Boletín Micológico Vol. 3 Nº 3 - 183 - 189 1987

ANTAGONISTIC ACTIVITY "IN VITRO" OF SOME SAPROPHYTIC FUNGI OCCURRING ON THE PHYLLOPLANE OF RICE, WHEAT AND MAIZE.

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SUMMARY

A total of 51 isolates belonging to 16 genera representative of fungi most frequently encountered on wheat-, rice- and maize-phylloplane were studied "in vitro" to isolate antagonistic activity.

Antagonism was examined with the dual cultures method on agar media among these fungi and against the pathogens **Drechslera maydis, Pyricularia oryzae** *and* **Helminthosporium maydis.** *The most antagonistic were* **Penicillium chrysogenum, P. thomii** *and* **Stachybotrys atra;** *they antagonize in particular* **Botrytis cinerea, Fusarium moniliforme** *and* **Alternaria alternata.** *The most successful antagonists against pathogens were* **P. chrysogenum** *and* **P. charlesii** *markedly active against* **Helminthosporium maydis, Pyricularia oryzae** *and* **Drechslera oryzae.** *Isolates of* **Chaetomium globosum, Gibberella zeae, Fusarium oxysporum, Trichothecium roseum** *and* **B. cinerea** *displays the antagonistic activity against* **H. maydis; Cladosporium cladosporioides** *against* **P. oryzae** *and* **D. oryzae; Aspergillus clavatus** *against* **D. oryzae.** *The results are discussed in relation to the biological control of these cereal pathogens.*

RESUMEN

[Actividad antagónica "in vitro" de algunos hongos saprofíticos presentes en el filoplano del arroz, centeno y maíz.]

Fue investigada la actividad antagónica in vitro de un total de 51 cepas pertenecientes a 16 géneros fúngicos detectados con mayor frecuencia en el filoplano del centeno, arroz y maíz.

El antagonismo fue observado con el método de los cultivos duales en medios con agar, entre estos hongos y los fitopatógenos: **Drechslera oryzae, Pyricularia oryzae** *y* **Helminthosporium maydis.** *Los más antagónicos son:* **Penicillium chrysogenum, P. thomii** *y* **Stachybotrys atra;** *ellos antagonizaron en particular con* **Botrytis cinerea, Fusarium moniliforme** *y* **Alternaria alternata.** *El antagonismo más exitoso contra los tres patógenos mencionados fue con* **P. chrysogenum** *y* **P. charlesii,** *quienes fueron notoriamente activos. Las cepas de* **Chaetomium globosum, Gibberella zeae, Fusarium moniliforme, Trichothecium roseum** *y* **B. cinerea** *presentan una actividad antagónica contra* **H. maydis; Cladosporium cladosporioides** *contra* **P. oryzae** *y* **D. oryzae; Aspergillus clavatus** *frente a* **D. oryzae.** *Los resultados son discutidos en relación al control biológico de estos patógenos de cereales.*

Several recent studies on the biocenosis of the aerial surfaces of plants have revealed the buffering effect of the natural saprophytic mycota which acts against pathogens (Fokkema, 1976; Dickinson, 1976). The importance of non-pathogenic fungi and the dangers inherent with their destruction, notably through the irrational use of fungicides, have been recognized (Fokkema, 1976), as well as their potential as biological controls of plant pathogens (Blackeman & Fokkema, 1982). More recently competition by antagonists for space, as well as competition for nutrients has been recognised as an essential mechanism necessary to protect plants against certain of their parasites. The most active in this respect includes species of the genera **Trichoderma, Gliocladium, Fusarium, Penicillium, Cladosporium, Alternaria** and "pink" yeasts, mainly **Sporobolomyces** spp., and "white" yeasts, mainly **Cryptococcus** spp. (Fokkema et al., 1979; Newhook, 1957; Tronsmo & Dennis, 1977). In certain cases, fungi may activate a host defence mechanism which inhibits the pathogen. It is current practice to carry out "in vitro" and "in vivo" estimations of the potential antagonistic ability of fungi, by some form of screening programme implementing a variety of different techniques.

