MESOPHILIC, THERMOPHILIC AND KERATINOPHILIC FUNGI IN A RICE FIELD SOIL AND PHYLLOPLANE FUNGI.

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SUMMARY

Mesophilic, thermophilic and keratinophilic fungi in the soil and mesophilic fungi on the phylloplane of rice (Oryza sativa) were isolated at various stages of cultivation and crop growth, from a field near Pavia (Northern Italy).

Mesophilic fungi isolated form the soil comprised 24 genera with 35 species, Acremonium strictum and Aspergillus fumigatus were dominant and present during all stages of growth of the rice. Cladosporium cladosporioides, Fusarium oxysporum, Penicillium brevicompactum, Phoma fimeti and Trichoderma harzianum were frequently isolated.

Thermophilic fungi were represented by 7 genera and 7 species. The thermotolerant Aspergillus fumigatus and the thermophilic Thermomyces lanuginosus were dominant.

Keratinophilic fungi included the genera Arthroderma, Chrysosporium, Keratinomyces, Microsporum and Trichophyton. Chrysosporium indicum and Ch. keratinophilum were dominant.

Fungi on the phylloplane comprised 26 genera with 48 species. The most The most frequent were Alternaria alternata, Cladosporium cladosporioides, Epicoccum purpurascens, Fusarium moniliforme and Gibberella acuminata.

Comparison with fungi recorded from our other studies of maize and wheat fields shows that among thermophilic and phylloplane fungi the dominant species were approximately the same, whereas the dominant mesophilic and keratinophilic species differed with each cereal.

INTRODUCTION

Fungi of rice fieldsoils and the rice phylloplane

RESUMEN

[Hongos mesofilicos, termofilicos y queratinofilicos en suelos de campos de arroz y del filoplano.]

Hongos mesofilicos, termofilicos y queratinofilicos en el suelo y hongos mesofilicos sobre el filoplano del arroz (Oryza sativa) fueron aislados en diversos estados de cultivo y desarrollo de los granos, a partir de un campo cercano a Pavia (Norte de Italia).

Los hongos mesofílicos aislados del suelo comprenden 24 géneros con 35 especies, Acremonium strictum y Aspergillus fumigatus fueron dominantes y estuvieron presentes durante todas las etapas de desarrollo del arroz.

Cladosporium cladosporioides, Fusarium oxysporum, Penicillium brevicompactum, Phoma fimeti y Trichoderma harzianum fueron aislados con frecuencia

Los hongos termófilos estuvieron representados por 7 géneros y 7 especies. La especie termotolerante Aspergillus fumigatus y la termofilica Thermomyces lanuginosus fueron dominantes.

Los hongos queratinofilicos incluyen los géneros Arthroderma, Chrysosporium, Keratinomyces, Microsporum y Trichophyton. Chrysosporium indicum y Ch. keratinophilum fueron dominantes.

Los hongos del filoplano abarcaron 26 géneros con 48 especies. Los más frecuentes fueron Alternaria alternata, Cladosporium cladosporioides, Epicoccum purpurascens, Fusarium moniliforme y Gibberella acuminata.

Comparando los hongos registrados en nuestros anteriores estudios sobre campos de maíz y trigo observamos que entre los hongos termofilicos y del filoplano las especies dominantes eran aproximadamente las mismas; en tanto que las especies dominantes mesofilicos y queratinofilicas difieren en cada cereal investigado.

have been studied in the USSR (10), France (2), India (7,9), Indonesia (12) and Italy (11).

Recent interest in the saprobic fungal populations of soils and of cereal phylloplane is due

principally to the search for a relationship between the plant and fungal populations. If the results of these searches are to be exploited efficiently proper analysis of the fungi occurring in soils and on the

phylloplane of each cereal is essential.

The present paper reports the results of a survey in 1983 of soil and phylloplane fungi in a rice field during the life cycle of this cereal. The soil fungi examined were mesophilic, thermophilic and keratinophilic, while those on the phylloplane were mesophilic. The rice (Oryza sativa linnaeus) cultivated was "Arborio" variety and 140 days passed between sowing and harvest.

