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Studies on the distribution of alkalophilic and alkali-tolerant soil fungi I

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Soil fungi were isolated from two different soil types using alkaline and slightly acidic media (alkaline cornmeal agar (ACMA), pH 9.7; cornmeal agar (CMA), pH 6.0) to study their distribution. Different species were obtained on each isolation medium. The number of species of *Acremonium* and *Fusarium* increased on ACMA, though many species growing well in acidic conditions were not detected on ACMA. Most of the fungi isolated on ACMA, especially from the alkaline soils, were alkalophiles or alkali-tolerants that can grow at pH 10. *Acremonium alternatum, A. furcatum, Acremonium* sp. 6, *Gliocladium cibotii* (YBLF 575), *Phialophora geniculata, Stachylidium bicolor* and *Stilbella annulata* were alkalophilic, of which *Acremonium* sp. 6 was the most pronounced alkalophile. Ability to grow under alkaline conditions, as well as under acidic conditions, was common in many *Acremonium* species. The use of alkaline medium facilitates the isolation of alkalophilic soil fungi.

Key Words——alkaline isolation medium; alkalophilic; distribution; soil fungi.

Most species of terrestrial fungi are considered to germinate and grow well in weakly acidic to neutral pH range (Park, 1968). Only a few investigators have reported on fungi growing under alkaline conditions. An alkali-tolerant black yeast, *Exophiala alcalophila* Goto & J. Sugiyama, was isolated from Japanese soils during a survey of alkalophiles (Goto et al., 1981). In the subsequent research by Aono (1990), several yeasts were found to be able to grow at pH above 10. Okada et al. (1993) recently described a new alkalophilic hyphomycete. The distribution of such fungi is still unknown.

Many studies on fungal communities in soils have been presented in the last several decades (Domsch et al., 1980; Carroll and Wicklow, 1992; Gams, 1992), and some alkaline soils were examined for their fungal flora (Warcup, 1951; Stenton, 1953; Nicholls, 1956; Mukerji, 1965; Pugh and Dickinson, 1965; Rai et al., 1971). In most of the studies, isolation media were used in acidic to neutral pH ranges. For some alkalophiles, these acidic media are presumably not suitable for growth. It is necessary to examine whether the pH of the isolation media affects the observed species diversity. Therefore, we used both alkaline and acidic media to isolate fungi from two different soil types, weakly acidic forest soils from Japan and alkaline soils from Indonesia.

This study was undertaken to investigate the distribution of alkalophilic and alkali-tolerant fungi in different soil types. To achieve this goal, the effect of isolation media was evaluated first. Using alkaline and acidic isolation media, we obtained different results on the number and composition of the species isolated. Then the effect of pH on growth of the isolates was examined, because the differences in species composition might be due to pH responses in different species.

Materials and Methods

Soil samples Two different soils, acidic and alkaline ones, were used. The acidic soil samples (pH 5 to 6 in H_2O) were randomly collected from ca. 10 cm depth in deciduous broad-leaved or evergreen oak forests at Oita Pref., Japan on 25 Sep. 1992. The alkaline soil samples (pH 7 to 8 in H_2O) were collected from 10–15 cm depth in grassland or cultivated areas at Jayapura, West Irian, Indonesia on 28 May 1992. Fifteen samples from each soil type were used for isolation.

Isolation of fungi Dilution plates were prepared from 1 g (fresh weight) of each soil sample, at dilutions of 10, 10^2, 10^3 in test-tubes with sterile physiological saline solution, on cornmeal agar (CMA, pH 6.0; Nissui, Tokyo) and alkaline cornmeal agar (ACMA, pH ca. 9.7) both containing 100 mg/l chloramphenicol.

ACMA was prepared with solution A (17 g CMA powder, 900 ml distilled water) and solution B (3 g Na_2CO_3, 3 g $NaH_2PO_4 \cdot 2H_2O$, 100 ml distilled water). After sterilization, 900 ml of solution A and 100 ml of solution B

Table 1. Composition of buffer solution for the pH-adjustment of malt extract and Sabouraud dextrose agars.

