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ANTAGONISTIC ACTIVITY "IN VITRO" OF SOME MESO-, THERMO- AND KERATINOPHILIC FUNGI OF WHEAT-, RICE, MAIZE-FIELD SOILS.

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SUMMARY

A total of 123 isolates belonging to 50 species representative of the meso-, thermo- and keratinophilic fungi most frequently encountered in wheat-, rice-, maize-field soils, were studied "in vitro" to isolate antagonistic activity.

Antagonism was examined within the dual cultures method on agar media between and among these fungi, and against strains of **Bacillus subtilis**. *Mesophilic isolates of* **Penicillium brevicompactum. P. rubrum, P. purpurogenum** *and* **Aspergillus ustus** *showed antifungal activity against keratinophilic fungi and species of the genera* **Fusarium, Acremonium** *and* **Trichoderma**. *The keratinophilic* **Chrysosporium queenslandicum** *also showed antifungal abilities against others keratinophilic fungi (***Microsporum gypseum, C. merdarium** *and* **C. keratinophilum**). *Antagonistic capacities against meso-, thermo- and keratinophilic fungi differ from species to species and between strains of the same species (***P. rubrum** *and* **P. brevicompactum**).

Marked antagonists of **B. subtilis** *were* **P. brevicompactum, P. purpurogenum, P. rubrum, P. thomii**, *the keratinophilic* **C. merdarium** *and* **M. gypseum**, *the thermophilic* **Humicola grisea** *var.* **thermophila** *and thermotolerant strains of* **Aspergillus fumigatus** *and* **A. niger**.

Bacillus subtilis *was however an active antagonist against keratinophilic isolates (***Arthroderma quadrifidum, C. evolceanui, C. indicum, C. queenslandicum** *and* **M. gypseum**) *and some isolates of* **Fusarium, Acremonium murorum, A. strictum, Trichoderma harzianum** *and* **Myceliophthora thermophila**.

The antagonistic capacities of fungi and bacteria, which differ from species to species and between strains of the same species, could be related to variabilities and their genetic ability to produce different active compounds which interfere with the metabolism of sensitive fungal or bacterial species.

RESUMEN

[Actividad antagónica "in vitro" de algunos hongos meso-termo y queratinofílicos en campos de centeno, arroz y maíz.]

En suelos de cultivo de centeno, arroz y maíz, se realizaron estudios "in vitro" para observar antagonismo fúngico de 123 cepas aisladas, que comprenden 50 especies representativas de hongos mesotermo y queratinofílicos frecuentemente presentes en estos terrenos. Este fue visualizado por el método de cultivos duales en agar contra cepas de **B. subtillis**. *Los mesofílicos tales como* **Penicillium brevicompactum, P. rubrum, P. purpurogenum** *y* **Aspergillus ustus** *mostraron actividad contra hongos queratinofílicos y especies de los géneros* **Fusarium, Acremonium** *y* **Trichoderma**. *Las especies queratinofílicas* **Chrysosporium queenslandicum** *también mostraron propiedades antifúngicas contra otros hongos queratinofílicos (***Microsporum gypseum, Chrysosporium merdarium** *y* **C. keratinophilum**). *Estos antagonismos entre hongos mesotermo- y queratinofílicos difieren de especie a especie y entre cepas de la misma especie (***Penicillium rubrum** *y* **P. brevicompactum**).

Antagonistas notorios de **B. subtilis** *fueron* **Penicillium brevicompactum, P. purpurogenum, P. rubrum, P. thomii**; *los queratinofílicos* **Chrysosporium merdarium** *y* **Microsporum gypseum**; *el termófilo* **Humicola grisea** *var* **thermophila** *y las cepas termotolerantes de* **Aspergillus fumigatus** *y* **A. niger**.

Bacillus subtilis *fue sin embargo un activo antagónico contra los aislamientos queratinofílicos (***Arthroderma quadrifidum, C. evolceanni, C. indicum, C. queenslandicum** *y* **M. gypseum**) *y alguno de* **Fusarium, Acremonium murorum, A. strictum, Trichoderma harzianum** *y* **Myceliophthora thermophila**.

Las capacidades antagónicas de los hongos y bacterias, las cuales difieren de especie a especie y entre cepas de la misma especie podrían estar relacionadas con la variación y su capacidad genética para producir diferentes compuestos activos los cuales interfieren con el metabolismo de las especies fúngicas o bacterianas sensibles.

INTRODUCTION

In surveys of phylloplane fungi and of meso, thermo and keratinophilic fungi of wheat-, rice-, maize- field soils, associations have been recorded between cereals and the above fungal groups (Caretta et al., 1985, 1986, 1987). Many difficulties related to these results were concerned with the assessment of single possible interactions between particular fungal species and cereal species, or with possible antagonisms among fungal species both between the three fungal groups. The aim of the research was to investigate the "in vitro" antagonistic activity between a range of these fungal saprobes isolated from the soil, and to evaluate if such antagonism affects metabolite production, for instance, antibiotics. This last aspect was evaluated "in vitro" against **Bacillus subtilis** (Ehrenberg) Cohn., the most frequently used test-organism for the evaluation of antibiotic properties.

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

MATERIALS AND METHODS

- **Fungal isolates** - A total of 123 isolates belonging to 50 species representative of meso-thermo- and keratinophilic fungi, and most frequently encountered on wheat-, rice-, maize-field soils were selected for investigation of antagonistic activity. These are listed in Table I.

- **Bacteria** - The strains of **Bacillus subtilis** and **Rhizobium japonicum** used in the experiments were:

B. subtilis:

a) strain ATCC 19659;
b) strain PB 1791 (rec E4) obtained from the Dipartimento di Genetica e Microbiologia of Pavia;
c) strain PB 1652 (parental rec^+) obtained as above;
d) strain (Firenze) isolated from soil, obtained from the Istituto Sperimentale Zoologia Agraria of Firenze;
e) **Rhizobium japonicum**, obtained as above.

All were individually tested for antagonism with fungal isolates.

- **"In vitro" fungal antagonism** - Antagonism between 50 species was evaluated in dual culture by inoculation of all possible pairs on cultural media.

With a needle, a small tuft of young culture is removed and planted 2,5 cm apart from a tuft belonging to a different strain onto a Petri dish (10 cm diam.) containing about 25 ml malt extract agar (MEA) medium.