MATERIALS AND METHODS

The soil studied

The rice field was at Certosa (Pavia), Lombardy. Its soil had a sandy silty texture (81.85 o/o sand, 11.80 o/o silt and 6.35 o/o clay), with a slightly acid reaction (pH 6.34). Other soil components measured included: nitrogen 0.155 o/o, organic carbon (bicromate oxidation method) 1.004 o/o, humus 1.729 o/o, C/N ratio 6.477, P2Os assimilable 33 ppm and K2O exchangeable 105 ppm.

The field had received a preliminary ploughing and levelling and was fertilized with N, P and K at 90, 130, 180 Kg/ha respectively. Weed control was by application of herbicide (Bentazon 1.5 Kg/ha) through boom-mounted hydraulic pressure mozzles

carried by a tractor.

Collection of soil samples and isolation of fungi.

All the soil samples were always collected in the same sides of the field during the search. The soil samples were collected in January (before ploughing), in May (before irrigation), in June-July-August (during inundation) and in September-October

(after drainage and harvest).

The monthly soil samples were: three for the meso-thermo and keratinophilic fungi isolation and other six for the thermo- and keratinophilic fungi isolation. The different number of soil samples for the different fungal groups was suggested because the thermophilic and keratinophilic population was scarcely representative in only three soil samples. The aim of this research was to evaluate the qualitative fungal population of soil during the life cycle of rice. Each soil sample was collected by inserting sterile plastic specimen tubes (length 15 cm and internal diameter 30 mm) into the soil. Each soil

sample was mixed and air dried.

Fungal analyses were made on the day of soil collection by three different methods,

- mesophilic fungi: fungal analysis was by the dilution method (1/5000): 10 g of mixed soil from each sample was added to 500 ml of sterile water and shaken for 30 min, to obtain maximum dispersion; 5 ml of this dilution was added to 495 ml of sterile water; 2 ml of this dilution was poured per 16 cm diameter plate into potato dextrose agar (PDA) to which rose Bengal (0.035 g/l), aureomycin (50 μ g/ml) and streptomycin (0.1 mg/ml) had been added, and acidified to pH 4.5. Three plates from each flask were prepared for a total of nine plates. The plates were incubated at 22.5° C. All fungal colonies recorded were counted.
- thermophilic fungi: two g of each soil sample were plated, in duplicate, directly on Petri dishes containing malt extract agar (MEA) with the addition of rose Bengal (0.035 g/l), auremycin (50 μ g/ml) and streptomycin (0.1 mg/ml) and acidified to pH 4.5. Half of these dishes were incubated at 45° C and the others at 50° C for three days. The thermophilic or thermotolerant nature of the species was determined by measuring their growth at 18° C and 50° C. For every soil sample, each fungal species was counted only once even if numerous isolates of this species were present in the plates of the same sample.
- keratinophilic fungi: Sixty g of each soil sample were transferred aseptically into sterile Petri dishes of 16 cm diameter and a total of nine dishes were prepared. These were moistened with distilled water containing cycloheximide at 2 o/oo and baited with autoclaved fragments of human and horse hair and fowl feathers. The Petri dishes were incubated at 25° C and examined at intervals for up to 12 weeks and remoistened when it was necessary. For every Petri dish, each fungal species was counted only once, even if it was present on all three kinds of baits.

Sampling procedures and cultural methods for isolation of phylloplane fungi.

Fungal analysis of leaf surface was made monthly from 1st May to 31st September 1983

At each sampling 10 leaves were randomly collected. Each leaf was aseptically cut into three segments and subjected to the following three methods:

A. leaf impression: the leaf surface of the first segment was temporarily pressed against malt extract agar (MEA), amended with CAF (0.5 o/oo) and streptomycin sulphate (1 o/oo), in Petri dishes. The dishes were incubated at 25° C for 7 days.