Basal medium (900 ml)	Final pH	Composition of buffer solution (mmol/100 ml)			
		Na_2CO_3	$NaHCO_3$	Na_2HPO_4	$NaH_2PO_4 \cdot 2H_2O$
MA	11.0	50.0[a]			
	10.0	27.5	22.5		
	9.0	3.0	47.0		
	8.0			49.0	1.0
	7.0			27.5	22.5
	6.0			5.0	45.0
	5.0				50.0[d]
SA	11.0	50.0[b]			
	10.0	50.0[c]			
	9.0	25.0	25.0		
	8.0	9.0	41.0		
	7.0			40.0	10.0
	6.0			10.0	40.0
	5.0				50.0[e]

[a]pH adjusted to 12.7 with 1 N NaOH. [b]pH adjusted to 13.3 with 1 N NaOH. [c]pH adjusted to 12.6 with 1 N NaOH. [d]pH adjusted to 3.8 with 1 N HCl. [e]pH adjusted to 3.2 with 1 N HCl.

were mixed. The final pH of the mixture was about 9.7.

Four plates were prepared for each dilution series, and all were incubated at 24°C for one to three weeks. The growing fungi were observed under the light microscope, and representative strains were isolated. All isolates are maintained in Yamanouchi Pharmaceutical Co., Ltd. (YBLF), some in the Japan Collection of Microorganisms (JCM) and some in the Centraalbureau voor Schimmelcultures (CBS).

Identification of fungi The following literature was mainly used for identification of the isolates: Gams (1971) for *Acremonium*; Booth (1971) and Gerlach and Nirenberg (1982) for *Fusarium*; Ellis (1971, 1976), Matsushima (1975), Barron (1968), Carmichael et al. (1980) and Domsch et al. (1980) for other deuteromycetes; and Gams (1977) for *Mortierella*.

Growth rates of the isolates at various pH In each species, one strain was selected at random as a representative. The strains were inoculated onto malt extract agar (MA: 10 g malt extract powder (Difco, Detroit), 2.5 g peptone, 20 g agar, 1 L distilled water) of which the initial pH was adjusted after autoclaving to 5.0, 6.0, 7.0, 8.0, 9.0, 10.0 and 11.0 with buffers. The media were prepared by mixing 900 ml of the above MA formula with 100 ml of one of the buffer solutions listed in Table 1. Growth patterns of the species were obtained by measuring twice the colony diameter of the representative strains after incubation for one to two weeks.

Strains which could not grow at pH 10 are regarded as alkalophobic, those which grew at pH 10 as alkali-tolerant, and those which could grow at pH up to 10, but not at pH 5-6, as alkalophilic. Growth patterns of the selected alkalophilic fungi were determined using Sabouraud dextrose agar (SA; Difco, Detroit) at various pHs. The pH of SA was also adjusted by mixing 900 ml of the SA formula with 100 ml of one of the buffer solutions shown in Table 1.

Results

Fungal species isolated from acidic soils The species and some unidentified taxa isolated from the weakly acidic soils from Japan are listed in Table 2. Fifty-eight taxa were recorded in total from CMA and ACMA. Thirty taxa were recorded on CMA and 35 taxa on ACMA. Only 7 taxa were common to both media. Twenty-three and 28 taxa were recorded only on CMA and ACMA, respectively. Many taxa isolated on CMA were species of *Mortierella, Mucor, Trichoderma, Gliocladium* and *Fusarium*. On ACMA, all the species of *Mortierella, Mucor* and *Trichoderma* and some *Gliocladium* species were absent. One species of *Acremonium* was common to both media, but the other two were isolated only on ACMA. In *Fusarium*, 3 taxa were common to both and 2 others appeared only on ACMA. The common *Fusarium* taxa were found more frequently on ACMA than on CMA. Other common species were *Gliocladium cibotii, G. roseum* and *Volutella ciliata*. The latter two species also showed higher frequencies on ACMA. *Cylindrocladium scoparium, Plectosporium tabacinum* and *Phoma* sp. 1 were isolated only on ACMA.

The optimum pH for the growth of representative strains of each species was established (Table 3). Thirty-nine (67.2%) of the 58 taxa grew at pH 10, and they were designated as alkali-tolerant or alkalophilic fungi. Although many taxa isolated from acidic soils showed the optimum growth in acidic conditions, a few species (*Acremonium furcatum, A. murorum, Wardomyces inflatus*, etc.) grew better on alkaline medium than acidic.

Fungal species isolated from alkaline soils Forty-seven taxa were isolated from the weakly alkaline soils from Indonesia (Table 4). Seventeen species were recorded on CMA and 33 taxa on ACMA. Only 3 species were common to both media. Comparing these results with those from acidic soils from Japan, the following conclusions are obvious: 1) no mucoralean fungi were isolated, 2) *Trichoderma* species were less abundant on CMA. The species common to both media were *Acremonium alternatum, Fusarium solani* and *Stachybotrys bisbyi*. Although 10 *Acremonium* species were recorded in total, only 1 species was obtained on CMA and all species on ACMA. Also in *Fusarium*, 1 species was found on CMA and 4 others on ACMA. These two genera showed increased levels on ACMA in both acidic and alkaline soils. *Phialophora geniculata, Phoma* sp. 2 and *Stilbella annulata* were isolated on ACMA at high frequencies. Most of the isolates (78.7%) can grow at pH 10 (Table 5). The optimum pH for growth of most *Acremonium* species was in the alkaline range (above pH 9).

