INCIDENCE OF ANTIPROTOZOAL AND ANTIVERMAL ANTIBIOTICS IN FUNGI. IV

FUNGI IMPERFECTI, ORDER *MONILIALES*, COLLECTED IN CHINA

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We have isolated cultures of fungi from soil samples collected in southern China and are now reporting the incidence of antibiotic activities in 54 isolates which were identified as 30 species belonging to the order *Moniliales* of the class *Fungi imperfecti*. This study of non-predacious *Moniliales* freshly isolated from nature indicates a strikingly high incidence of antiprotozoally active substances in this order of microorganisms. These data are in good accordance with our earlier observations[1] based on screening of predacious *Moniliales* from culture collections.

In our search for new antibiotics having an antiprotozoal and/or antinematodal activity we have screened members of the order *Moniliales* isolated from soil samples collected at altitudes between 100~700 meters above sea level in subtropical forests about 100 km north-west of Canton, China. Fifty-four isolates were identified as 30 species belonging to the order *Moniliales* and were subjected to screening for antiprotozoal, antivermal, antibacterial and antifungal activities using the following test organisms:

Protozoa: *Trypanosoma cruzi*, *Leishmania brasiliensis*, *Euglena gracilis*, *Euglena gracilis* (depigmented), *Astasia chattoni*, *Tetrahymena piriformis*

Nematode: *Anguillula aceti*

Bacteria: *Bacillus subtilis*, *Escherichia coli*

Fungi: *Candida pseudotropicalis*, *Aspergillus fumigatus*.

Materials and Methods

The screening procedure used in this work was identical with that described in a previous paper[1].

Results

In Table 1 the list of *Moniliales* tested and their activities are presented; Fig. 1 gives the percentual incidence of various antagonisms in the studied order of fungi.

Ninety-eight percent of the tested *Moniliales* were active against protozoa, 28 % showed antinematodal activity and 39 % were active against bacteria and/or fungi.

Discussion

In the first paper of this series[1] we have discussed in more detail our methods and aims in detecting cultures having a specific antiprotozoal or antinematodal activity. We are looking

Table 1. Antibiotic spectrum of 54 species of *Moniliales* from China. (For explanation of abbreviations see footnote)

Order, family, genus, species (variety)	Activity against										
	Protozoa after 48 hours						Bacteria and fungi after 24 hours				Nematodē after 72 hours
	Euglena gracilis	*Euglena gracilis* (depigmented)	*Astasia chattoni*	*Tetrahymena piriformis*	*Trypanosoma cruzi*	*Leishmania brasiliensis*	*Bacillus subtilis*	*Escherichia coli*	*Candida pseudotropicalis*	*Aspergillus fumigatus*	*Anguillula aceti*
Order: Moniliales											
Family: *Dematiaceae*											
Alternaria alternata (Fr) Keissler	0	0	0	0	##	D	0	0	±	0	0
Alternaria tenuissima (Fr) Wiltshire	D	D	D	0	D	D	##	0	##	0	0
Cladosporium cladosporioides (Fres) de Vries	D	D	##	##	D	D	0	0	###	0	0
Cladosporium herbarum (Pers) Link ex Fr.	###	0	0	0	###	D	0	0	0	0	0
Rhinocladiella sp. Nannf. in Melin *et* Nannf.	0	0	0	0	##	D	0	0	0	0	0
Family: *Moniliaceae*											
Acremonium bactrocephalum W. Gams	0	0	0	0	##	##	0	±	0	0	0
Acremonium strictum W. Gams (2 isolates)	0	0	0	0	##	D	0	0	0	0	0
	0	0	0	0	###	D	0	0	0	0	0
Aspergillus petrakii Vörös	0	0	0	0	0	0	0	0	0	0	0
Aspergillus sydowii (Bain. *et* Sartory) Thom *et* Church	##	0	0	0	0	D	###	0	0	0	0
Penicillium allahabadense Mehrotra *et* Kumar	0	0	0	0	D	D	±	0	+	0	0
Penicillium janthinellum Biourge (2 isolates)	0	0	0	0	##	D	##	0	0	0	0
	0	0	0	0	D	D	±	0	0	0	0
Penicillium ochrochloron Biourge	0	0	0	0	D	D	0	###	0	0	0