- B. leaf washing: the second segment of each leaf was shaken for 20 min, in 50 cm3 sterile distilled water. The suspension was diluted at 1/10 and 1/100 and, in duplicate, 0.1 cm3 of the dilution was spread on Petri dishes containing MEA. The dishes were incubated at 25°C for 15 days.
- C. damp chamber: each third segment was placed individually in 16 cm diameter dishes containing a 12 cm diameter sterile filter paper moistened with 5 ml of sterile distilled water containing streptomycin sulphate (40 μ g/ml). The dishes were incubated at 25°C for 14-20 days.

Each fungal species was counted only once per dish, even if more than one colony of the same species was present.

RESULTS

Mesophilic soil fungi.

A total of 625 isolates representative of 35 species belonging to 24 genera were found from a total of 21 rice field soil samples (table 1). These fungi formed four main groups:

- those isolated in the soil only before ploughing;
- those which occurred in the soil both before ploughing and in the flooded field throughout the life cycle of the rice;
- those which developed in the submerged field during the growth of rice;
- 4) those isolated only once.

A great number of isolates were obtained in July and August before panicle formation in water-logged condition and during active growth of the rice. Acremonium strictum and Aspergillus fumigatus were present all months.

Thermophilic soil fungi,

A total of 165 isolates of 7 species belonging to 7 genera were obtained from 63 samples of rice field soil (table 2). Prominent species were the thermotolerant Aspergillus fumigatus and the thermophilic Thermomyces lanuginosus which were isolated both before and after ploughing; the other species were isolated only from submerged and drained soil. Aspergillus fumigatus was isolated from 100 o/o of the soil samples. We recorded also a great number of different sterile fungi producing colourless or brown to grey mycelium. More thermophilic species occurred in summer especially when the soil was submerged, and these species disappeared from the soil in October after the rice harvest.

· Keratinophilic soil fungi.

The total population of keratinophilic fungi isolated from a total of 63 soil samples collected by the rice field soil was small (table 3). Keratinophilic fungi were more common before ploughing: Arthroderma quadrifidum (and its anamorph Trichophyton terrestre), Chrysosporium pannicola, Ch. indicum and Microsporum gypseum were isolated. Of these only Ch. indicum and M. gypseum appeared after ploughing: the former from June to October, the latter only in June. Among species isolated after ploughing Ch. queenslandicum was present only in June, but in all the samples.

• Fungi of the phylloplane

A total of 48 species belonging to 26 genera were isolated from the rice phylloplane (table 4). Five species were extremely common on the rice phylloplane: Alternaria alternata, Cladosporium cladosporioides, Epicoccum purpurascens, Fusarium moniliforme and Gibberella acuminata.

Few fungi were associated with expanding leaves during vegetative growth in May. Populations were high during initial growth in June, flowering in July and ripening in August and decreased in September (harvest).

DISCUSSION

The microfungi isolated from the soil in this study differed in some aspects from those reported by Dutta & Ghosh in Orissa (9) and by Das in West Bengal, India (7). In part these differences are explained by the use of different techniques and by the fact that we explored, in addition to mesophilic fungi, the thermophilic and keratinophilic population. Among the mesophilic fungi, we noticed some similarities with the data reported by Montemartini Corte in Lombardy (11) and by Egorova & Oksenyuk in the Maritime area of the USSR (10). In comparison with the latter's studies, the genera Mucor and Chaetomium, which were wi-despread in the Primor'e region, were not found in Pavia region and, on the contrary, Epicoccum, which appeared frequently at Pavia, was absent in the Primor'e region. Besides the mesophilic fungi, these authors (7, 9, 10) recorded some thermophilic and keratinophilic fungi, even if they did not specifically look from them. Our results may suggest that conditions for the growth of these two particular groups of fungi in rice soil are not completely unfavourable. The occurrence of particular species could be affected by the presence or absence in the rice ecosystem of other taxa, which might inhibit their growth. The species of crop plant could also significant by influence soil fungal populations (9).