In this study, several fungi from wheat-, rice- and maize-phylloplane isolates were tested "in vitro" by the dual culture method, with a view to assessing:

a) the antagonistic ability between different isolates of the three cereals;
b) the antagonistic activity of the phylloplane fungi against the pathogens Drechslera

oryzae (Breda de Haan) Subram. & Jain, **Pyricularia oryzae** Cav. and **Helminthosporium maydis** Nisikado.

The results of the two sets of experiments are here reported, and their significance is discussed.

MATERIAL AND METHODS

Fungi used in this study were species obtained during a previous studies, of rice- wheat- and maize-phylloplane mycota (Caretta et al., 1985; 1986; 1987), and maintained in potato dextrose agar (PDA) and Czapek Dox agar kept in slanted test tubes.

These are listed in Table 1. The list includes some species (**P. chrysogenum, G. zeae** and **F. oxysporum**) not recorded from the fungi of rice-field soil in that long after have been identified. A total of 51 isolates representative of 16 genera were examined in paired cultures, and evaluated for their potential antagonism. These fungi were also compared "in vitro" against the following pathogens:
Pyricularia oryzae, RCC482 and 481 (strains obtained from the Ente Risi Mortara);
Drechslera oryzae, RRC479 (strain obtained from Istituto di Patologia Vegetale of Milano);
Helminthosporium maydis, A95 (strain obtained from Istituto di Patologia Vegetale of Milano).

The interactions between the phylloplane saprobes were examined by inoculating all possible paired combinations; while the interactions among saprobes and pathogens by direct opposition.

Dual cultures were established 3.5 cm apart on PDA (pH 5) in 10 cm Petri dishes, and incubated in the dark for 7 days at 22º C.

Interactions were assessed using a key based on the observations of Porter (1924), Skidmore & Dickinson (1976) and Johnson & Curl (1978). On the five separate modes of interacting colony growth, only the following modes of interactions were recorded:

a) mutual inhibition of more than > 2 mm;
b) unilateral inhibition with a cleary visible zone between the colonies.

Table 1

RICE

Acremonium kiliense Grutz
Aspergillus flavus Link ex Gray
Aspergillus fumigatus Fresen
Aspergillus niger van Tieghem
Aspergillus ochraceus Wilhelm
Botrytis cinerea Pers.: Fr.
Cladosporium cladosporioides (Fresen.) de Vries
Drechslera state of **Pyrenophora avenae** Jto & Kuribayashi
Epicoccum purpurascens Ehrenb. ex Schlecht.
Fusarium moniliforme Sheldon
Fusarium oxysporum Schlecht. emend.Sny & Hans
Gibberella acuminata Wollenw.
Gibberella zeae (Schw.) Petch
Penicillium brevicompactum Dierckx
Penicillium charlesii Smith
Penicillium chrysogenum Thom
Penicillium thomii Maire
Stachybotrys atra Corda
Trichoderma viride Pers.
Trichothecium roseum Link

MAIZE

Alternaria alternata (Fr.) Keissler
Aspergillus flavus Link ex Gray
Aspergillus fumigatus Fresenius
Aspergillus ochraceus Wilhelm
Chaetomium globosum Kunze ex Fries
Cladosporium cladosporioides (Fres.) de Vries
Doratomyces stemonitis (Pers.: Fr.) Morton & Smith
(= **Cephalotrychum stemonitis** (Pers.) Link)
Drechslera state of **Pyrenophora avenae** Jto & Kuribayashi
Epicoccum purpurascens Ehrenb. ex Schlecht.
Fusarium moniliforme Sheld.
Fusarium oxysporum Schlecht. emend.Sny. & Hans
Penicillium chrysogenum Thom
Penicillium purpurogenum Stoll
Stachybotrys atra Corda
Trichoderma viride Pers. ex Gray

WHEAT

Alternaria alternata (Fr.) Kessler
Aspergillus clavatus Desm.
Aspergillus fumigatus Fres.
Aspergillus niger van Tieghem
Botrytis cinerea Pers: Fr.
Doratomyces stemonitis (Pers: Fr.) Morton & Smith
(= **Cephalotrichum stemonitis** (Pers.) Link)
Chaetomium globosum Kunze ex Fries
Cladosporium cladosporioides (Fresen.) de Vries
Fusarium moniliforme Sheld
Fusarium oxysporum Schlecht. emend. Sny. & Hans
F. solani (Mart.) App. & Woll.
Gibberella acuminata Wollenw.
Penicillium brevicompatum Dierckx

Penicillium charlesii Smith
Penicillium chrysogenum Thom
Penicillium variabile Sopp
Sporobolomyces roseus Kluyver & Van Niel
Trichoderma viride Pers. ex Gray
Trichurus spiralis Hasselbr.

