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Alkalophily Among Some Filamentous Fungi Isolated from Saudi Arabian Soils

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ABSTRACT. The growth, both radial and as biomass expressed by 13 fungal species from alkaline soils at two localities in the Makkah district was studied. No obligate alkalophily was found but facultative alkalophily was shown by *Cylindrocarpon heteronemum* and *Acremonium strictum*, while *Fusarium moniliforme* appeared to be highly alkaline-tolerant. Fast growing fungi such as *Rhizopus nigricans, Cunninghamella elegans, Rhizoctonia solani* and *Arthrobotrys oligospora* were alkaline-sensitive. Slower growing species were, on the other hand, alkaline-tolerant. A positive correlation was found between the level of alkalophily and the frequency of occurrence in Saudi soils of the Makkah District.

Growth studies of alkalophilic microorganisms are of great importance from the physiological, ecological and industrial standpoints. Most studies of alkalophiles so far have focussed on bacteria, especially *Bacillus* spp. (Guffanti *et al.* 1978, 1980, 1986, Gee *et al.* 1980, Koyama *et al.* 1983, Mclaggan *et al.* 1984). Alkalophily among fungi in general and within filamentous fungi in particular has clearly found less interest. So far only alkaline-tolerant organisms have been recorded. Thus, Battley and Barlett (1966) gave data for several yeasts which revealed upper pH limits of growth at 9. Matsushima *et al.* (1980) could isolate only alkaline-tolerant fungi and yeasts from soil and poultry excrements.

In previous work (Khodair *et al.*1991,Salama *et al.*1993)several filamentous fungi were isolated on alkaline media (pH 10.5) from alkaline soils (pH 8.5-9) of the Makkah District. Their appearance on isolation plates was not indicative as to whether they were obligate alkalophiles, facultative alkalophiles or alkaline-tolerant. In the present investigation the growth of 13 species belonging to 12 genera isolated from the aforementioned soils, were studied with respect to their level of alkalophily and to correlate, if possible, such levels with their frequency of occurrence in different soils.

Materials and Methods

Growth was estimated gravimetrically in liquid cultures and as radial growth rates on solid media. Sabouraud's medium was used consisting of (g/l) glucose. 40; peptone, 10; and agar, 20 when needed for solidification, Preliminary investigations on growth had proved the superiority of this medium over Czapek's medium and over that recommended by Horikoshi and Akiba (1982) for isolation of alkalophilic microorganisms. Two buffer systems, $Na_2CO_3 - NaHCO_3$ and $NaH_2PO_4 - NaOH$, were used to buffer the medium over a pH range of 6-11. The medium was adjusted at intervals of single pH units from 6-8 with phosphate buffer and pH 9-11 using carbonate buffer. The two buffers were separately sterilized before addition in suitable aliquots to the basal sterile medium just before inoculation. The carbonate buffer was sterilized by filtration. The final buffer concentration was 0.2M. Conical flasks (100 ml), each containing 25 ml buffered medium, were used for biomass estimations after 7 days growth at 25 °C on a reciprocal shaker (Model KS500, Janke and Kunkel, Ika-werk). Preliminary trials had proved the superiority of shaken over static cultures. For estimation of radial growth, Petri dishes (8.5 cm diam.), each poured with 20 ml medium, were used. Inoculation was by a 0.5 cm diam. disc from a 7 day-old agar culture of each fungus transferred to each flask or to the centre of each plate. Triplicate flasks or dishes were used for each treatment. Biomass was filtered off (whatman 41) at the end of the experimental period, washed several times with distilled water and then oven-dried at 80 °C to constant weight. On agar, colony diameters were measured daily for either one week or less according to growth rate. The initial and final pH values of the liquid media were measured by a Corning pH meter (Model 120). Fungal identification was accomplished through the references of Raper and Thom (1949), Raper and Fennel (1965), Barron (1968), Booth (1971), Barnett and Hunter (1972), Domsch and Gams (1972) and Domsch *et al.* (1980).