The plates were incubated in the dark at 25º C. Controls were single and dually-inoculated cultures of the same fungus; when growth of each fungus achieved maximal growth (in general after 20 days) the inhibition zone in the dual culture and the radial growth of each colony was measured. In dual cultures the maximum diameter was measured. According to the classification of Porter (1924) and Skidmore and Dickinson (1976) relative to the reaction types occurring between fungal isolates, only the following modes of inhibition of a distance colony growth, were recorded:

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

Antagonism among mesophilic and thermophilic fungi was evaluated in dual cultures by first inoculating the thermophilic fungus; after incubation at 42º C and growth of the fungus, the mesophilous fungus was inoculated, and plates incubated in the dark at ambient room temperature at 25º C. This experiment was repeated with two fungi in a reversed situation.

Competitive nutritional ability was comparatively utilizing the evaluated percentage value of growth rate inhibition between fungi in paired cultures and each fungus in single cultures.

- **Antibiotic properties of fungi** - the strains of B. subtilis and the strain of R. **japonicum** were used in this experiment as follows:

Cultures of the tester strains were grown at 37º C in Difco nutrient agar and Sabouraud agar.

A stock spore suspension of each strain of **B. subtilis** and **R. japonicum** was prepared according to the method of Grove and Randall (1955).

This was diluted one hundred times with sterile distilled water so as to obtain a working-strength suspension.

Assay plates were prepared by adding 0.2 ml of this working-strenght suspension to 25 ml of cooled 45º C Sabouraud agar.

After solidification, a small tuft of a young fungal culture was removed with a needle and planted onto a central point of the plate, and left to incubate at 25º C for 1-3 weeks (Method A).

For the thermophilic fungi, the plates were incubated at 42º C.

This experiment was repeated with the two organisms in reverse situations; that is to say the fungus was placed in the agar of a Petri dish and the bacterium planted in the center of the dish (Method B). Reults were recorded as "inhibition zones" in millimeters (mm).

In another set of experiments Bacteria and fungi showing antagonistic actions on agar plates

were screened against the same organism grown in a broth cultural medium. Liquid cultures of the bacterium and fungus were prepared by inoculating a potato-dextrose broth (50 ml, pH 6.8) into 250 ml flasks containing approximatively 10^6 bacteria or fungal conidia; these were left for 7 days at 25º C on a reciprocating shaker operating at 120 strokes per minute.

Culture filtrates were then prepared by filtering through Whatman n.1 filter paper, contrifuged at 10.000 rpm for 20 minutes and then sterilized with a Millipore (pore size 0.22 um) procedure. The effect of the bacteria on the growth of fungal isolates incorporate into the agar medium and the effect of fungal isolates on the growth of bacteria also incorporated in agar medium was determined as follows: a well of 6 mm diameter was practiced in the center of solidified PDA in 10 cm Petri plates. The well was filled either with 100 ul of a bacterial culture in broth or with 100 ul of a fungal culture in broth.

In the first case a suspension of conidia lead previously been mixed with 30 ml of agar medium on Petri dish; in the second, 0.5 ml of bacterial culture had been mixed with 30 ml of agar medium.

Intensity of antagonistic activity is here indicated with a (+) sign. Each + sign is used to indicate antagonism and an inhibition zone of 5 mm; +++ indicates very strong antagnism and an inhibition zone of 15 mm.

Table 1

Fungal isolates examined. (In parentheses the number of some fungal strains examined)

WHEAT

Acremonium furcatum Moreau ex Gams
A. murorum (Corda) W. Gams
A. strictum W. Gams
Aspergillus ustus (Bain.) Thom & Church
A. fischeri Wehmer
A. wentii Wehmer
A. flavipes (Bain. & Sart.) Thom & Church
A. nidulans (Eidam) Winter
A. candidus Link ex Link
A. fumigatus Fres. (no.10 strains)
Beauveria bassiana (Bals.) Vuill.
Bahusakala olivaceonigra (Berk. & Br.) Subram.
Chrysosporium indicum (Randawa & Sandhu) Garg
Ch. keratinophilum (Frey) Carmichael (no.2 strains)
Ch. merdarium (Link ex Grey) Carmich.
Ch. queenslandicum Apinis & Rees
Ch. tropicum Carmichael
Ch. pannicola (Corda) v. Oorschot & Stalpers
Chrysosporium sp.
Fusarium graminearum Schwabe
Fusarium merismoides Corda
F. moniliforme Sheldon
F. oxysporum Schlecht. emend. Snyd & Hans.
Gliocladium penicillioides Corda
G. roseum Bain
Gliocladium sp.
Microsporum gypseum complex (Bodin) Guiart & Grigorakis
Myceliophthora vellerea (Sacc. & Speg.) v. Oorschot
M. thermophila (Apinis) v. Ooschot.
Mucor sp.
Nectria inventa Pethybr.
Penicillium brevicompactum Dierckx (no.2 strains)
P. frequentans Westling
P. janthinellum Biourge
P. purpurogenum Stoll
P. rubrum Stoll
P. restrictum Gilman & Anbbot
Penicillium sp.
Rhizopus stolonifer (Ehrenb. ex Link) Lind
Scopulariopsis carbonaria Morton & Smith
Trichophyton terrestre Durie & Frey

MAIZE

Arthrinium state of **Apiospora montagnei** Sacc.
Arthroderma cuniculi Dawson (no.2 strains)
A. quadrifidum Dawson & Gentles
Aspergillus fumigatus Fres. (no.4 strains)
A. niger v. Tiegh. (no. 3 strains)
Aspergillus sp.
Chrysosporium indicum (Rand. e Sandh.) Garg
C. pannicola (Corda) v. Oorschot & Stalp.
C. queenslandicum Apinis e Rees
Fusarium sp. (no.5 strains)
Gliocladium sp.
Keratinomyces ajelloi Vanbreusegh (no.3 strains)
Myceliophthora thermophila (Apinis) v. Oorschot
Myrothecium roridum Tode ex Stendel
Penicillium purpurogenum Stoll (no.2 strains)
Trichoderma harzianum Rifai (no. 2 strains)

RICE

Aspergillus fumigatus Fres. (no.2 strains)
Aspergillus sp. (no.4 strains)
Arthroderma quadrifidum Dawson & Gentles
Chrysosporium indicum (Rand & Sandh.) Garg (no.2 strains)
C. keratinophilum (Frey) Carmichael (no.2 strains)
Ch. merdarium (Link ex Grey) Carmichael
Ch. queenslandicum Apinis & Rees