Phylloplane fungi are saprobes or parasites. Their antagonism and biological control on aerial surfaces of this host and the resistance induced in rice plants by immunization and by treatment with various fungal fluids have been investigated (1.15).

As the number of colonies recorded on the rice phylloplane was low, it is not possible to point out any differences among the three different methods for fungal isolation. It is possible to draw some conclusions only for the genus Fusarium, mostly isolated by damp chamber method, and for the yeast-like colonies, isolated frequently by leaf washing method, yet never by damp chamber method. We recorded more frequently fungi which are common on phylloplane of various plants and which are the first colonizer of leaves (8). Alternaria alternata, Aureobasidium pullulans, Botrytis cinerea, C. cladosporioides and E. purpurascens are considered the most common fungi of the phylloplane (13). The abundance of A. alternata, C. cladosporioides and E, purpurascens on the rice phylloplane is confirmed by our study but we rarely recorded Botrytis and Aureobasidium, genera common in our previous studies on maize and wheat fields and in the air around Pavia (3, 4, 5, 6). Among the other fungi which colonized the soil and the phylloplane, it is interesting to note the high presence of the genus Fusarium, with F. oxysporum in the

soil and F. moniliforme on the phylloplane. It is known that F. oxysporum as a soil-borne pathogen or saprobe plays an important role in the microbiological processes of soil as an antagonist and competitor with many organisms for nutrients (14, 16).

Many fungi recorded during this research were also found in our previous studies on a maize

field and on a wheat field (4, 5, 6).

The dominant species isolated from these three cereals are compared in table 5. We note that the dominant thermophilic and phylloplane species were almost always the same, while the dominant mesophilic and keratinophilic species differed with each cereal. It is interesting to note the particular distribution of A. fumigatus which was found dominant among thermophilic fungi in the rice, wheat and maize fields, but dominant among the mesophilic fungi only in the rice field. This could suggest that A. fumigatus is a true colonizer of rice fields.

In conclusion, the species present in these three cereal fields probably play an important role in the plant growth. Their occurrence in soil and on the phylloplane at various stages of the plant growth may be indicative of commensalism, competition or antibiosis among the components of each fungal population.

Investigations about antagonism or synergy

of these three groups of fungi are in progress.

TABLE 1

Occurrence of mesophilic fungi in a rice-field soil.

JA	NUARY	MAY	JUN	JUL	AUG	SEPT	ост	TOTAL
Acremonium fusidioides (Nicot) W.Gams	1				1			2
A. strictum W. Gams	2	3	1	1	11	15	26	59
Alternaria alternata (Fr.) Keissler	Ī	1	2	ī	1	ALTERNATION .	1971/00	6
Aspergillus fischeri Wehmer	_	_	1		_			1
A. fumigatus Fresen.	3	5	18	1	3	2	5	37
A. niger van Tieghem	41			16		-		17
A, ochraceus Wilhelm				ī				l
A. ustus (Bain) Thom & Church				1				ī
Botrytis cinerea Pers.: Fr.			1	-				Ī
Byssochlamys nivea Westling			85	1.				
Cephalotrichum stemonitis (Pers.) Link				•0	2			1 2 1
Cephalotrichum sp			1					ĩ
Cladosporium cladosporioides			**					
(Fresen.) de Vries		5		71	12	3	1	92
C. herbarum Link		5 2	1	7.1	12	J	1	32
		2	3.		1			3 1
Drechslera poae (Baudys) Shoemaker					1		1	
Emericellopsis minima Stolk							1	1
Epicoccum purpurascens Ehremb.		2	13		•			1/
Schlecht.	240	2 5	12	1	1	~	540	16
Fusarium oxysporum Schlecht	1	3		1	6	2	1	16
Geotrichum candidum Link					13			13
Hansenula saturnus (Klöcker) H.					1.00			
& P. Sydow	540			1	160			161
Paecilomyces lilacinus (Thom) Samson	2	4	4					2
Penicillium brevicompactum Dierckx	40	3	1		3	1		48
P. glabrum (Wehmer) Westling	,				10			10
P. janthinellum Biourge	6							6
P. rugulosum Thom				22				22
P. thomii Maire			_				1	1 3 8
P. variabile Soop	122		3		<u> </u>			3
Penicillium sp	5			220	3			
Petriella setifera (Schm.) Curzi				1				1
Phialophora cyclaminis van Beyma		News	1		Contra	orania.		1
Phoma fimeti Brun.		2		1	3	10	16	32
Pseudallescheria boydii (Shear)								
Mc Ginnis. Padhye & Ajello					1	1	2	4
Rhizopus stoloniser (Ehrenb,: Fr.) Lind	11	4			20	2 2		17
Rhodotorula glutinis (Fresen.) Harrison		2		2	⁶ 3	2	1	10
Talaromyces flavus (Klöcker) Stoll & Samso	n				3			3
Torulomyces lagena Delitsch			1					1
Trichoderma harzianum Rifai	8	2	1			3	2	16
Sterile cultures	2	1	3			3 1	2 1	8
Total colonies	83	37	47	122	237	40	59	625
Number of genera	8	11	11	12	15	9	11	24
Number of species	11	12	12	15	17	9	11	35