Growth patterns of alkalophilic and alkali-tolerant fungi Some isolates on CMA were alkalophobic: i.e., *Trichoderma* spp., *Mortierella* spp., *Mucor* spp. and *Gliocladium* spp. (except for *G. cibotii* and *G. roseum*). *Trichoderma*

Table 2. Frequency of occurrence of fungi in fifteen acidic soil samples.

Species/Taxon	Frequency (Max. 15)	
	CMA	ACMA
Acremonium furcatum (F. & V. Moreau) ex W. Gams		5
A. murorum (Corda) W. Gams	1	3
A. persicinum (Nicot) W. Gams		1
Alternaria sp. 1		1
Alternaria sp. 2		1
Alternaria sp. 3		1
Aspergillus sp. 1	1	
Aspergillus sp. 2	1	
Beauveria bassiana (Bals.) Vuill.		2
B. brongniartii (Sacc.) Petch		1
Chloridium virescens var. *chlamydosporum* (van Beyma) W. Gams & Hol.-Jech.	1	
Cladosporium sp. 1	5	
Cladosporium sp. 2		1
Cordana pauciseptata Preuss	2	
Cunninghamella sp.	1	
Cylindrocladium scoparium Morgan		4
Dactylella musiformis (Drechsler) Matsushima		3
Fusarium acuminatum Ellis & Everh.	1	5
F. lateritium Nees		4
F. sacchari var. *subglutinans* (Wollenw. & Reinking) Nirenberg	1	10
F. solani (Mart.) Sacc.	6	12
Fusarium sp. 1		1
Gliocladium cibotii van Beyma	1	1
G. roseum Bain.	4	12
G. virens Miller, Giddens & Foster	1	
G. viride Matr.	3	
Isaria sp.		1
Metarhizium anisopliae (Metschn.) Sorok.		2
Microthecium retisporum Udagawa & Cain	1	
Monacrosporium bembicodes (Drechsler) Subram.		1
Mortierella isabellina Oudem.	2	
M. vinacea Dixon-Stewart	5	
Mucor sp. 1	4	
Mucor sp. 2	2	
Paecilomyces sp. 1	4	
Paecilomyces sp. 2		1
Papulaspora sp.		1
Penicillium sp. 1	1	
Penicillium sp. 2	1	
Penicillium sp. 3	1	
Penicillium sp. 4		1
Penicillium sp. 5		1
Phoma sp. 1		6
Plectosporium tabacinum (van Beyma) M. Palm et al.		6
Sesquicillium buxi (Schmidt: Fr.) W. Gams		1
Sporothrix sp. 1	1	
Sporothrix sp. 2	1	
Trichoderma hamatum (Bon.) Brain.	3	
T. harzianum Rifai	2	
Trichoderma sp. 1	6	
Trichoderma sp. 2	3	
Verticillium bulbillosum W. Gams & Malla		2
Verticillium sp. 1		1
Verticillium sp. 2		2
Volutella ciliata (Alb. & Schw.: Fr.) Fr.	1	3
Wardomyces inflatus (Marchal) Hennebert		1
Unidentified hyphomycete 1		1
Unidentified hyphomycete 2		1

Table 3. Optimum growth pH on MA for the isolates from acidic soil.