The strain of **C. cladosporioides** was also antagonistic against **P. oryzae** and **D. oryzae**, while **A. clavatus** was a strong antagonist against **D. oryzae**.

Some strains of these pathogen-antagonist fungi were recorded among those fungi showing mutual or unilateral inhibition capacities.

RESULTS

Table 2 shows the antagonism observed on direct opposition plates between wheat-, rice- and maize-phylloplane fungi.

In Table 3 are given the fungi having a good inhibitory action on the growth of pathogens.

Among the tested phylloplane saprobes, only a few fungi were mutually antagonistic.

Most of these were isolates of the wheat-phylloplane and involved prevalently interactions with fungi of same phylloplane.

Many more numerous were fungi strongly antagonistic against saprobes of other phylloplanes. These result in unilateral inhibition with saprophytes inhabiting the same and other phylloplanes. A strong inhibition of **Acremonium kiliense, Alternaria alternata, Fusarium moniliforme** and **Gibberella zeae** occurred in direct opposition plates with a strain of **Penicillium chrysogenum** isolated from rice phylloplane. **Penicillium thomii** (from rice phylloplane) was also antagonistic against **A. ochraceus** and **Botrytis cinerea** present on wheat-phylloplane. A strong competitive fungus was **Stachybotrys atra** present on maize phylloplane, which inhibits isolates of **A. alternata, B. cinerea** and **F. moniliforme.**

Of the other numerous saprophytes tested, a relatively small number was found to be antagonistic generally against one fungal species only, for instance in **A. niger** against **B. cinerea, A. fumigatus** against **A. niger, C. cladosporioides** against **Doratomyces (=Cephalotrichum) stemonites, Drechslera sp.** against **T. viride, F. oxysporum** against **A. flavus** and **Sporobolomyces roseus** against **D. stemonitis.**

Data relative to the "in vitro" ability of phylloplane saprobes fungi to inhibit pathogens, highlight the antagonistic ability of nine fungi of the 51 tested. Seven out of these nine, and precisely **P. chrysogenum, P. charlesii, C. globosum, T. roseum, G. zeae, F. oxysporum** and **B. cinerea** significantly inhibited **Helminthosporium maydis. Penicillium chrysogenum** and **P. charlesii** also inhibited **Pyricularia oryzae** and **Drechslera oryzae.**

Table 2
Interactions between opposed colonies of phylloplane fungi growing on PDA.

A - Mutual inhibition:	
A. fumigatus (W)	**F. solani** (W)
E. purpurascens (R)	**B. cinerea** (W)
D. stemonitis (W)	**A. clavatus** (W)
	A. niger (W)
P. chrysogenum (R)	**T. spiralis** (W)
P. purpurogenum (M)	**F. oxysporum** (M)
B - Unilateral inhibition:	
A. niger (R)	**B. cinerea** (W)
A. fumigatus (W)	**A. niger** (W)
C. cladosporioides (W)	**D. stemonitis** (W)
Drechslera sp. (M)	**T. viride** (M)
P. chrysogenum (R)	**A. kiliense** (R)
	A. alternata (W)
	F. moniliforme (R)
	G. zeae (R)
P. thomii (R)	**A. ochraceus** (R)
	B. cinerea (W)
Sp. roseus (W)	**D. stemonitis** (=**C. stemonitis**) (W)
S. atra (M)	**A. alternata** (W)
	B. cinerea (W)
	F. moniliforme (R)
F. oxysporum (R)	**A. flavus** (R)

W = Wheat
R = Rice
M = Maize

Table 3
Interactions between opposed colonies of phylloplane fungi and pathogens.