Results

Mycelial dry weight

The tested fungi responded differently to pH values. Increase in dry mycelial weight with increasing pH from 6 to 7. followed by a clear statistically significant drop was shown by *Rhizoctonia solani, Rhizopus nigricans, Scopulariopsis brevicaulis* and *Beauveria bassiana* (Table 1). A second group of fungi showed sensitivity to alkalinity at pH 9. A gradual increase in the amount of growth of such fungi (*Fusarium moniliforme, Paecilomyces divaricata, Aspergillus flavipes, Cunninghamella elegans, Penicillium notatum, Arthrobotrys oligospora* and *Acremonium murorum*) was evident with increased alkalinity until pH 8 after which a drop in dry mycelial weight accurred. A third group of fungi was less sensitive, responding negatively usually at pH 11 (*Cylindrocarpon heteronemum* and *Acremonium strictum*). These two fungi

Table 1. Mycelial dry weight (mg) after 7 days growth at different pH values

Fungal Species	Dry weight (mg) at initial pH					
	6	**7**	**8**	**9**	**10**	**11**
Cylindrocarpon heteronemum (Barkely and Broome) Wollenweber.	240	245	241	290	279	74
Acremonium strictum W. Gams.	132	138	166	170	182	75
Fusarium moniliforme Sheld.	230	315	295	150	140	145
Acremonium murorum (Corda) W. Gams.	485	680	580	292	183	62
Paecilomyces divaricata (Thom) Gillman and Abbott	118	129	150	98	96	28
Aspergillus flavipes Bain. and Sart.	265	295	345	225	170	57
Cunninghamella elegans Lendner.	305	285	300	132	47	42
Penicillium notatum Westling.	240	250	250	180	135	125
Arthrobotrys oligospora Fres.	145	165	225	82	81	81
Rhizoctonia solani Kuhn.	113	201	84	58	35	20
Rhizopus nigricans Ehrenb.	247	257	227	52	54	42
Scopulariopsis brevicaulis (Sacc.) Bain.	173	165	125	90	92	86
Beauveria bassiana *Vuill.	170	180	158	98	62	64

L.S.D.	**Between treatments**	**Within treatments**
at 0.05	19	16
at 0.01	22	21

* Isolated for the first time from Saudi soils.

produced a higher biomass at pH 9-10 than at pH 6. *Fusarium moniliforme* showed sensitivity at pH 9, which is comparatively earlier than the two aforementioned alkalophilic fungi, yet it could grow at the three highest pH values of 9, 10 and 11. When compared to dry weight at pH 6, a drop of 37% in biomass of *F. moniliforme* was evident at pH 11, whilst a drop of 69% in biomass of *Cylindrocarpon heteronemum*, the most alkalophilic organism, accurred at pH 11, indicating a lower sensitivity of the former to high alkalinity levels.

PH variation

The change in pH in liquid medium from initial values was generally limited (Table 2). Three exceptions were evident, *Rhizopus nigricans, Penicillium notatum* and *Beauveria bassiana,* where a significant fall in pH (from 6, 7, or 8 to 2.3 in case of *R. nigricans*; about 3.5 in case of *P. notatum* and to about 3.4 in case of *B. bassiana*) had occurred by the end of the experiment in media adjusted to pH values of 6,7 and 8. The final pH values in these media ranged from 2.3 to 3.75.

Radial growth

The fast growing fungi (*R. nigricans, A. oligospora, C. elegans, R. solani*) were significantly alkaline sensitive generally from pH 8 to 9 (Table 3 and Fig. 1). As indicated in Table 3 and Fig. 1 *R. nigricans* could not grow at pH 9, 10 or 11. Growth of *A. oligospora* at pH 9 and 10 was about 40% and 18% of that obtained at pH 6 respectively. *C. elegans* showed very feeble growth at pH 9 and could not grow at pH 10 or 11. Almost the same pattern of growth was exhibited by *R. solani.* Slow growing fungi (*A. murorum, P. divaricata, A. flavipes, P. notatum, S. brevicaulis, B. bassiana*) showed less sensitivity, being able to grow at even pH 11 at rates ranging from 1.9 - 3.1 mm/24h. Moderately growing species (*C. heteronemum, A. strictum, F. moniliforme*) were more tolerant to higher pH values. Their growth rates gradually declined with increase in alkalinity from 9 to 11. However they revealed higher growth rates than all other tested fungi at pH 11. indicating their prominent alkaline-tolerance (Table 3 and Fig. 1).