Doratomyces stemonitis (Pers. ex Stend.) Morton & Smith
Fusarium sp. (no. 4 strains)
Humicola grisea Traaen v. **thermoidea** Cooney & Emerson
Mucor sp. (no.2 strains)
Microsporum gypseum complex (Bodin) Guiart & Grigorakis (no.3 strains)
Penicillium rubrum Stoll
P. thomii Maire
Penicillium sp.
Phoma fimeti Brun.
Petriella setifera (Schm.) Curzi
Thermomyces lanuginosus Tsiklinsky
Trichoderma harzianum Rifai (no.4 strains)

Table 2

Radial growth inhibition (expressed in percentage) of fungal isolates in dual culture (inhibited species)

	Field-soil	%
Aspergillus ustus	W	1
Penicillium thomii	R	1
P. purpurogenum	M	2
P. rubrum	W	3
Chrysosporium keratinophilum	R	4
Aspergillus fischeri	W	5
Myceliophthora vellerea	W	5
Gliocladium roseum	W	6
Penicillium brevicompactum (strains ß)	W	6
Penicillium sp.	R	8
Thermomyces lanuginosus	R	9
Aspergillus wentii	W	10
Chrysosporium indicum	R	11
Fusarium graminearum	W	11
Aspergillus flavipes	W	13
Acremonium strictum	W	16
Aspergillus nidulans	W	17
Penicillium sp.	W	18
P. janthinellum	W	19
P. purpurogenum	W	23
Petriella setifera	R	25
Acremonium murorum	W	27
Penicillium restrictum	W	32
Apiospora montagnei	M	33
Gliocladium sp.	W	34
G. nigrum	M	35
Acremonium furcatum	W	37
Fusarium sp.	M	40
Nectria inventa	W	41
Fusarium oxysporum	W	43
Gliocladium penicillioides	W	46
Fusarium sp.	R	52
Gliocladium sp.	M	54
Beauveria bassiana	W	67

Table 3

Inhibitory fungal species. Radial growth inhibition expressed in percentage.

Fungal species	Field-soil	%
Aspergillus fumigatus	M	78
Rhizopus stolonifer	W	68
Fusarium sp.	M	66
Trichoderma harzianum	R	66
Fusarium merismoides	W	58
Fusarium sp.	M	51
Trichoderma harzianum	R	50
T. harzianum	M	48
Fusarium moniliforme	W	46
Fusarium sp.	R	44
Fusarium sp.	M	40
Phoma fimeti	R	27
Penicillium purpurogenum	M	24
Fusarium sp.	M	20
Mucoracea	R	18
Microsporum gypseum	W	6
Penicillium frequentans	W	6
P. rubrum	R	6
Chrysosporium queenslandicum	R	3
Keratinomyces ajelloi	R	3
Chrysosporium merdarium	R	1

RESULTS

Results obtained from the dual plates experiments using 50 fungal species against each other and against bacteria, highlight different behaviours of colony growth.

A. FUNGAL ANTAGONISM

The fungi wich showed a growth reduction in dual cultures are listed in Table 2. Those fungi whose the growth rate was more inhibited were mostly strain of enthomogenous **B. bassiana**, and certain strains of **Fusarium, Gliocladium, A. furcatum** and **N. inventa**. The highest percentage inhibition of growth was observed in **B. bassiana** (-67%, as compared to the normal growth of a single colony) and in **Fusarium** and **Gliocladium**. In these isolates the inhibition ranged from 40 to 54%. This is probably due to nutriet impoverishment rather than inhibitory metabolites.

Isolates on direct dual opposition plates which proved to be inhibitory strains, that is to say, strains which caused significant reduction in the growth of other fungi of between 40 - 78% (see Table 3) were strains of **A. fumigatus** (thermotolerant), **Rh. stolonifer, T. harzianum, F. merismoides, F. moniliforme** and other strains of **Fusarium**, all isolated from maize-, wheat- and rice-field soils. On direct dual opposition plates the rapid overgrowth observed in isolates of **Trichoderma** and **Rhizopus** is probably responsible for the significantly slower growth rate of opposing fungal inoculi.

As far as reaction types a mutual inhibition of more than 2 mm, these occurred particulary in isolates of species belonging to genera Aspergillus and **Penicillium** (see Table 4). Some species were observed to be intrageneric antagonists, i.e. antagonists to others species of the same genus, for example both **A. flavipes** and **A. nidulans** acted against **A. ustus**.

Species of the genera Aspergillus (thermotolerant strains. **Fusarium** and **Trichoderma** proved to be particularly active. Strains of **Rhizopus stolonifer** and **T. harzianum** isolated from wheat and rice field soils respectively, also showed a marked hyperparasitic activity.

In Table 5 and 6 are listed those fungal species showing a significant antagonistic behaviour of direct opposition as far as the following modes of interacting colony are concerned:

a) mutual inhibition at a distance of > 2 mm (Table 4);
b) unilateral inhibition with a clearly visible zone between the colonies (Table 5 and illustrated in Figures of Plate 1).

Table 4
Fungal isolates showing mutual inhibition at a distance > 2 mm on agar plates.

P. restrictum	W	**F. oxysporum**	W
		P. janthinellum	W
		A. wentii	W
P. thomii	R	**P. janthinellum**	W
A. flavipes	W	**A. ustus**	W
P. purpurogenum	M	**A. ustus**	W
		A. flavipes	W
A. nidulans	W	**A. ustus**	W
A. fischeri	W	**F. graminearum**	W
A. ustus	W	**A. fischeri**	W
		F. graminearum	W
		Penicillium sp.	W
		M. gypseum	R
Penicillium sp.	W	**A. fisheri**	W
		F. graminearum	W
Penicillium sp.	R	**P. brevicompactum**	W
P. rubrum	W	**C. keratinophilum**	R
		C. merdarium	R

Table 5
Fungal isolates showing unilateral inhibition with a cleary visible zone.

Inhibitory species		Inhibited species	
P. brevicompactum (strain α)	W	**F. moniliforme**	W
		A. murorum	W
		T. harzianum	M
P. purpurogenum	M	**F. oxysporum**	W
		M. gypseum	W
		A. fumigatus	M
P. brevicompactum (strain ß)	W	**Fusarium sp.**	R
		A. montagnei	M

		P. setifera	R
		P. fimeti	R
		Mucor (?) sp.	R
		K. ajelloi	M
		A. strictum	W
		A. fumigatus	M
		C. queenslandicum	R
		M. gypseum	W
		M. vellerea	W
P. rubrum (0061)	R	**A. fumigatus**	M
		T. lanuginosus	R
C. queenslandicum	M	**M. gypseum**	W
		C. merdarium	R
		C. keratinophilum	R
P. rubrum (0040)	R	**K. ajelloi**	M
		C. queenslandicum	M
		C. keratinophilum	R
		M. gypseum	W
		M. vellerea	W
A. ustus	W	**K. ajelloi**	R
		M. vellerea	W
		C. keratinophilum	R
		C. indicum	R
		C. queenslandicum	M
		M. gypseum	W

Some especies acting as mutual inhibitors at a distance of > 2mm seemed to be intrageneric antagonists of other species belonging to the same genus, f. e. **A. flavipes** and **A. ustus**. Most antagonistic fungi were species belonging to the genera **Penicillium** and **Aspergillus**, isolated prevalently from wheat field-soil.