Optimum pH	Species/Taxon	Isolated on CMA/ACMA		Strain No.
>10	***Acremonium furcatum***[a]		○	YBLF 573 (=JCM 9210)
9	***Wardomyces inflatus***		○	YBLF 654
	Acremonium murorum	○	○	YBLF 586 (=JCM 9204)
8	**_Alternaria_ sp. 3**		○	YBLF 593
	Monacrosporium bembicodes		○	YBLF 610
	Verticillium sp. 2		○	YBLF 578
	Fusarium solani	○	○	YBLF 562
	Gliocladium cibotii	○	○	YBLF 588 (=JCM 9206)
7	***Acremonium persicinum***		○	YBLF 583 (=JCM 9212)
	Beauveria bassiana		○	YBLF 596
	Dactylella musiformis		○	YBLF 611
	Metarhizium anisopliae		○	YBLF 627
	Paecilomyces sp. 2		○	YBLF 619
	Plectosporium tabacinum		○	YBLF 570 (=JCM 9207)
	Verticillium bulbillosum		○	YBLF 582 (=JCM 9213)
	Volutella ciliata	○	○	YBLF 572 (=JCM 9201)
	Aspergillus sp. 1	○		YBLF 594
	Aspergillus sp. 2	○		YBLF 595
6	***Beauveria brongniartii***		○	YBLF 597
	Fusarium lateritium		○	YBLF 565
	Penicillium sp. 4		○	YBLF 632
	Phoma sp. 1		○	YBLF 661
	Sesquicillium buxi		○	YBLF 641
	Verticillium sp. 1		○	YBLF 652
	Unidentified hyphomycete 1		○	YBLF 671
	Unidentified hyphomycete 2		○	YBLF 568
	Fusarium sacchari var. _subglutinans_	○	○	YBLF 563
	Cladosporium sp. 1	○		YBLF 602
	Paecilomyces sp. 1	○		YBLF 628
	Mucor sp. 2	○		YBLF 656
<5	**_Alternaria_ sp. 1**		○	YBLF 591
	Alternaria sp. 2		○	YBLF 592
	Cladosporium sp. 2		○	YBLF 601
	Cylindrocladium scoparium		○	YBLF 608
	Fusarium sp. 1		○	YBLF 569
	Isaria sp.		○	YBLF 623
	Penicillium sp. 5		○	YBLF 633
	Papulaspora sp.		○	YBLF 567
	Fusarium acuminatum	○	○	YBLF 564
	Gliocladium roseum	○	○	YBLF 614
	Cunninghamella sp.	○		YBLF 657
	Penicillium sp. 2	○		YBLF 634
	Chloridium virescens var. *chlamydosporum*	○		YBLF 599
	Cordana pauciseptata	○		YBLF 603
	Gliocladium virens	○		YBLF 616
	G. viride	○		YBLF 618
	Microthecium retisporum	○		YBLF 658
	Mortierella isabellina	○		YBLF 659
	M. vinacea	○		YBLF 625
	Mucor sp. 1	○		YBLF 655
	Penicillium sp. 1	○		YBLF 631
	Penicillium sp. 3	○		YBLF 635
	Sporothrix sp. 1	○		YBLF 643
	Sporothrix sp. 2	○		YBLF 642
	Trichoderma hamatum	○		YBLF 649
	T. harzianum	○		YBLF 650
	Trichoderma sp. 1	○		YBLF 648
	Trichoderma sp. 2	○		YBLF 651

[a] Bold characters indicate the species that can grow at pH 10.

Table 4. Frequency of occurrence of fungi in fifteen alkaline soil samples.

Species/Taxon	Frequency (Max. 15)	
	CMA	ACMA
Acremonium alternatum Link: Fr.	1	2
A. persicinum		1
A. polychromum (van Beyma) W. Gams		1
Acremonium sp. 1		2
Acremonium sp. 2		1
Acremonium sp. 3		1
Acremonium sp. 4		2
Acremonium sp. 5		1
Acremonium sp. 6		2
Acremonium sp. 7		1
Aspergillus sp. 1	4	
Cercospora sp.		1
Cladosporium sp. 3	2	
Cylindrocarpon sp.		2
Dactylaria sp.	1	
Fusarium buxicola Sacc.		1
F. oxysporum Schlecht.: Fr.		1
F. solani	9	9
F. ventricosum Appel & Wollenw.		3
Fusarium sp. 2		1
Geotrichum sp.	1	
Gliocladium cibotii		2
G. roseum		1
G. virens	2	
G. viride	2	
Gonytrichum macrocladum (Sacc.) S. Hughes	1	
Graphium sp. 1		1
Graphium sp. 2		1
Idriella lunata P. E. Nelson & Wilhelm		1
Myrothecium roridum Tode: Fr.		1
Penicillium sp. 6	1	
Penicillium sp. 7		1
Pestalotiopsis sp.	1	
Phialophora geniculata van Emden		6
Phialophora sp.		1
Phoma sp. 2		6
Pseudobotrytis terrestris (Timonin) Subram.	2	
Pseudogliomastix protea (Sacc.) W. Gams & Boekhout		1
Rhinocladiella sp.	1	
Scopulariopsis brumptii Salvanet-Duval		1
Stachybotrys albipes (Berk. & Br.) Jong & Davis	3	
S. bisbyi (Srinivasan) Barron	2	1
Stachylidium bicolor Link: Fr.		2
Stilbella annulata (Berk. & M. A. Curtis) Seifert		4
Trichoderma sp. 2	2	
Tritirachium sp.	1	
Unidentified hyphomycete 3		2

Table 5 Optimum growth pH on MA for the isolates from alkaline soil.

