum (Drechsler) Leonard & Suggs	36	27
Fusarium	34.67	26
F. moniliforme Sheld	12	9
F. oxysporum Schlecht	32	24
Paecilomyces	13.33	10
P. variotii Bainier	13.33	10
Penicillium	30.67	23
P. chrysogenum Thom	30.67	23
Phoma	52	39
P. laminariae Cooke et Massee	52	39
Stachybotrys	57	43
S. atra Corda	57	43
Trichoderma	18.67	14
T. viride Pers : Fr.	18.67	14
Ulocladium	38.67	29
U. atrum Preuss	21.33	16
U. botrytis Preuss	6.67	5

The present results indicated that five fungal genera isolated from mud and soil showed higher occurrence; *Aspergillus* (98.67%), *Alternaria* (82.67%), *Cladosporium* (69.33%), *Stachybotrys* (57%) and *Phoma* (52%). Most of these fungi were belonging to dematiaceous hyphomycetes which are characterized by the presence of melanin in their cell wall, facilitate

them to tolerate unfavorable environmental conditions like the variable changes in temperature between daytime and night and between summer and winter (Bell and Wheeler, 1986; Butler *et al*., 2001). The fungal species: *Aspergillus terreus, A. flavus, A. niger, Cladosporium cladosporioides, Alternaria alternata, and Stachybotrys atra* represent the mostly occurred species with percentage of occurrence of 74.67%, 69.33%, 62.67%, 69.33%, 57.33% and 49.33%, respectively. Moreover, species of *Aspergillus* in aquatic environments of Iraq were previously isolated with high percentage of occurrence (Abdulah *et al*., 2000; Al-Salehy, 2002; Abdulah *et al*., 2010).

The average of the present fungal colony counts were ranging from 1550-4150 colony/gram of soil, whereas Abdullah and Abbas (2008) found lower numbers of fungal colonies in the sediments of Shatt Al-Arab River (550-750 colonies/gram), so as the soil of the intertidal zone of Khour Al-Zubair canal, salinity (15-31 ppt), contain fungal colonies of about 760-2000/gram (Al-Salehy, 2002). However, Al-Nasarawy (2006) mentioned that 200-2633 colonies/gram were isolated from Al-Razaza lake sediments, while in Umm Al-Naag marsh the number of the isolated fungal colonies increased to 3177 to 6300 colonies/gram of mud.

Table (2) showed that most of the tested fungal isolates had enzymatic activity and the enzyme, cellulase, was secreted by all the tested strains in solid media. The fungal species; *C. cladosporioides, A. flavus, A. candidus*, and *B. specifera* were common producers of cellulase, while *A. fumigatus* and *T. viride* exhibit lower activity. This result is in agreement with the results of Muhsin and Al-Helfi (1982) and Khalid *et al*. (2006) since they indicated that several fungal species had the ability to grow on cellulose medium and species of the genera, *Aspergillus* and *Penicilium* showed higher enzymatic activity. Also all the tested strains produce amylase and species of the genera: *Alternaria, Aspergillus* and *Curvularia* exhibited higher enzymatic activity (*Alternaria chlamydospora*, 9.38%; *Aspergilus candidus*, 7.69%; *A. flavus*, 7.02%; *A. fumigatus*, 7.94%; *A. niger*, 5.26% and *Curvularia lunata*, 8.57%). Starch is a polymer of glucose and is an essential component of plant cells and regarded as a reserves of glucose in different plant parts such as seeds, fruits, leaves, bulbs and tubers (Baum and John-Hill, 1995) and is digested by amylase to simple sugars, so fungi by their production of amylase play important role in the fermentation process of carbohydrates. Therefore, this result is in support to the conclusion of Norouzian *et al*. (2006) that *Aspergillus* species are starch hydrolyser in nature. Furthermore, Sohail *et al*. (2009) suggested higher production of amylase by the species; *A. niger, A. flavus*, and *Alternaria* sp.

Soil may contain fatty substances as one of its constituents, and because soil microorganisms such as bacteria, actinomycetes and fungi play important role in the utilization and degradation of fatty materials by the production of an extracellular enzyme, the lipase (Kow *et al*., 2006), so the ability of some fungal isolates to produce lipases were studied on solid media and out of 14 species produce this enzyme, *A. flavus, A. niger, F. oxysporum, Trichoderma viride*, and *Ulocladium atrum* showed higher enzymatic activity. Previous studies referred to the ability of fungi to

Table 2. The enzymatic activity of the tested fungal isolates.