It is important to note that the antagonized fungal species had been all isolated from wheat field-soils, and among these were isolates of **F. graminearum** (strains not listed in the previous study).

A significant difference in the antagonism occurred between isolates showing unilateral inhibition. Strong antagonistic abilities were shown by isolates of **P. brevicompactum** (strains alpha and beta isolated from wheat field-soils) against **Fusarium** and other keratinophilic fungi isolated from maize- and rice-field soils.

Of the two strains of **P. rubrum**, both isolated from rice field-soils, one showed antagonism to **A. fumigatus** and to **T. lanuginosus**, while the other antagonism to geo- keratinophilic species of the genera **Chrysosporium, Keratinomyces, Microsporum** and **Myceliophthora**.

Among the numerous keratinophilic fungi, only the strains of **C. queenslandicum** isolated from maize field-soil showed an antagonism to isolates of **M. gypseum, C. merdarium** and **C. keratinophilum**, all isolated from rice field-soil.

Of the isolated thermophilic fungi none strains showed an antagonistic behaviour against other fungi.

A comparison between the frequency of isolation of the most antagonistic species with the total number of recorded colonies from the considered soils, highlights that, where highly antagonistic fungi are more frequent (as maize and rice fields), the total number of genera and species is lower (Table 6). Instead, in wheat field-soil, where different strains of fungi are more abundant, antagonistic species are very few.

Table 6

Antagonistic species occurring in wheat-, maize- and rice-field soils.

Field soils	Total		Antagonist species	%
Wheat	Isolates	1772	P. brevicompactum	0.5
	Genera	49	Rh. stolonifer	0.9
	Species	83	T. harzianum	0.5
Maize	Isolates	913	C. queenslandicum	19.1
	Genera	39	P. brevicompactum	7.8
	Species	54	P. purpurogenum	27.3
			T. harzianum	17.3
Rice	Isolates	869	P. brevicompactum	7.7
	Genera	34	T. harzianum	2.6
	Species	51		

B - ANTAGONISM BETWEEN B. subtilis, R. japonicum AND OPPOSING FUNGAL SAPROBES.

On the basis of "in vitro" interactions following method A, with fungal inoculates placed in the center of the plate (see Table 7 and 8), 16 fungal isolates, representative of 10 species, were found to be strong antagonists to the four strains of **B. subtilis**.

Many of those fungi which showed marked antibacterial antagonism, were species of the genus **Penicillium (P. brevicompactum, P. purpurogenum, P. rubrum)**, already included in Table 4

for their antifungal activity. They also caused significant reductions in the growth of **B. subtilis** with an inhibition ability resulting in an uncolonized zone of 5-10 mm around the inoculum. This antagonistic effect is probably due to an antibiotic or an inhibitory metabolite production.

Strong antagonists of **B. subtilis** were also strains of **P. thomii,** the keratinophilic **C. merdarium** and **M. gypseum,** and the thermophilic **H. grisea** var. **thermophila,** all isolated from rice field-soils.

Numerous strains of **A. fumigatus** (a thermotolerant species), mainly isolated from rice- and maize-field-soils, and **A. niger** isolated from maize field-soils, all showed strong antagonists to **B. subtilis.** Individual isolates of **T. harzianum** were antagonists to two strains of **B. subtilis.** Antibacterial activity only occurred in the cultural filtrates of the following fungi: **P. purpurogenum, A. niger, Penicillium** sp. and two thermotolerant strains of **A. fumigatus.**

Method B of cultural growth, in which the bacteria are inoculated in the center of the plate, the results of "in vitro" interaction between **B. subtilis** and isolates of fungal species showed significantly different responses (see Table 6 and 7, illustrated in Figures of Plate 2).

All strains of **B. subtilis,** particularly the Firenze strain isolated from soils, inhibited the growth of keratinophilic **A. quadrifidum, C. evolceanui, C. indicum, C. queenslandicum** and **M. gypseum** isolates.

On agar plates the inhibition appeared to be associated with one or more diffusable substances, since inhibition occurred at a certain distance away from the central inoculum of **B. subtilis.**

A reduction in fungal growth also occurred when culture filtrates of **B. subtilis** were placed in wells on agar plates.

Bacillus subtilis was also an active antagonist against isolates of **Acremonium murorum, A. strictum,** certain isolates of **Fusarium, M. thermophila** and **T. harzianum.** some isolates of **A. fumigatus** extracted from wheat field-soils showed a marked susceptibility to diffusible inhibitory substances produced by **B. subtilis.**

The antagonistic activity of **B. subtilis** was also observed against isolates of **M. thermophila** and **H. grisea** var. **thermoidea,** two species previously included among those fungal species showing antibacterial activity.

DISCUSSION

The results of the tests here reported demonstrate or confirm several facts concerning the antagonistic ability of particular fungi occurring in soil.

Of the meso-, thermo- and keratinophilic fungi screened the most active in this respect were mesophilic isolates of **P. brevicompactum, P. rubrum, P. purpurogenum** and **A. ustus** extracted from wheat- than rice- and maize-field soils.

These ubiquitous species occupying numerous habitats in the soil and decaying vegetation of many areas, are well-known producers fungistatic and fungicid metabolites, in many cases already isolated and identified (Moss, 1971; Wogan & Mateles, 1968; Natori et al., 1980; Bartman et al., 1981). **Penicillium rubrum** and the closely related **P. purpurogenum,** both produce rubratoxin A and B. **P. brevicompactum** is known to produce a number of metabolites, including mycophenolic acid during antifungal activity for example against **R. solani** (Domsch & Gams, 1968), **C. cladosporioides** and **C. herbarum** (Magan & Lacey, 1984)

Toxinogenic strains of **A. ustus** are reported to have been isolated from stored foodstuffs (Steyn & Vleggaar, 1974), and among the toxic metabolites produced, most important toxin is reported to be austiol, of gastro-instestinal (Vleggaar et al., 1974). The strains of these fungal species by us isolated, have confirm their antagonistic ability, but they also exhibit specific antagonistic capacities, presumably related to the production of different metabolites.