Optimum pH	Species/Taxon	Isolated on CMA/ACMA		Strain No.
>10	**_Acremonium alternatum_**[a)]		○	YBLF 581
	Acremonium sp. 6		○	YBLF 726 (=CBS 681.94)
	Gliocladium cibotii		○	YBLF 575
	Phialophora geniculata		○	YBLF 587 (=CBS 658.94)
	Stachylidium bicolor		○	YBLF 646
	Stilbella annulata		○	YBLF 647
9	**_Acremonium_ sp. 1**		○	YBLF 579 (=CBS 682.94)
	Acremonium sp. 2		○	YBLF 574 (=CBS 737.94)
	Acremonium sp. 3		○	YBLF 580 (=CBS 683.94)
	Acremonium sp. 4		○	YBLF 576
	Graphium sp. 1		○	YBLF 620
	Graphium sp. 2		○	YBLF 621
	Myrothecium roridum		○	YBLF 626
	Scopulariopsis brumptii		○	YBLF 640
	Stachybotrys albipes	○		YBLF 645
8	**_Acremonium persicinum_**		○	YBLF 590
	A. polychromum		○	YBLF 725
	Acremonium sp. 5		○	YBLF 577 (=CBS 741.94)
	Acremonium sp. 7		○	YBLF 723 (=CBS 685.94)
	Cylindrocarpon sp.		○	YBLF 606
	Gliocladium roseum		○	YBLF 613
	Phialophora sp.		○	YBLF 665
	Phoma sp. 2		○	YBLF 660
	Pseudogliomastix protea		○	YBLF 719 (=CBS 656.94)
	Fusarium solani	○	○	YBLF 551
	Penicillium sp. 6	○		YBLF 629
7	**_Cercospora_ sp.**		○	YBLF 598
	Fusarium oxysporum		○	YBLF 557
	Unidentified hyphomycete 3		○	YBLF 666
	Aspergillus sp. 1	○		YBLF 728
6	**_Fusarium buxicola_**		○	YBLF 558
	F. ventricosum		○	YBLF 554
	Fusarium sp. 2		○	YBLF 555
	Idriella lunata		○	YBLF 622
	Stachybotrys bisbyi	○	○	YBLF 644
	Dactylaria sp.	○		YBLF 609
	Rhinocladiella sp.	○		YBLF 639
	Gonytrichum macrocladum	○		YBLF 637
	Pestalotiopsis sp.	○		YBLF 636
<5	_Penicillium_ sp. 7		○	YBLF 630
	Cladosporium sp. 3	○		YBLF 600
	Geotrichum sp.	○		YBLF 612
	Gliocladium virens	○		YBLF 615
	G. viride	○		YBLF 617
	Pseudobotrytis terrestris	○		YBLF 638
	Trichoderma sp. 2	○		YBLF 729
	Tritirachium sp.	○		YBLF 670

[a)]Bold characters indicate the species that can grow at pH 10.

sp. 2, *G. virens* and *G. viride* were isolated from both acidic and alkaline soils, and the isolates of these species showed the same alkalophobic growth patterns (Tables 3, 5). On the other hand, many isolates grew at pH 10 as well as in acidic conditions. *Fusarium* species isolated from acidic soils, for instance, grew well in acidic to neutral range and considerably at pH 10. They are considered as typical alkali-tolerant fungi (Fig. 1A). The isolates from alkaline soils showed similar patterns, but they were much more alkali-tolerant than those from acidic soils (Fig. 1B). Two strains of *F. solani* (YBLF 562 and YBLF 551) isolated from different soil types showed the same growth patterns.

Many *Acremonium* species were isolated, and they are classified into three or more groups (Table 6). All the *Acremonium* isolates grew very well under alkaline conditions and three species were found to be alkalophilic. The optimum growth for these alkalophilic *Acremonium*