The main observation was that, on liquid media, *C. heteronemum* and *A. strictum* performed as facultative alkalophiles (Table 1) while, on solid media, they appeared as highly alkaline-tolerant (Table 3). A further difference between media was that the negative growth of *R. nigricans* and *C. elegans* (alkaline-sensitive) on agar media at pH 9-11 became positive, although limited, when cultured at the same pH values but under shaking conditions in liquid medium.

Table 2. Final pH Values after 7 days growth on buffered liquid media

Fungal Species	Final pH Values					
	6	7	8	9	10	11
Cylindrocarpon heteronemum (Barkely and Broome) Wollenweber.	6.34	7.03	7.23	9.61	9.45	10.14
Acremonium strictum W. Gams.	6.10	6.80	7.04	9.37	9.38	9.91
Fusarium moniliforme Sheld.	6.01	6.77	7.15	9.35	9.70	9.78
Acremonium murorum (Corda) W. Gams.	6.12	6.81	7.18	9.63	9.56	10.12
Paecilomyces divaricata (Thom) Gillman and Abbott	5.89	6.59	6.86	9.44	9.54	10.00
Aspergillus flavipes Bain. and Sart.	6.12	6.80	7.22	9.72	10.06	10.17
Cunninghamella elegans Lendner.	6.14	7.08	7.48	9.61	10.07	10.18
Penicillium notatum Westling.	3.27	3.54	3.75	9.39	10.20	10.35
Arthrobotrys oligospora Fres.	6.28	6.96	7.03	9.55	10.08	10.21
Rhizoctonia solani Kuhn.	6.09	6.65	7.40	10.00	9.58	10.19
Rhizopus nigricans Ehrenb.	2.30	2.30	2.30	10.10	9.63	10.27
Scopulariopsis brevicaulis (Sacc.) Bain.	6.25	6.99	7.54	9.62	10.03	10.09
Beauveria bassiana *Vuill.	3.09	3.36	3.81	9.56	10.12	10.20

Table-3. Rate of growth (colony diameter in mm/24h) of test fungi at different pH values

Fungal Species	**Rate of growth (mm/24 h) at pH**					
	6	**7**	**8**	**9**	**10**	**11**
Cylindrocarpon heteronemum (Barkely and Broome) Wollenweber.	10.6	10.4	10.1	6.3	5.0	3.9
Acremonium strictum W. Gams.	11.6	11.4	11.3	4.7	3.4	3.3
Fusarium moniliforme Sheld.	12.1	12.1	12.1	8.3	8.7	5.6
Acremonium murorum (Corda) W. Gams.	4.6	4.4	4.3	3.4	3.1	2.6
Paecilomyces divaricata (Thom) Gillman and Abbott	7.0	6.9	4.4	4.0	3.0	2.4
Aspergillus flavipes Bain. and Sart.	6.4	5.6	5.1	3.6	2.4	2.0
Cunninghamella elegans Lendner.	28.3	21.3	17.0	1.4	0.0	0.0
Penicillium notatum Westling.	9.1	9.4	8.0	2.9	2.0	2.6
Arthrobotrys oligospora Fres.	28.3	28.0	28.3	11.1	4.9	2.6
Rhizoctonia solani Kuhn.	21.3	16.8	16.6	2.9	1.3	0.0
Rhizopus nigricans Ehrenb.	41.5	28.3	28.3	0.0	0.0	0.0
Scopulariopsis brevicaulis (Sacc.) Bain.	7.9	8.1	8.9	4.5	3.7	3.1
Beauveria bassiana *Vuill.	4.6	4.6	4.1	2.6	2.5	1.9

L.S.D.	**Between treatments**	**Within treatments**
at 0.05	1.1	1.1
at 0.01	1.4	1.3