TABLE 2

Thermophilic fungi in rice-field soil expressed as number of Petri dishes on which each fungal species was recorded.

	JANUARY	MAY	JUN	JUL	AUG	SEPT	OCT	TOTAL
TT Aspergillus fumigatus Fres.	9	9	9	9	9	9	9	63
TT Aspergillus sp.			2		9 6	9 2		10
TP Chaetomium thermophile La Touche			1					1
TP Malbranchea pulchella var. sulfurea								
(Miehe) Cooney & Emerson			3	2		1		6
TT Paecilomyces variotii Bain.			1	11 ET-41		257A		1
TP Rhizomucor pusillus (Lindt) Schippe	Г	6	5	1	3			15
TP Scytalidium thermophilum (Cooney								70,47
& Emerson) Austwick				1				1
TP Thermomyces lanuginosus Tsiklinsky	8		4	1	7	7	8	35
TP Sterile cultures	1000 mag		9	9	7	8		33
Total colonies	17	15	34	23	32	27	17	165
Number of genera	2	2	6	5	3	3	2	
Number of species	2	2	6 6	5	3	3	2	

TT = thermotolerant

TP = thermophilic

TABLE 3

Keratinophilic fungi recorded in rice field soil expressed as the number of Petri dishes on which each fungal species was found.

	JANUARY	MAY	JUN	JUL	AUG	SEPT	OCT	TOTAL
Arthroderma quadrifidum Dawson								
& Gentles	4							4
Arthroderma sp.	4							4
Chrysosporium pannicola (Corda) van								
Oorschot & Stalpers	6							6
Ch. indicum (Randhawa & Sandhu) Garg	2		5	4	4	4	3	22
Ch. keratinophilum (Frey) Carmichael		4	4			2	2	12
Ch. queenslandicum Apinis & Rees				9				9
Ch. tropicum Carmichael		4	2		2			8
Keratinomyces ajelloi Vanbreuseghem					1	4		5
Microsporum gypseum fulvum complex (Bodin)							
Guiart & Grigorakis	2		4					6
Trichophyton terrestre confplex Durie &								
Frey	3							3
Total colonies	21	8	15	13	7	10	5	79
Number of genera	4	1	2	1	2	2	1	
Number of species	5	2	4	2	3	3	2	

TABLE 4

Fungi isolated from rice-phylloplane by three different methods: Leaf impresion (A), leaf washing (B), damp chamber (C).