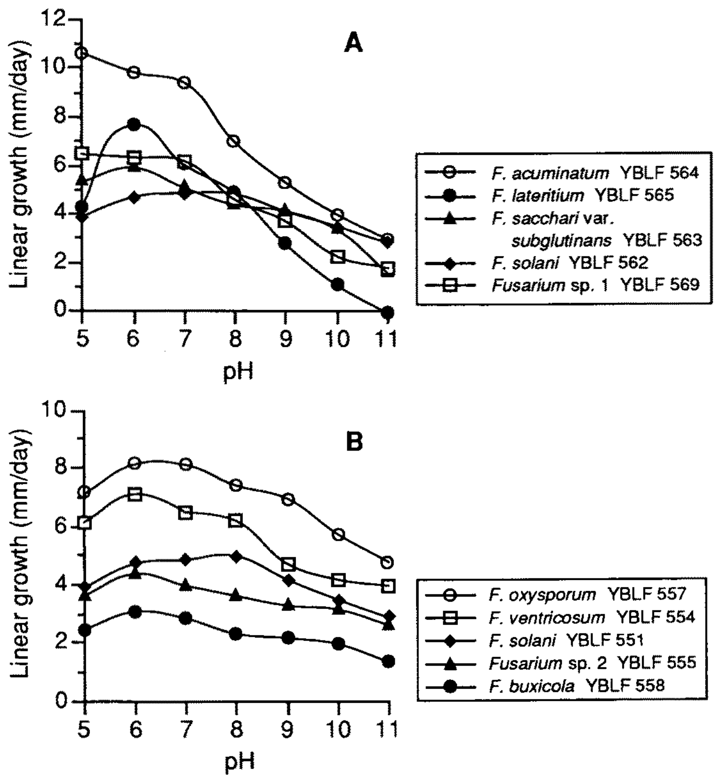

Fig. 1. Effect of pH on the growth on MA of *Fusarium* species. A. Species isolated from acidic soils. B. Species isolated from alkaline soils.

species was observed on the alkaline side (above pH 8), but they showed slightly different growth rates on MA and SA (Figs. 2A-2C). *Acremonium furcatum* (Sect. *Nectrioidea*) grew faster than other alkalophilic *Acremonium* species, and it grew constantly well between pH 7 and pH 11 (Fig. 2A). *Acremonium alternatum* (Sect. *Acremonium*) grew best at pH 8 and declined slightly above pH 8 on SA (Fig. 2B). *Acremonium* sp. 6 (Sect. *Acremonium*) grew well above pH 10 on MA and SA, and this is the most strictly alkalophile among the isolates (Fig. 2C).

Acremonium furcatum was isolated from acidic soils, while *Acremonium alternatum* and *Acremonium* sp. 6 were isolated from alkaline soils. The growth patterns of these strains are the same, though they belong to different species.

We isolated four other alkalophiles from alkaline soils: *Gliocladium cibotii, Phialophora geniculata, Stachy-*

Table 6. Classification of *Acremonium* isolates.

Section	Species	Strain No.
Nectrioidea	*Acremonium furcatum*	YBLF 573 (=JCM 9210), YBLF 708 (=JCM 9211), YBLF 709, YBLF 710, YBLF 711
	Acremonium sp. 1	YBLF 556 (=CBS 655.94), YBLF 579 (=CBS 682.94), YBLF 727
	Acremonium sp. 2	YBLF 574 (=CBS 737.94)
	Acremonium sp. 3	YBLF 580 (=CBS 683.94)
Acremonium	*A. alternatum*	YBLF 581, YBLF 584, YBLF 714
	Acremonium sp. 4	YBLF 576, YBLF 717 (=CBS 684.94)
	Acremonium sp. 5	YBLF 577 (=CBS 741.94)
	Acremonium sp. 6	YBLF 585 (=CBS 630.94), YBLF 726 (=CBS 681.94)
Gliomastix	*A. murorum*	YBLF 586 (=JCM 9204), YBLF 703, YBLF 704 (=JCM 9205), YBLF 730
	A. persicinum	YBLF 583 (=JCM 9212), YBLF 590
	A. polychromum	YBLF 725
	Acremonium sp. 7[a]	YBLF 723 (=CBS 685.94)

[a]Included tentatively in the Section *Gliomastix*.