Pathogens	H. maydis	P. pryzae	D. oryzae	
Saprobes	RCC481	482	479	
P. chrysogenum (R)	+	+	+	+
P. charlesii (R)	+	-	+	+
C. cladosporioides (R)	-	+	-	+
A. clavatus (W)	-	-	-	+
Ch. globosum (M)	+	-	-	-
T. roseum (M)	+	-	-	-
G. zeae (R)	+	-	-	-
F. oxysporum (M)	+	-	-	-
B. cinerea (W)	+	-	-	-

DISCUSSION

The results of these tests confirme antagonistic ability of some saprophytic fungi occurring on the rice-, wheat- and maize-phylloplane. Some of these fungi for instance **A. niger, A. fumigatus, C. cladosporioides, E. purpurascens, S. roseus** and **F. oxysporum** has been recently recognized as antagonists against other saprobes (Heuvel, 1971; Pace & Campbell, 1970; Sharma et al., 1979; Singh et al., 1983).

Some has been reported to be antagonists against "in vivo" pathogens and in agar media (Newhook, 1957; Skidmore & Dickinson, 1976; Rai & Singh, 1980). Nevertheless among those fungi screened in this research, the most antagonistic were **P. chrysogenum, P. thomii** and **S. atra**; they antagonize several fungal species, in particular **B. cinerea, F. moniliforme** and **A. alternata**. These antagonistic species were occasionally isolated respectively on the phylloplane of maize, rice and wheat.

The antagonistic ability of each of strain fungi differs against pathogens. In this respect the most successful antagonists were isolate of **P. chrysogenum** and **P. charlesii**, markedly active against **H. maydis, P. oryzae** and **D. oryzae**.

Penicillium chrysogenum is perhaps the most ubiquitous of all Penicillia, occupying a wide range of habitats. It is a source of much of the raw material of the penicillin industry, and is the best known penicillin producing fungus. This fungus tested against plant pathogenic fungi showing a specific and strong inhibitory action against **Phytium ultimum** (Domsch & Gams, 1968) and **Monilinia laxa** (Melgarejo et al., 1985).

Penicillium charlesii (which PITT 1978 places in synonymy with P. fellutanum Biourge) is predominantly inhabits in soils, but was also been isolated in a wide variety of cereal crops and seeds. The itaconic and carolic acids produces (Turner & Aldridge, 1983) and limited antifungal activity against dermatophytes has been recorded (Bilay et al., 1964).

Our screening also displays the antagonistic activity of the following isolates against plant-pathogenic fungi: **C. globosum, G. zeae, F. oxysporum, T. roseum** and **B. cinerea** against **H. maydis**; **C. cladosporioides** against **P. oryzae** and **D. oryzae**; **A. clavatus** against **D. oryzae**

Antagonistic activity of all these isolates against maize, rice and wheat pathogenic fungi may be due to their production of metabolites, toxins or antibiotics. The fact that only certain isolates of the same species display an antagonistic ability, could be the result of genetic variability and or individual capacities.

In this field of reseach it is important to identify the antagonists, but it is also important to describe their distribution in different areas of the world, and on the phylloplane of which host plant they are found.

This marked levels of antagonism by us observed in certain fungi, particularly **P. chrysogenum** and **P. charlesii**, suggests their possible use in the control of plant pathogens.

Blends of these antagonists may have wider applications.

ACKNOWLEDGEMENTS

Research work supported by CNR, Italy, Special grant I.P.R.A. Subproject 1. Paper Nº 1556. We wish to thank Mrs. G. Della Volpe Sorrentini and Mr. L. Morandi for their thecnical assistence.