In the current set of experiments this was noted in two strains of **P. rubrum** which appears to be particularly antagonistic one against keratinophilic fungi, and the other against isolates of thermophilic **T. lanuginosus** and thermotolerant **A. fumigatus.**

A pronounced inhibitory activity towards a wider range of mesophilic and keratinophilic fungi was observed in two strains of **P. brevicompactum.**

Strains of **A. ustus** and the keratinophilic **C. queenslandicum,** the most common and widespread fungi distributed in soils are antagonists towards keratinophilic fungi. the data-set obtained in this study highlight that keratinophilic fungi are particularly sensitive to the antifungal metabolites produced by the fungal population currently inhabiting the soil.

Keratinophilic fungi, the so-called "substrate group" in Garrett's terminology, are the ultimate colonizers in the degadation of process keratin in all soils (Griffin, 1972). Although these fungi are common and have been isolated in a wide range of soils in different parts of the world, they do not appear to be evenly distributed.

Infact some types of soils may be very rich in terms of their content of keratinophilous fungi, while other may be extremely poor or completely devoid.

The antagonistic action against meso-, thermo- and keratinophilic fungi differs from species to species and between strains of the same species could be related to their genetic variability and ability to produce different active compounds, or alternatively, similar active compounds which interfere with the metabolism of sensitive fungal species.

In the genera **Aspergillus** and **Penicillium**, the antagonistic mechanism is antibiosis. Nevertheless it is difficult to determine an order of importance between antibiotic production and the production of other chemical and physical factors, on which the occurrence of fungi in soils, is often closely related.

The presumable antibiotic production by these Aspergilli and Penicilli was prove by us in inhibition "in vitro" test against strains of **B. subtilis**. Antibacterial antibiotic-producing fungi were again **P. rubrum, P. purpurogenum, P. thomii, P. brevicompactum**, all known for their antifungal activity, and also isolates of **M. thermophila, C. merdarium, M. gypseum** and certain thermophilous strains of **A. fumigatus.**

In these "in vitro" experiments, **B. subtilis** (specially the Firenze strain) was found to be mostly antagonist to the keratinophilic species of the genera **Arthroderma** (**A. cuniculi, A. quadrifidum**), **Chrysosporium** (**C. evolceanui, C. indicum, C. keratinophilum, C. queenslandicum, C. merdarium**), the **Keratinomyces ajelloi** and **M. gypseum** and also to isolates of **Fusarium, T. harzianum** and **M. thermophila.** Sensibility of different species varied among strains of the same species and from soil type to soil type.

A different "in vitro" behaviour was observed in the case of **Rhizobium japonicum**; this Gram-negative nitrogen fixing organism is insensitive to fungal activity, and does not produce any antifungal metabolites.

The genus **Bacillus** includes species producing toxic substances or antifungal antibiotics (Sharon et al., 1954; Berdy, 1980) particularly investigated were bacilysin and fengymycin, obtained by cultures of different strains of **B. subtilis** (Loeffler et al., 1986). Several authors have reported the antifungal activity of **B. subtilis** and its use in controlling a number of plant pathogens (Aldrich & Baker, 1970; Broadbent et al., 1971; Dunleavy, 1955; Swinburne & Brown, 1976; Utkhede, 1983; Vapinder Singh & Deverall, 1984).

Seed bacterization and application of specific bacteria among which **B. subtilis**, or fungi to soil have been carried out extensively in order to achieve biological control of plant pathogens, or an enhancement of plant growth (Brown, 1974; Papavizas & Lumsden, 1980; Merriman et al., 1974; Alstrom, 1983; Gerhardson et al., 1985).

Suslow et al. (1979) have termed "rhizobacteria" these specific root-colonizing bacteria, while Schroth & Hancock (1982) have classed groups of such bacteria as "beneficial", deleterious" and "neutral" on the basis of their interaction with plants.

It is interesting to note that **B. subtilis** is an antagonist to keratinophilic fungi and to the mesophilic **Acremonium, Fusarium** and **Trichoderma** spp., but it is also an organism sensitive to fungal metabolites produced by Penicilli and Aspergilli.

The screening also highlighted the antagonistic activity of **C. queenslandicum**, a keratinophilic fungal species whose behaviour in this respect was very little known. This screening above all showed the antifungal activity of strains of **P. brevicompactum** and **P. purpurogenum** against keratinophilic fungi and to species of the genera **Fusarium, Acremonium** and **Trichoderma.**

These results would seem to suggest different ways of approaching research on fungal antagonism and their possible use in the control of plant pathogens.

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Table 7
"In vitro" antagonism between meso- and thermophilic fungi and bacteria

				method A					method B				
				a	b	c	d	r	a	b	c	d	r
1)	**Acremonium murorum**		W	±	-	-	+	-	+	+	+	++	-
2)	**A. strictum**		W	-	-	-	±	-	++	++	++	++	-
3)	**Arthrinium** state of **Apiospora montagnei**		M	-	±	-	±	-	±	-	-	+	-
4)	**Aspergillus candidus**		W	-	-	-	+	-	-	+	±	++	-
5)	**A. fumigatus**	TT	W	++	++	-	±	-	-	-	-	-	-
6)	**A. fumigatus**	TT	W	-	-	-	-	-	+	+	+	++	-
7)	**A. fumigatus**	TT	W	-	+	±	++	-	-	-	-	-	-
8)	**A. fumigatus**	TT	M	++	++	+	+++	-	-	-	-	-	-
9)	**A. fumigatus**	TT	R	-	++	-	-	-	-	±	-	±	-
10)	**A. niger**	TT	M	+++	+++	+++	++++	-	-	-	-	-	-
11)	**A. ustus**		W	-	-	-	-	-	-	-	-	-	-
12)	**Aspergillus** sp.	TT	M	+	++	-	+	-	-	-	-	-	-
13)	**Aspergillus** sp.	TT	R	-	+++	+	+++	-	-	-	-	-	-
14)	**Aspergillus** sp.	TT	R	+++	++	+	+	-	+	+	-	+	-
15)	**Chaetomium globosum**		W	-	+	-	+	-	+	+	+	+	-
16)	**Chaetomiun** sp.		W	+	+	+	+	-	+	+	+	++	-
17)	**Doratomyces stemonitis**		R	-	-	-	-	-	±	±	±	±	-
18)	**Fusarium** sp.		M	±	-	-	±	-	-	-	-	-	-
19)	**Fusarium** sp.		R	-	-	-	-	-	++	++	+++	++	-
20)	**Gliocladium roseum**		W	-	-	-	-	-	-	-	-	-	-
21)	**Humicola grisea** var. **thermoidea**	TT	R	++	+++	++	++	-	++	++	-	++	-
22)	**Mucor** sp.	TT	W	+	-	+	+	-	-	-	-	-	-
23)	**Mucor** sp.		R	-	-	-	+	-	-	-	-	±	-
24)	**Myceliophthora thermophila**	TT	W	++	++	++	++	-	-	-	-	-	-
25)	**M. thermophila**	TT	M	++	++	++	++	-	++	++	++	++	-
26)	**Penicillium brevicompactum**		W	+	+	+	+++	-	-	-	-	-	-
27)	**P. purpurogenum**		M	+++	+++	++	+++	-	-	-	-	-	-
28)	**P. rubrum**		W	++	+++	+++	+++	-	-	-	-	±	-
29)	**P. rubrum**		R	+++	+++	+++	++	-	-	-	-	-	-
30)	**P. thomii**		R	++	++	++	+++	-	-	-	-	-	-
31)	**Penicillium** sp.	TT	R	++	++	+++	+	-	+	+	-	+	-
32)	**Rhizopus nigricans**		W	-	-	-	-	-	-	-	-	-	-
33)	**Scopulariopsis carbonaria**	TT	W	-	-	-	-	-	-	-	-	-	-
34)	**Thermomyces lanuginosus**	TT	R	+	+	+	+	-	±	-	-	-	-
35)	**Trichoderma harzianum**		M	±	±	-	-	-	++	++	++	++	-
36)	**T. harzianum**		R	-	-	±	+++	-	-	-	-	+	-