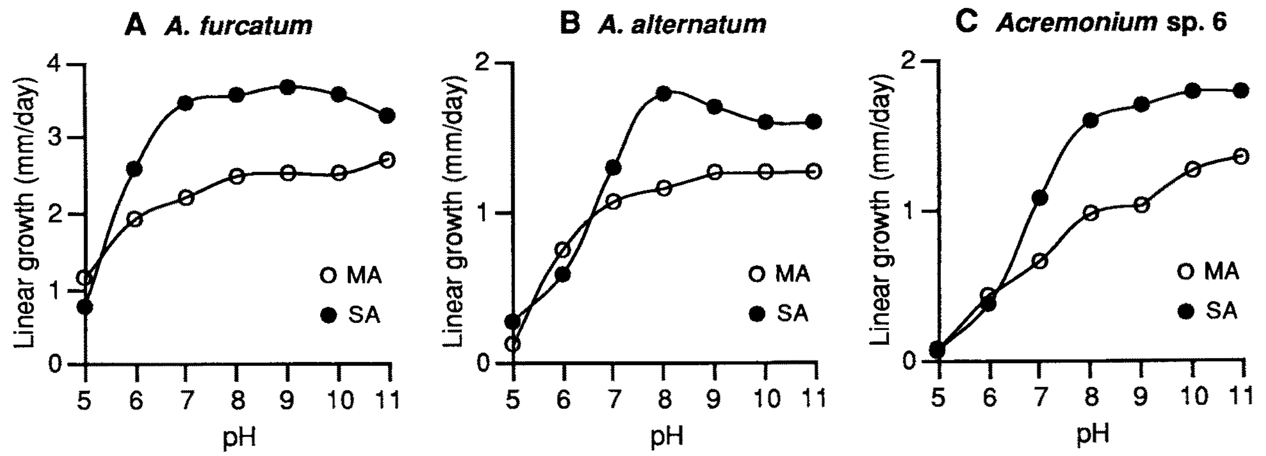

Fig. 2. Growth patterns on MA and SA of alkalophilic *Acremonium* species. A. *A. furcatum* (YBLF 573). B. *A. alternatum* (YBLF 581). C. *Acremonium* sp. 6 (YBLF 726).

lidium bicolor and *Stilbella annulata* (Figs. 3A-3D). They showed somewhat different growth patterns on MA and SA, and their growth declined rapidly at pH 5. *Gliocladium cibotii* was also isolated from acidic soils. In contrast to the strain from alkaline soil (YBLF 575), the optimum growth pH of that from acidic soil (YBLF 588) was slightly lower (Tables 3, 5).

Discussion

Effect of isolation media on the isolates On dilution plates with an alkaline medium (ACMA), some alkalophil-

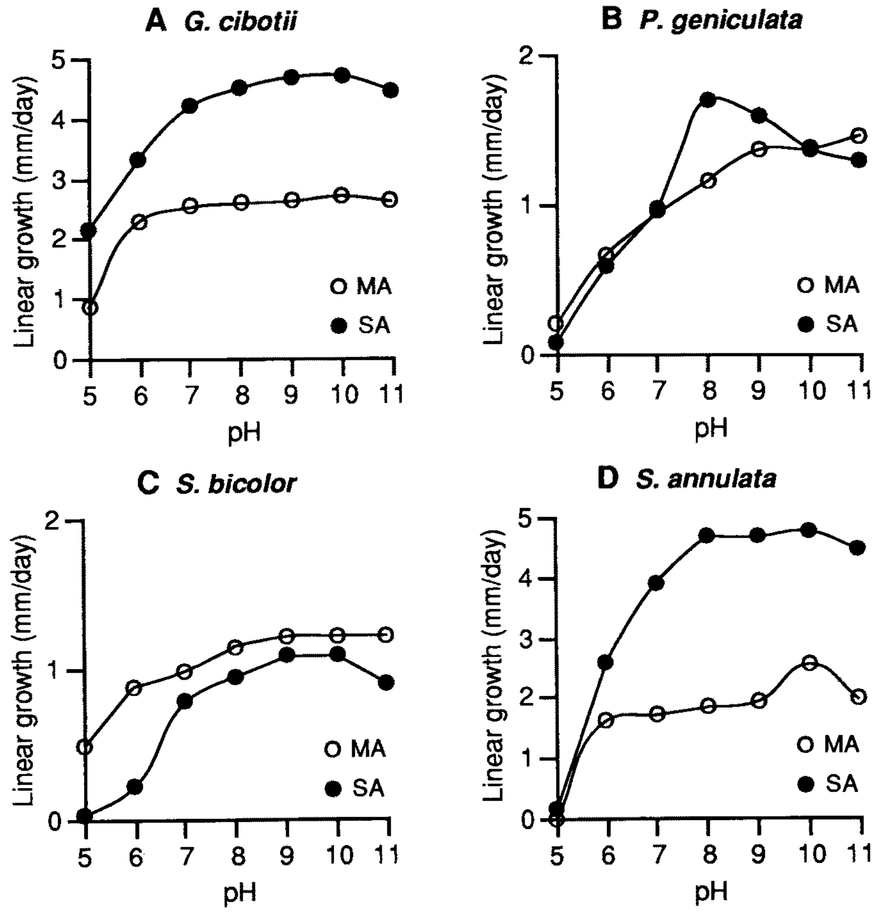

Fig. 3. Growth patterns on MA and SA of alkalophilic hyphomycetes. A. *Gliocladium cibotii* (YBLF 575). B. *Phialophora geniculata* (YBLF 587). C. *Stachylidium bicolor* (YBLF 646). D. *Stilbella annulata* (YBLF 647).