	٧	MAY B	ຸບ	_ ∢	JUNE	ာ	JULY	В	AUGUST C A	IST A	B Si	SEPTEMBER C A	3ER A	В	၁	TOTAL A B	, #	ပ
Acremonium kiliense Grutz Alternaria alternata (Fr.) Keissler Alternaria brassicicola (Schw.) Wiltshire Alternaria longipes (Elis & Everh.) Mason				4 v	1 1	4	⊶ 1	- 11	7.7	1	1	7	1.1	- 1	3.2	lots	-111	2 2 2
Alternaria anamorph of Pleospora infectoria Fuckel Alternaria sp Arthrinium caricicola Kunze Aspergillus flavus Link ex Gray		1	ţ	-4-0	1111	1 1 1 1	4	1	-	4	ı	1	7	1	4	14-6	1 1 1 1	1011
Aspergillus fumigatus Fresen. Aspergillus niger van Tieghem Aspergillus ochraceus Wilhelm Aureobasidium pulluians (De Bary) Am. var pullulans				- 2 -	1 1 -	i i i	% - 1	2	1 1 1	1.1 1		11 1				0 m 1	14- 4	
Botrytis cinerea: Pers: Fr. Candida guilliermondii (Castellani) Langeron et Guerra Chaetomium dolichotricuma Ames Chaetomium globosum. Kunze ex Stewd.				1 11	8 11	1 71	1		7	[]	- 1	1 -				1 111	1 7 7	1 144
Cladosporium cladosporioides (Fresen.) de Vries. Cladosporium herbarum Link. Cladosporium oxysporum Berk. & Curt. Cladosporium sp Coprinus radiatus(Bolt.: Fr.) Fr.	4	1	Ľ	v 11	4	1 -1	s o 1	. 01 1	. 1 %	1 -	9 I	1 1	۱m	- 4	1.1	0 00 1	124	11-18
Drechslera anamorph of Pyrenophora avenae. Ito & Kuribayashi Epicoccum purpurascens Ehrenb. ex Schlecht Fusarium moniliforme: Sheidon Gibberella acuminata Wollenw.				1 2 1	l 4 l	10 10	v II	иll	وها	101	811	10	5 5	111	1019	1 25	1 7 11	1 17 223 223
Gliocladium roseum, Bain. Gliomastix murorum (Karst.) Dickinson Mucor racemosus Fresen Nigrospora anamorph of Khuskia oryzae Hudson Penicillium brevicompactum. Dierckx				П	1	1		į į	1 1	 1	t i - t	m I -	7	1 1	1 1	446		mll l
Penicillium fellutanium · Biourge Penicillium janthinellum Biourge Penicillium purpurogenum Stoll Penicillium thomii Maire Penicillium variabile Sopp	4	1 -	1 6	6	1	1.1	- 1 m	11 2	101	21 1	m -	1 1	-	Î	1	-4 w Uw	118119	111616

3	3 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	9.		MAIZE	Trichoderma harzianum Penicillium purpurogenum	Keratinomyces ajelloi Chrysosporium queenslandicum	Aspergillus fumigatus Thermomyces lanuginosus	Alternaria alternata Cladosporium cladosporioides Fusarium moniliforme Gibberella acuminata
1 1 2 1 3 3 1 1 1 1 1 1 1 1 1 1 1 1 1 1	1 1 2 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	£ 5	rom rice, wheat and maize fields.	WHEAT	Fusarium oxysporum Penicillium janthinellum Lipomyces starkeyi	Chrysosporium merdarium Myceliophthora anamorph of Crenomyces cereatus	Aspergillus fumigatus Rhizomucor pusillus	Alternaria alternata Aureobasidium pullulans var. pullulans Epicoccum purpurascens Cladosporium cladosporioides
2 - 1 - 1 - 1 - 1 - 1 - 1 - 1 - 1 - 1 -	9 1 2 1 1 1 1 1	TABLE	Dominant fungal species isolated from rice, wheat and maize fields.	RICE	Acremonium strictum Aspergillus fumigatus	Chrysosporium indicum Ch. keratiniphilum	Aspergillus fumigatus Thermomyces lanuginosus	Alternaria alternata Cladosporium cladosporioides Epicoccum purpurascens Fusarium moniliforme
Phoma herbarum Westdend Rhizopus oryzae Went & Prinsen Geerligs Rhizopus stolonifer: (Ehrenb.: Fr.) Lind Rhodotorula gutinis (Fresen.) Harrison Rhodotorula graminis. Di Menna Rhodotorula pallida Lodder Rhodotorula sp Sporobolomyces gracilis: Kluyver & Van Niel Sporobolomyces hispanicus Pelàez	et Ramirez Sporobolomyces roseus Kluyver & Van Niei Stachybotrys atra Corda Torula herbarum Pers. Trichoderma harzianum Rifai Trichothecium roseum Link Ulocladium alternariae (Cooke) Simmons Xylohypha nigrescens, (Pers.: Fr.) Mason	,			Mesophilic	Soil Keratinophilic	Тhетморhilic	Phylloplane