Legend:
W = wheat M = maize R = rice
a = **B. subtilis** ATCC 19659
b = **B. subtilis** PB 1791
c = **B. subtilis** PB 1652
d = **B. subtilis** from Florence soil
r = **Rhizobium japonicum**
TT = thermotolerant

Table 8
"In vitro" antagonism between isolates of keratinophilic fungi and bacteria.

			method A					method B			
			a	b	c	d	r	a	b	c	d
1)	**Arthroderma cuniculi**	M	-	-	-	-	-	++	+	+	++
2)	**A. cuniculi**	M	-	-	-	-	-	-	±	-	-
3)	**A. quadrifidum**	M	-	-	-	-	-	±	±	±	±
4)	**A. quadrifidum**	R	-	-	-	-	-	++	++	++	+++
5)	**Arthroderma sp.**	R	-	-	±	-	-	++	++	++	++
6)	**Chrysosporium evolceanui**	W	-	-	-	+	-	-	-	-	-
7)	**C. evolceanui**	M	-	-	-	+	-	++	++	++	+++
8)	**C. indicum**	W	-	±	±	±	-	±	±	±	++
9)	**C. indicum**	M	-	±	-	-	-	++	++	++	+++
10)	**C. indicum**	R	-	±	-	±	-	++	++	++	++++
11)	**C. keratinophilum**	W	-	+	-	-	-	±	-	-	++
12)	**C. keratinophilum**	W	-	-	-	-	-	-	-	-	++
13)	**C. keratinophilum**	R	-	-	-	-	-	++	++	++	++
14)	**C. merdarium**	W	-	-	-	-	-	-	-	-	++
15)	**C. merdarium**	R	++	++	++	++	-	+	±	±	++
16)	**C. queenslandicum**	W	-	-	-	±	-	-	-	-	++
17)	**C. queenslandicum**	M	-	-	-	++	-	++	++	+	+++
18)	**C. quenslandicum**	R	-	-	-	-	-	++	++	++	++
19)	**C. tropicum**	W	-	-	-	-	-	±	±	±	++
20)	**Keratinomyces ajelloi**	M	-	-	-	±	-	+	+	+	+
21)	**Microsporum gypseum**	W	+	±	-	±	-	+	+	+	±
22)	**M. gypseum**	R	-	-	-	-	-	±	±	±	-
23)	**M. gypseum**	R	-	-	-	-	-	++	++	++	+++
24)	**M. gypseum**	R	++	±	++	+	-	±	±	+	++
25)	**Myceliophthora vellerea**	W	++	-	-	-	-	±	±	+	++
26)	**Trichophyton terretre**	W	-	-	-	-	-	-	+	-	++

Legend:
W = wheat M = maize R = rice
a = **B. subtilis** ATCC 19659
b = **B. subtilis** PB 1791
c = **B. subtilis** PB 1652
d = **B. subtilis** from Florence soil
r = **Rhizobium japonicum**