Fungal isolates	Protease			Lipase			Amylase			Cellulase		
	EA	HD	CD	EA	HD	CD	EA	HD	CD	EA	HD	CD
Alternaria alternata	-	-	40	3.23	3	31	4.64	45	43	5.71	37	35
A.chlamydospora	4	26	25	2.78	37	36	9.38	35	32	3.45	30	29
A. phragmospora	-	-	25	-	-	27	2.13	48	47	7.41	29	27
A. radicina	3.33	31	30	-	-	24	2.63	39	38	3.22	32	31
Aspergillus candidus	7.14	30	28	-	-	29	7.69	28	26	8	27	25
A. flavus	16.67	35	30	4.26	49	47	7.02	61	57	16.28	50	43
A. fumigatus	1.78	57	56	1.52	67	66	7.94	68	63	1.62	63	62
A. niger	-	-	27	3.77	55	53	5.26	40	38	2.78	37	36
A. terreus	5.26	40	38	2.33	44	43	1.67	61	60	4.76	44	42
Bipolaris spicifera	6.67	32	30	3.33	31	30	4.88	43	41	9.38	35	32
Cladosporium cladosporioides	-	-	20	2.88	18	17	3.78	55	53	20	18	15
Curvularia lunata	3.33	31	30	-	-	23	8.57	83	35	7.14	30	28
Exserohilum rostratum	6.89	31	29	2.86	36	35	5	42	40	3.33	31	30
Fusarium moniliforme	3.92	53	51	-	-	43	4	52	50	4.44	47	45
F. oxysporum	2.33	44	43	4.88	43	41	4.76	44	42	2.08	49	48
Paecilomyces variotii	2.94	35	34	-	-	31	7.41	29	27	3.57	29	28
Penicillium chrysogenum	-	-	51	2.33	44	43	1.89	54	53	4	25	50
Phoma laminariae	-	-	41	-	-	37	2.56	40	39	4.65	45	43
Stachybotrys atra	3.39	61	59	-	-	47	1.64	62	61	6	53	50
Trichderma viride	12.82	44	39	3.17	65	63	1.45	70	69	1.67	61	60
Ulocladium atrum	-	-	37	4.88	43	41	5.26	40	38	4.26	49	47
U. botrytis	2.44	42	41	2.22	46	45	2.44	42	41	2.22	46	45

produce lipases which degrade fats and convert them to triglycerides, glycerol, and free fatty acids (Fadiloglu and Erkmen, 1999). Shukla Gupta (2007) mentioned that 13 fungal species out of 20 species isolated from soil enriched with fatty materials and decayed organic matters, were able to produce lipases and *A. niger, Fusarium sp., Rhizopus oryzae,* and *Candida* sp. showed the higher activity.

The present results showed that 15 fungal species were protease producers with higher activity exhibited by *A. flavus, A. candidus, and T. viride*. This is concurred with the study of Al-Bader (1986) who stated that three fungal species instead of other isolates of thermophilic and thermo-tolerant species also gave positive result to protease.

Conclusion

The present study revealed that most of the isolated fungi belonged to fungi imperfecti, in concomitant with other studies carried out in Iraq and other part of the World (Abdulla and Abbas, 2008; Abdullah *et al.*, 2010 a & b), while ascomycetous species were not isolated for they need special procedure for their isolation (Abdullah *et al.*, 2007).

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فطريات تربة أهوار محافظة ذي قار وفعاليتها الأنزيمية

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المستخلص - تم عزل وتشخيص 22 نوعاً من الفطريات التي تعود لـ 13 جنساً من تربة أهوار محافظة ذي قار، وتراوح معدل أعداد المستعمرات الفطرية بين 1550 – 4150 مستعمرة/غم تربة. وحققت *الأجناس Aspergillus* و *Alternaria* و *Cladosporium* و *Stachybotrys* و *Phoma* نسب ظهور عالية في عينات التربة وبلغت 98.67 % و 82.67 % و 69.33 % و 57.0 % و 52.0 % على التوالي. أما الأنواع الفطرية التي ظهرت بنسب عالية في عينات التربة فهي: *A. flavus* و *A. terreus* و *C. cladosporioides* و S. *atra*، في حين أن الأنواع *A. radicina* و *Fusarium moniliforme* و *Paecilomyces variotii* و *Ulocladium botrytis* سجلت أدنى نسب للظهور وبلغت 8% و 12% و 13.33% و 6% على التوالي. أعطت 9 أنواع من الفطريات المختبرة كشفاً موجباً لأنزيمات Cellulase و Amylase و Lipase و Protease وهي: *Alternaria chlamydospora* و *Aspergillus flavus* و *A. fumigatus* و *A. terreus* و *Bipolaris spicifera* و *Fusarium oxysporum* و *Exserohilum rostratum* و *Trichoderma viride* و *Ulocladium botrytis*. وتميزت *الفطريات Cladosporium cladosporioides* و *A. candidus* و *A. flavus* و *B. spicifera* بإنتاج مميز لأنزيم Cellulase، أما الأنواع *A. flavus* و *Stachybotrys atra* و *T. viride* فقد كانت متميزة بإنتاج أنزيم Amylase. كما حققت الأنواع *A. flavus* و *F. oxysporum* و *U. atrum* أعلى فعالية في أنتاج أنزيم Lipase، وأظهرت الأنواع *A. candidus* و *A. flavus* و *T. viride* فعالية في إنتاج أنزيم Protease.