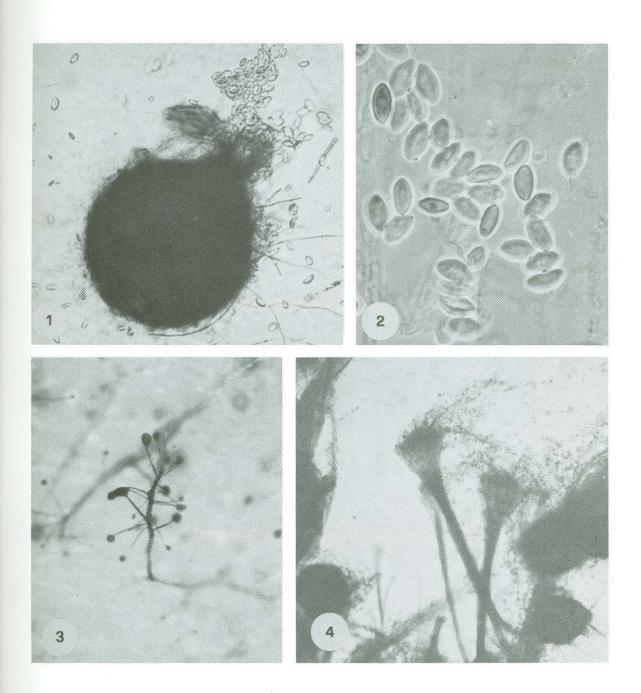


PLATE A. Petriella setifera: 1, ascoma (X250); 2, ascospores (X1.000); 3 and 4, the Graphium-like anamorph (X20 and X100).

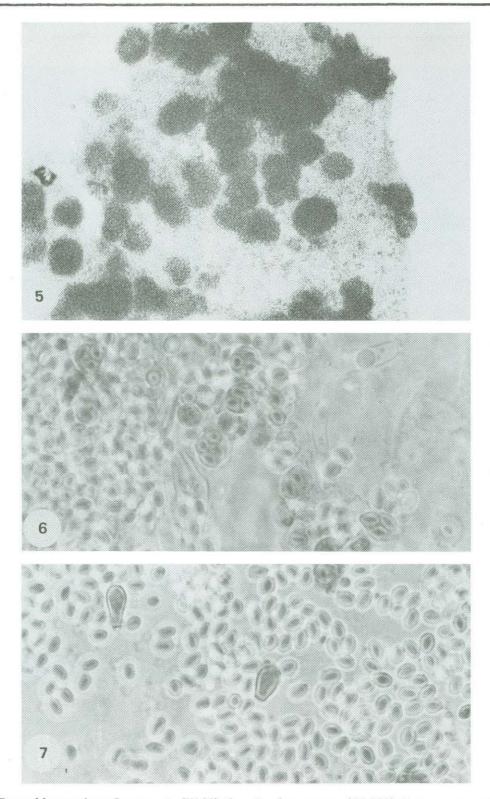


PLATE B. Byssochlamys nivea: 5, ascomata (X100); 6, asci and ascospores (X1.000); 7, ascospores and chlamydospores (X1.000).

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