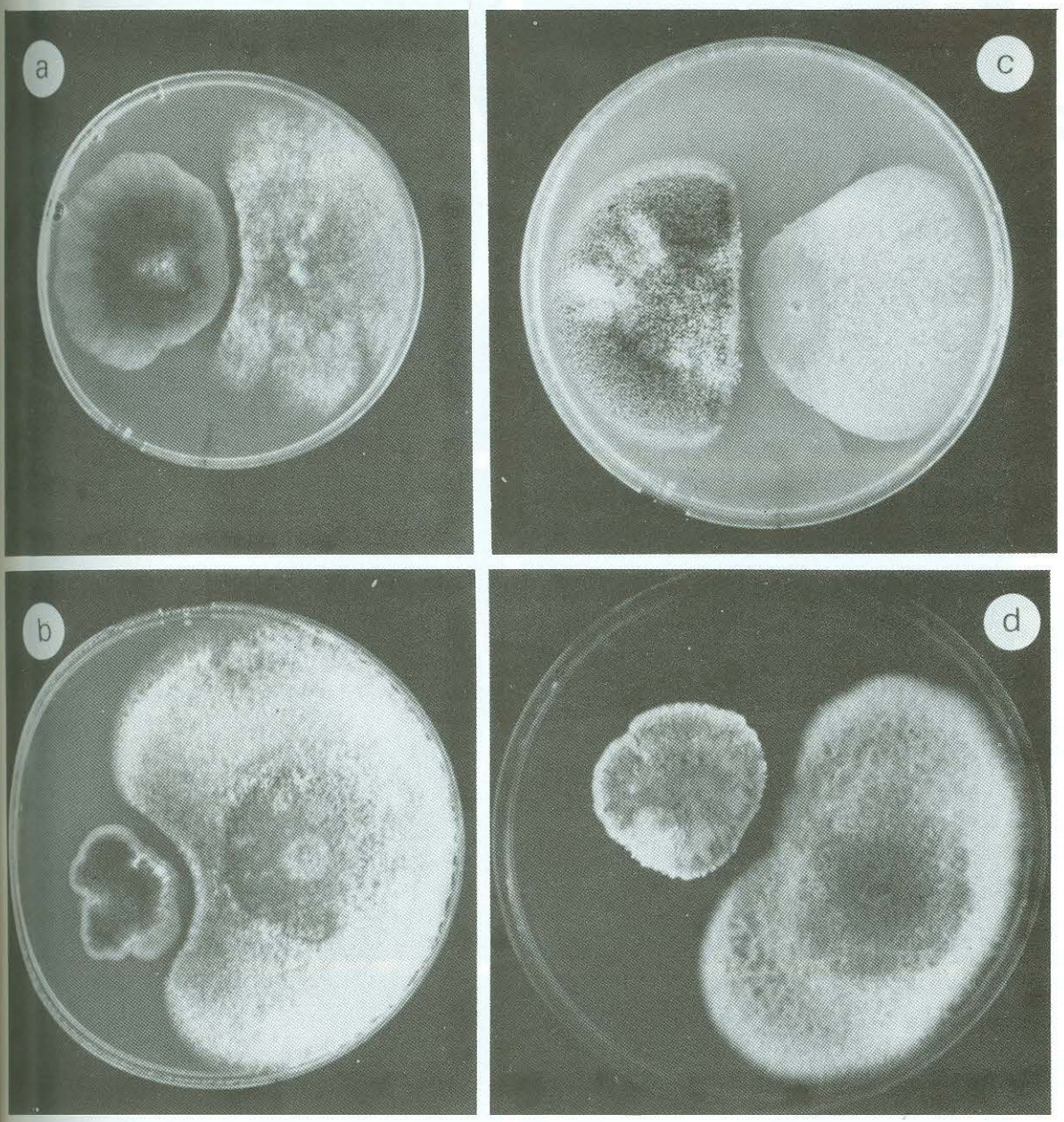
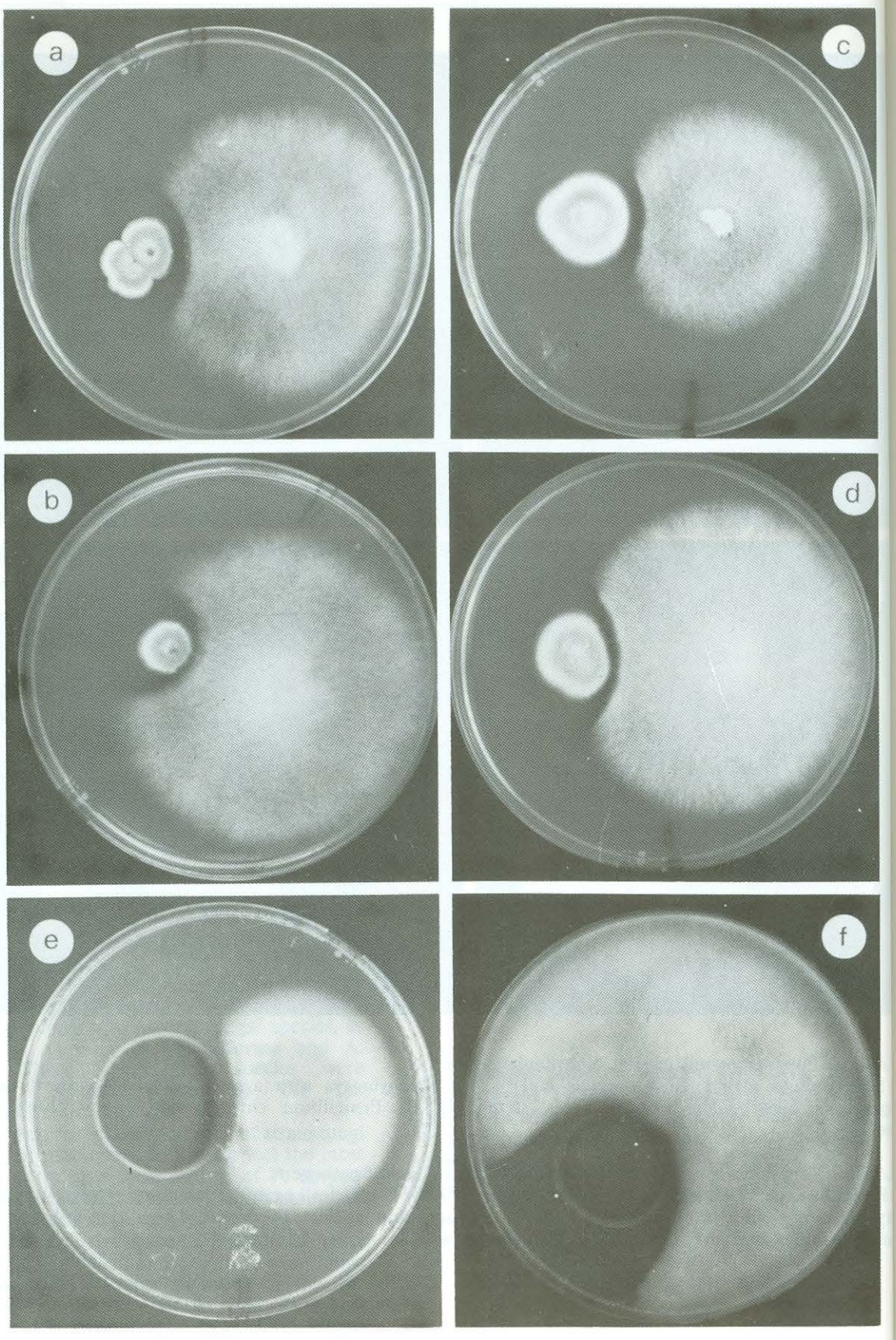


PLATE 1: Fungi antagonistic against phylloplane saprobes.
a) **Stachybotrys atra** against **Botrytis cinerea**.. b) **Stachybotrys atra** against **Alternaria alternata**. c) **Aspergillus niger** against **Doratomyces stemonitis**. d) **Penicillium chrysogenum** against **Fusarium moniliforme**.

PLATE 2: Antagonistic activity of phylloplane saprobes against pathogens.
a) **Penicillium charlesii** against **Drechslera oryzae** 479. b) **P. charlesii** against **Pyricularia oryzae** 482. c) **P. chrysogenum** against **P. oryzae** 482. d) **P. chrysogenum** against **D. oryzae** 479. e) **Cladosporium cladosporioides** against **P. Oryzae** 481. f) **C. cladosporioides** against **D. oryzae** 479.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