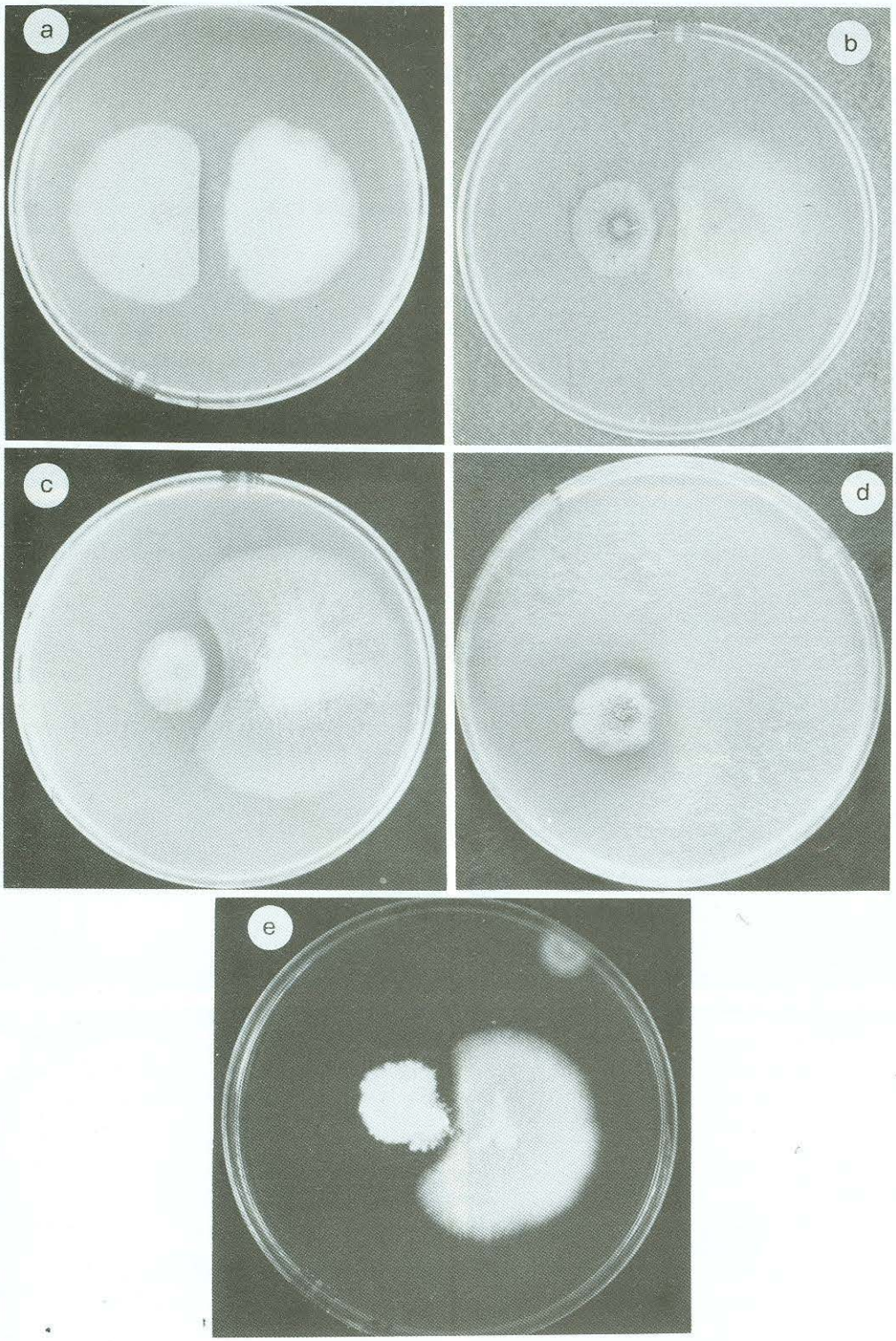
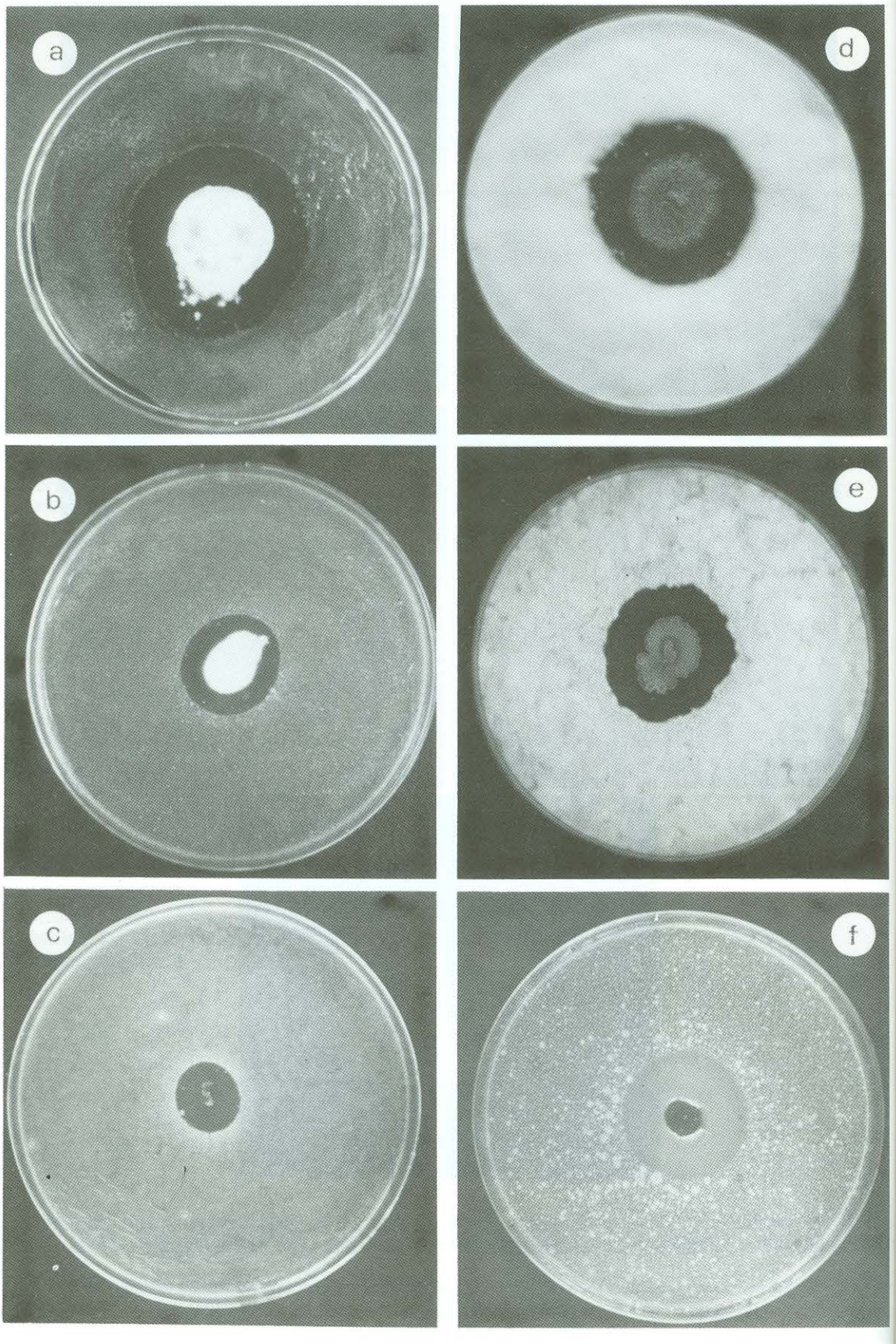


PLATE 1 - Antagonism among fungi. a) P. rubrum against A. ustus; b) P. brevicompactum against M. vellerea; c) P. brevicompactum against A. flavipes; d) P. brevicompactum against A. montagnei; e) Ch. queenslandicum against M. gypseum.

PLATE 2 - Antagonism among fungi and bacteria. Method A: a) P. purpurogenum (maize) and B. subtilis 1731 (rec); b) Ch. merdarium (Rice) and B. subtilis (A) ATCC 19659; c) M. thermophila (Maize) and B. subtilis (A) ATC 19659. Method B: d) B. subtilis (strain Firenze) and Ch. evolceanui (maize); e) B. subtilis (strain Firenze) and M. thermophila (maize); f) Inhibition halo produced by culture filtrate of P. purpurogenum (maize) against B. subtilis 1662 (rec^+).


a
d
b
e
c
f

REFERENCES

1. Aldrich, J.; Baker, R. (1970). Biological control of Fusarium roseum f.sp. dianthi by Bacillus subtilis. Plant Dis.Rep. 54:446-448.