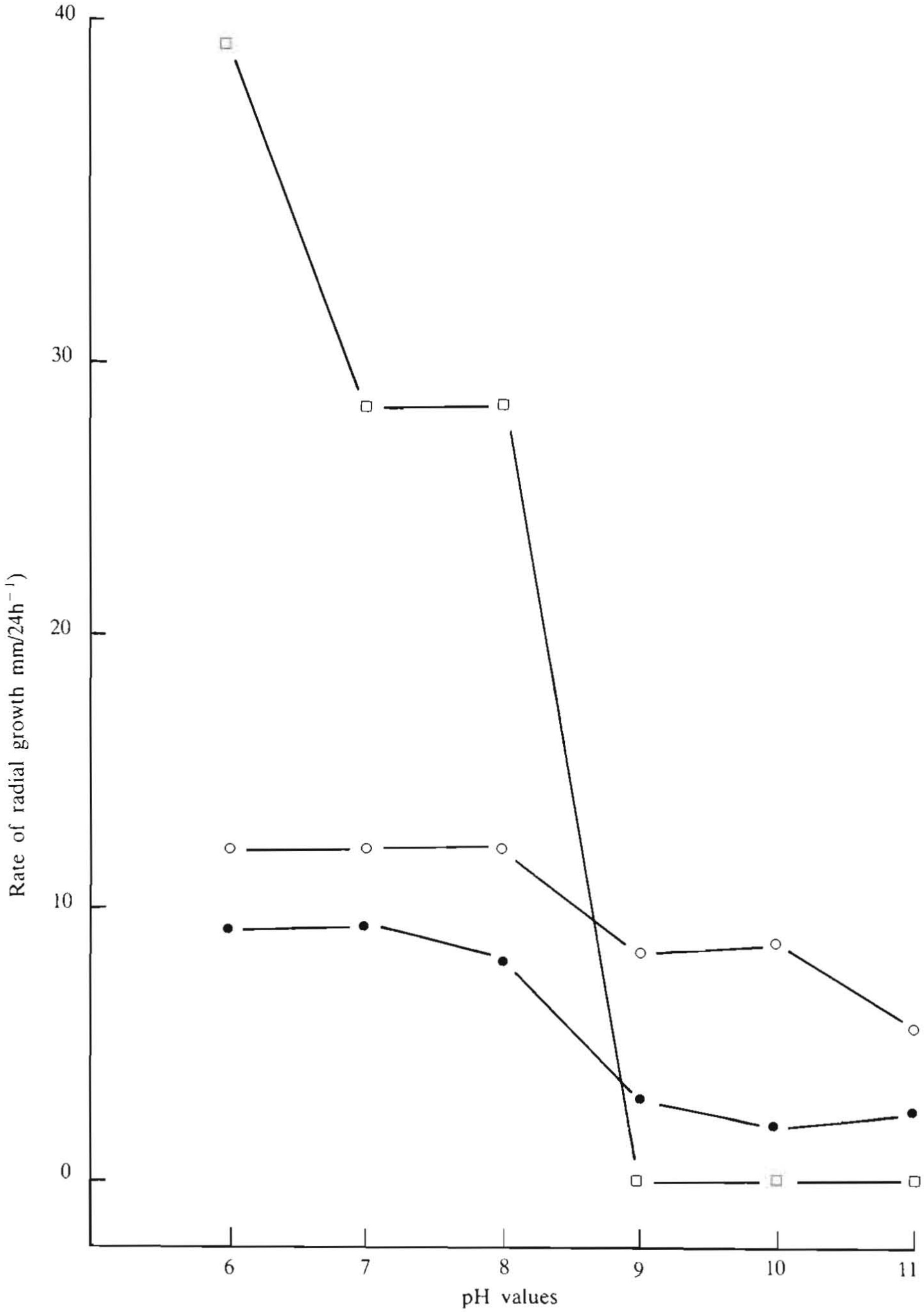

Fig. 1. Rate of radial growth (mm/24h) of 3 fungi representing fast rate of growth (*Rhizopus nigricans*, □), moderate growth (*Fusarium moniliforme*, ○) and slow growth (*Penicillium notatum*, ●) at different PH values.