Table 1. (Continued)

Order, family, genus, species (variety)	Activity against										
	Protozoa after 48 hours						Bactetia and fungi after 24 hours				Nematode after 72 hours
	Euglena gracilis	*Euglena gracilis* (depigmented)	*Astasia chattoni*	*Tetrahymena piriformis*	*Trypanosoma cruzi*	*Leishmania brasiliensis*	*Bacillus subtilis*	*Escherichia coli*	*Candida pseudotropicalis*	*Aspergillus fumigatus*	*Amguillula aceti*
Penicillium oxalicum CURRIE *et* THOM	0	0	0	0	D	D	##	±	0	+	0
Penicillium purpurrescens (SOPP) RAPER *et* THOM	0	0	0	0	D	D	##	0	0	0	0
Penicillium rolfsii THOM	0	0	0	0	D	D	0	0	0	0	0
Penicillium simplicissimum (OUDEM.) THOM	0	0	0	0	0	D	0	0	0	0	##
Penicillium soppii ZALESKI	#	##	0	0	0	D	0	0	0	0	+
Trichoderma aureoviride RIFAI	##	##	0	0	D	D	0	0	0	0	#
Trichoderma pseudokoningii RIFAI	0	0	0	0	#	D	0	0	0	0	0
Verticillium indicum (PETCH.) W. GAMS	0	0	0	0	#	D	0	0	0	0	##
Verticillium cellulosae DASZEWSKA	#	0	0	0	0	D	0	0	0	0	##
Family: *Tuberculariaceae*											
Fusarium acuminatum ELL. *et* EVERHART	D	##	##	##	D	D	#	0	0	0	+
Fusarium equiseti (CORDA) SACC.	0	#	0	0	D	D	0	0	0	0	0
Fusarium moniliforme SHELDON (4 isolates)	0	0	0	0	#	D	0	0	0	0	##
	0	0	0	0	D	D	0	0	0	0	0
	D	0	0	0	D	D	##	0	0	0	0
	0	0	0	0	D	D	0	0	0	0	+
Fusarium moniliforme SHELDON var. *minus* WOLLENW. (10 isolates)	#	0	0	0	0	D	0	0	0	0	0
	0	0	0	0	0	D	+	0	0	0	0
	0	0	0	0	#	D	0	0	0	0	0
	0	0	0	0	#	D	0	0	0	0	+
	0	0	0	0	D	D	0	0	0	0	0
	0	0	0	0	D	D	0	0	0	0	0

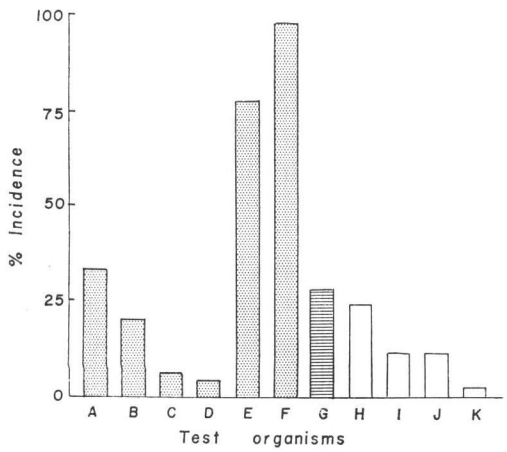
Table 1. (Continued)