2. Alström, S. (1983). A non-pathogenic bacterium affecting plants and phytopathogenic fungi. Abst., Third Int. Symp. Microbial.Ecology; August 7-12, 1983, Michigan State Univ.

3. Bartman C.D., Doerfler D.L., Bird B.A., Remaley A.T., Peace J.N., Campbell I.M. (1981). Mycophenolic acid production by Penicillium brevicompactum on solid media. Appl. Environm. Microb. 41:729-736.

4. Berdy J. (1980). CRC Handbook of antibiotic compounds. CRC Press, Boca Raton, Florida.

5. Broadbent P., Baker K.F., Waterworth Y. (1971). Bacteria and actinomycetes antagonistic to fungal root pathogens in australian soils. Austr. J. Biol. Sci. 24:925-944.

6. Brown M.E. (1973). Soil bacteriostasis limitation in growth of soil and rhizosphere bacteria. Can. J. Microbio. 15:195-199.

7. Brown M.E. (1974). Seed and root bacterization. Ann. Rev. Phytopath. 12: 181-197.

8. Caretta G., Del Frate G., Della Franca P., Guglielminetti M., Mangiarotti A.M., Savino E. (1985). Flora fungina del mais: funghi del terreno, del filoplano e spore dell'aria. Arch. Bot. Biogeogr. Ital. 61:143-168.

9. Caretta G., Del Frate G., Della Franca P., Guglielminetti M., Mangiarotti A.M., Savino E. (1986). Studies on the occurrence of fungi in a wheat-field. I. Mesophilic, thermophilic and keratinophilic fungi in soil. Bol. Mic. 3:55-70.

10. —. (1986) Studies on the occurrence of fungi in a wheat-field. II. Fungi on the phylloplane and fungal air-spora. Bol. Mic. 3:71-79.

11. —. (1987) Mesophilic, thermophilic and keratinophilic fungi in a rice field soil and phylloplnae fungi. Bol. Mic. 3: 117-127.

12. Domsch K.H., Gams W. (1986). Die Bedeutung Vorfruchtabhaengiger Verschiebungen in der Bodenmikroflora. 2. Antagonistische Einfluesse auf Pathogene Bodenpilze. Phytopath. Z. 63:165-176.

13. Dunleavy, J. (1955). Control of damping-off of sugar beet by Bacillus subtilis. Phytopath.45:252-258.

14. Gerhardson, B., Alström, S., Ramert, B. (1985). Plant reactions to inoculation of roots with fungi and bacteria. Phytopath. Z. 114:108-117.

15. Griffin, D.M. (1972). Ecology of soil fungi. Chapman and Hall, London. pp. 193.

16. Grove, D.C., Randall, W.A. (1955). Assay Methods of Antibiotics - a laboratory manual. New York: Medical Enciclopaedia Inc.

17. Loeffler W., Tschen, J.S.-M, Vanittanakom Nongnuch, Kugler, M., Knorpp, E., Hsieh T.-F., Wu T.-G. (1986). Antifungal effects of Bacilysin and Fengymycin from Bacillus subtilis F-29-3. A comparison with activities of other Bacillus antibiotics. J. Phytopath. 115:204-213.

18. Magan, N., a Lacey J. (1984). Effect of water activity, temperature and substrate on interactions between field and storage fungi. Trans. Br. Mycol. Soc. 82:83-93.

19. Merriman P.R., Price, D.R., Kollmorgen J.P., Piggott, T., Ridge, E.H. (1974). Effects of seed inoculation with Bacillus subtilis and Streptomyces griseus on the growth of cereals and carrots. Aust. J. Agric. Res. 25:219-226.

20. Moss, N.O. (1971). The rubratoxins, toxic metabolites of Penicillium rubrum Stoll., Chapter 5, in Microbial toxins, fungal toxins, vol. VI eds. A. Ciegler, S. Kadis and S.J. Ajl (New York, Acad. Press).

21. Natori S., Sakaki S., Kurata H., Udagawa S.-I., Ichinoe M., Saito M., Umeda M., Ohtsubo K. (1970). Production of rubratoxin B by Penicillium purpurogenum Stoll. Appl. Microbiol. 19:613-617.

22. Papavizas G.C., Lumsden R.D. (1980). Biological control of soil-borne fungal propagules. Ann. Rev. Phytopathol. 18:389-413.

23. Porter, C.L. (1924). Concerning the characters of certain fungi as exhibited by their growth in the presence of other fungi. Amer. J. Bot. 11:168-188.

24. Schroth, M.N., Hancock (1981). Selected topics in biological control. Ann. Rev. Microbiol. 35:453-476.

25. —, — (1982). Disease-suppressive soil and root-colonizing bacteria. Science 216:1376-1381.

26. Sharon N., Pinsky A., Turner-Graff R., Babad J., Cercos A.P. (1954). Classification of the antifungal antibiotics form Bacillus subtilis. Nature, London 174:1190-1191.

27. Skidmore, A.M., Dickinson, C.H. (1976). Colony interactions and hyphal interference between Septoria nodorum and phylloplane fungi. Trans. Br. Mycol. Soc. 66:57-64

28. Steyn, P.S., Vleggaar R. (1974). Austocystins. Six novel dihydrofurd (3', 2':4,5) furo (3, 2-6) xanthenones from Aspergillus ustus. J. Chem. Soc., Perkin Trans. I:2250-6

29. Suslow, T.V., Kloepper, J.W., Schroth, M.N., Burr, T.J. (1979). Beneficial bacteria enhance plant growth. California Agric. 33:15-17.

30. Swinburne T.R., Brown A.E. (1976). A comparison of the use of Bacillus subtilis with conventional fungicides for the control of apple canker Nectria galligena. Ann. Appl. Biol. 82:365-368.

31. Utkhede, R.S. (1983). Antagonism of isolates of Bacillus subtilis to Phytophthora cactorum. Can. J. bot. 62:1032- 1035.

32. Vapinder Singh, Deverall B.J. (1984). Bacillus subtilis as a control agent against fungal pathogens of Citrus fruit. Trans. br. Mycol. Soc. 83:487-490.

33. Vleggaar R., Steyn, P.S., Nagel, D.W. (1974). Constitution and absolute configuration of austdiol, the main toxic metabolite from A. ustus. J. Chem. Soc. Perkin Trans. I:45-9.

34. Wogan, G.N., Mateles, R.I. (1968). Mycotoxins, Prog. industrial microbiol. 7:149.