Discussion

Horikoshi and Akiba (1982) defined alkalophilic microorganisms as those having growth optima at pH 9-10.5 and scarcely growing near the neutral range. Krulwich and Guffanti (1983) distinguished between alkaline-tolerant bacteria and obligate alkalophiles. They stated that alkaline-tolerant organisms grow well at pH 8.5 to 9, generally do not grow above 9.5, but grow at neutral pH values. By contrast, obligate alkalophiles generally can not grow below 8.5, do not grow at neutral pH but often exhibit optimal growth at pH 10 or above. On the other hand, facultative alkalophiles can grow both in the neutral and highly alkaline pH ranges (Gee *et al.* 1980, Guffanti *et al.* 1986).

Very few characterizations of alkalophiles have been conducted using chemostat conditions in which a constant pH value is maintained (Krulwich and Guffanti 1983). In the present study, the growth media remained at nearly constant pH till the end of the experimental period except for *Rhizopus nigricans, Penicillium notatum* and *Beauveria bassiana*, where a significant drop (pH 2.3-3.75) was finally evident in media initially buffered to pH values of 6, 7 and 8. No correlation was found between the drop in pH and the amount of biomass produced. The incapability of the buffering system, most probably, was due to a relatively high production of organic acids unbalanced by alkaline substances such as ammonia. However, the pH of growth media of all other test fungi could be maintained practically constant during the whole experimental period, thus fulfilling one of the most important requirements in the study of alkalophily.

The two methods that were used for measuring growth, biomass and radial measurements, both revealed that most test organisms could grow at all pH values from 6 to 11 but with different yields. According to the definitions of alkalophiles (mostly bacilli) given by Horikoshi and Akiba (1982), Krulwich and Guffanti (1983) and Guffanti *et al.* (1978, 1980) none of the fungi can be defined as obligate alkalophiles, but rather as facultative alkalophiles or alkaline-tolerants.

Cylindrocarpon heteronemum and *Acremonium strictum* gave, in the present investigation, statistically higher biomass on the alkaline side (pH 9 and 10) than that produced on the acidic side. In case of *C. heteronemum* there was about 21% increase in biomass at pH 9 relative to pH 6 or 7 and in case of *A. strictum* there was about 38% increase in biomass at pH 10 relative to pH 6 or 7 (Table 1). They can thus be considered as facultative alkalophiles. This is a new record since, as far as the authors are aware, filamentous fungi showing facultative alkalophily have not earlier been described.

On agar media, these two fungi appeared as alkaline-tolerant. Their capability to grow better at higher pH values in liquid cultures under shaken conditions than on solid media may be due to factors such as higher availability of oxygen and of nutrients in liquid culture. A similar situation was found with *Rhizopus nigricans* which failed completely to grow on agar media at pH 9 and above, while it grew slowly in shaken conditions at pH values ranging from 9-11. Accordingly, biomass estimation is suggested as a more sensitive method. In the case of *Fusarium moniliforme*, both methods indicated that it is alkaline-tolerant. Johnson (1923) reported that *Penicillium variable, Fusarium bullatum* and *F. oxysporum* could grow at a pH value of 11. Recently, alkaline-tolerant filamentous fungi and yeasts were also isolated from soils and poultry excrements (Matsushima *et al.* 1980).

In the present study and as a first record, a relationship between rate of radial growth on solidified media and alkalophily could be established. Fast growing fungi on plates, represented by *R. nigricans, C. elegans, Rhizoctonia solani* and *A. oligospora* appeared to be alkaline-sensitive. On the other hand, moderately growing fungi, represented by *F. moniliforme, C. heteronemum* and *A. strictum,* and slow growing fungi, represented by *A. murorum, P. divaricara, A. flavipes* and *P. notatum,* appeared to be alkaline-tolerant although on different levels.

The alkaline-tolerance of the fungi analyzed in the present study agrees well with earlier records of occurrence frequencies in Saudi alkaline soils; the more tolerant fungi were more commonly inrated (Khodair *et al.* 1991 Salama *et al* 1993).