ic fungi were recovered that cannot grow on the acidic medium (*Acremonium alternatum, A. furcatum, Acremonium* sp. 6, *Gliocladium cibotii, Phialophora geniculata, Stachylidium bicolor* and *Stilbella annulata*). While the growth of fast-growing fungi such as *Trichoderma* and zygomycetes was retarded on ACMA, alkali-tolerant species got a chance to develop. Very different isolates were obtained on the two media. From each of the two different soil types, we detected many more species on ACMA than on CMA. As only a few species were isolated on both media, the utilization of alkaline and acidic media is very effective to isolate various soil fungi. Only a few bacteria grew on ACMA, and they did not prevent us from isolating fungi.

Characteristics of the isolates Species differences between the two isolation media were considered to be based on the fungal responses to pH. Fungi are generally considered to grow well on acidic substrata (below pH 7) and do not grow under alkaline conditions. Many isolates obtained on CMA showed the same characteristics. On the other hand, isolates obtained on ACMA were alkali-tolerant or, rarely, alkalophilic, especially in alkaline soils.

From a taxonomical point of view, all isolates of *Acremonium* that belong to different sections were alkalophilic or alkali-tolerant. They consisted of 12 species belonging to at least three sections: 2 species from acidic soils and 9 species from alkaline soils, and 1 species (*A. persicinum*) from both soils. They could grow well over a wide alkaline range. Although Okada et al. (1993) suggested that *Acremonium alcalophilum* was a unique alkalophilic species, the ability to grow under alkaline conditions is now found to be common to many *Acremonium* species.

All isolates of *Fusarium* species showed a high tolerance of alkaline conditions, and they appeared on ACMA at high frequencies. *Stilbella annulata* and *Stachylidium bicolor* were newly found to be alkalophilic.

Our data (Tables 2–5) confirm previous observations that *Trichoderma* and *Gliocladium* species are usually favoured by acidic soils (Papavizas, 1985). *Gliocladium cibotii* and *G. roseum*, however, are thought to be alkali-tolerant or alkalophilic species.

It has been reported that most of the alkalophilic microorganisms belong to bacteria (*Bacillus, Micrococcus*, etc.) and cyanobacteria, inhabiting soils and salt lakes (Horikoshi and Akiba, 1982; Grant and Tindall, 1986). Only a few reports concern alkalophilic fungi. We found that many alkalophilic hyphomycetes can be preferentially isolated from alkaline soils. Many more alkalophilic species are expected to exist in nature, which can be isolated on alkaline media.

Distribution of alkalophilic and alkali-tolerant fungi Most alkalophilic or alkali-tolerant fungi were isolated from alkaline soils. To date, the physiological characteristics of isolates from alkaline soils have not been examined in most of the floristic studies. So, the relationship between soil types and fungal physiological characteristics is still unknown. According to our results, soil fungi are likely to be selected by their substrata, and physiological characteristics of soil fungi might reflect their habitats (Tables 3, 5). From acidic soil samples, however, we isolated not only alkali-tolerant fungal strains but also a few alkalophiles (e.g., *Acremonium furcatum*). Similar results were reported for alkalophilic bacteria (Horikoshi and Akiba, 1982). Although fungi can generally change the pH of their surroundings during growth (Cooke and Whipps, 1993), it is still unresolved whether alkalophiles in acidic soils are inactivated forming spores or other resting structures, or change the microenvironment to suit their growth. Using alkalophilic isolates, we made a preliminary study of pH changes in the liquid media of which the basic components were the same as those of the agar media (MA, Table 1). The pH values of the liquid media remained basically stable with fungal growth, though they decreased slightly by about 0.3 to 0.5 units in most cases.

In this study, we found that different species of soil fungi were obtained on the two isolation media. Our results show a considerable diversity of soil microorganisms and suggest that only part of the species present in a soil can grow on a particular isolation medium. The pH of the medium is not the only factor in fungal isolation. Other physical and environmental factors such as temperature, incubation time and isolation methods also affect the recovery of soil fungi. Each of them is important to investigate the soil flora. Using alkaline isolation media will facilitate the isolation of alkalophilic soil fungi.

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