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حب القلوية بين بعض الفطريات الخيطية المعزولة من تربة سعودية

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تتصف دراسات النمو للكائنات الدقيقة المحبة للقلوية بأهميتها الكبيرة من الوجهة الفسيولوجية، البيئية والصناعية. ولم تلق الفطريات وخصوصاً الخيطية منها - بعكس البكتيريا - الاهتمام الكافي. والى الآن لم يسجل إلا القليل من الفطريات المتحملة للقلوية العالية. ولقد تمكن عدد من الباحثين (خضير، رمضاني وعقاب ١٩٩١م، سلامة، عقاب ورمضاني ١٩٩٢م) من عزل عدد من الفطريات الخيطية على وسط غذائي قلوي (رقم إيدروجيني ١٠,٥) من عينات من التربة القلوية (رقم ايدروجيني ٨,٥ - ٩) في منطقة مكة. ولم يكن ظهور هذه الفطريات على أطباق العزل تحت هذه الظروف دالاً على مدى حبها للقلوية من حيث كونها محبة للقلوية اجباراً أو محبة لها اختياراً أو متحملة للقلوية. وبناء على ذلك، تم في البحث الحالي دراسة نمو ١٣ نوعاً فطرياً تنتمي الى ١٢ جنساً عزلت كلها من التربة السابق ذكرها بهدف التعرف على مدى محبتها للقلوية، ومحاولة ربط هذا المدى بمستوى تواجد هذه الفطريات في عينات التربة المختلفة. وقدر نمو كل من هذه الفطريات على وسط سابورو السائل (للحصول على وزن الميسليوم الجاف) وعلى نفس الوسط متصلبا (لتعيين النمو القطري) تحت ظروف منضبطة من الرقم الايدروجيني (من ٦ الى ١١). وأثبتت الدراسات المبدئية تميز المزارع السائلة المهتزة عن الساكنة.

وأظهرت النتائج غياب حب القلوية اجباراً من الفطريات المدروسة (لا تنمو عادة تحت الرقم الايدروجيني ٥,٨، ولا تنمو اطلاقاً عند الرقم المتعادل، ولكنها تنمو مثاليا عند الرقم ١٠ فأكثر). أما حب القلوية اختياراً (نمو أكبر عند الأرقام ٩، ١٠ منه تحت الظروف الحامضية عند رقم ٦) فقد تمثل في الأنواع *Cylindrocarpon heteronemum* (Barkely and Broome) Wollenweber. ، و *Acremonium strictum* W. Gams. وهي نتيجة غير مسجلة فيما سبق من دراسات في هذا المجال. أما الفطرة *Fusarium moniliforme* Sheld. فقد أظهرت قدرة عالية على تحمل القلوية (تنمو جيداً عند الرقم ٥,٨ ـ ٩، ولا تنمو عادة فوق ٥,٩ ولكنها تنمو عند رقم التعادل).

كما أوضحت الدراسة الحالية ـ ولأول مرة ـ علاقة وثيقة بين معدل النمو القطري للفطريات الخيطية على الأوساط المتصلبة ومستوى حبها للقلوية، حيث اتصفت الفطريات سريعة النمو مثل *Rhizopus nigricans* Ehrenb. و *Cunninghamella elegans* Lendner. و *Scopulariopsis brevicaulis* و *Rhizoctonia solani* Kuhn. و *Arthrobotrys oligospora* Fres. بحساسيتها الواضحة للقلوية.

وأظهر البحث أيضاً أفضلية استخدام الكتلة الحية على الأوساط السائلة المهتزة (للحصول على الوزن الجاف للعزل الفطري) عند استخدام الامتداد القطري على الأوساط المتصلبة كوسيلة لقياس نمو الفطريات الخيطية بغرض تحديد مدى حبها للقلوية نظراً لما توفره الطريقة الأولى من أوكسجين وأغذية متاحة عن الطريقة الأخيرة.

وأوضح البحث الحالي علاقة موجبة بين مستوى حب القلوية للفطريات المدروسة ومدى تواجدها في التربة السعودية.