Order, family, genus, species (variety)	Activity against										
	Protozoa after 48 hours						Bacteria and fungi after 24 hours				Nematodē after 72 hours
	Euglena gracilis	*Euglena gracilis* (depigmented)	*Astasia chattoni*	*Tetrahymena pirifformis*	*Trypanosoma cruzi*	*Leishmania brasiliensis*	*Bacillus subtilis*	*Escherichia coli*	*Candida pseudotropicalis*	*Aspergillus fumigatus*	*Anguillula aceti*
Fusarium moniliforme SHELDON var. *minus* WOLLENW. (10 isolates)	0	0	0	0	D	D	0	±	0	0	0
	0	0	0	0	D	D	0	0	0	0	0
	0	0	0	0	D	D	0	0	0	0	0
	##	#	0	0	D	D	0	0	0	0	0
Fusarium moniliforme SHELDON var. *subglutinans* WR. *et* REINK (2 isolates)	D	#	0	0	D	D	#	0	0	0	0
	D	(##)	0	0	D	D	±	0	+	0	##
Fusarium oxysporum SCHLECHT., emend. SNYDER *et* HANSEN p.p. (3 isolates)	0	0	0	0	0	D	0	0	0	0	0
	0	0	0	0	#	D	0	0	0	0	+
	+	0	0	0	##	D	+	0	0	0	0
Fusarium sambucinum FUCK. (3 isolates)	0	0	0	0	#	D	0	0	0	0	0
	+	0	0	0	#	D	0	0	0	0	0
	##	#	0	0	0	D	0	0	0	0	+
Fusarium solani (MART.) SACC. emend. SNYDER *et* HANSEN p.p. (6 isolates)	0	0	0	0	0	D	0	0	0	0	0
	0	0	0	0	0	D	0	0	±	0	+
	#	0	0	0	#	D	0	0	0	0	0
	#	##	0	0	0	D	0	0	0	0	+
	0	0	0	0	D	D	0	±	0	0	0
	0	0	0	0	D	D	0	0	0	0	+
Fusarium moniliforme SHELDON var. *lactis* (PIR. *et* RIB.) BILAI	0	0	0	0	##	D	0	±	0	0	0

Protozoa: 0 No activity, + about 25% of organisms dead, # about 50% of organisms dead, ## about 75% of organisms dead, (##) only single organisms living, D all organisms dead, L pronounced lysis. Nematode: 0 No activity, + about 25% of nematodes dead, # about 50% of nematodes dead, ## about 75% of nematodes dead, D all nematodes dead. Bacteria, Fungi: 0 No activity, ± diffuse zone, not more than 2 mm from edge of disc, + zone diameter up to 15 mm, # zone between 15~20 mm, ## zone more than 20 mm.

Fig. 1. Percentual incidence of various antagonisms in *Moniliales* (Percentages calculated without regard to degree of activity)

Protozoa:
A-*Euglena gracilis*
B-*Euglena gracilis* (depigmented)
C-*Astasia chattoni*
D-*Tetrahymena piriformis*
E-*Trypanosoma cruzi*
F-*Lishmania brasiliensis*
Nematode:
G-*Anguillula aceti*
Bacteria and Fungi:
H-*Bacillus subtilis*
I-*Escherichia coli*
J-*Candida pseudotropicalis*
K-*Aspergillus fumigatus*



for groups of microorganisms which could serve as abundant sources of antibiotics of these types and are also engaged in the isolation of antiprotozoal antibiotics. For example, from *Dactylaria lutea* ROUTIEN, the antiprotozoal activity of which we described previously[1], we have recently isolated a specifically active antiprotozoal antibiotic[2,3,4].

The incidence of *Moniliales* which produce compounds active against protozoa is strikingly high. Whether predacious[1] or non-predacious, practically all species belonging to this order showed such activity. Ninety-five percent of Oomycetes (mostly *Saprolegniales* have been studied) also produced compounds active against protozoa[7]. A substantially lower frequency of cultures active in this way was found in *Aspergillaceae*[5] (28%) and *Penicillia*[6] (60%).

Comparing the antibiotic activities of predacious *Moniliales* described previously[1] with that of non-predacious *Moniliales* recently isolated from nature gives some interesting indications:

1. Even non-predacious *Moniliales* are rich sources of antiprotozoal antibiotics. Ninety-eight percent of the species studied exhibited a pronounced antiprotozoal activity. In predacious *Moniliales* 100% of the screened cultures showed activity of this type[1].

2. Antinematodal activity was found in 28% of the microorganisms screened in this study. This is significantly less than that found in nematode-trapping predacious fungi (82%) described previously[1]. Taking into account the unusual living habits of nematode-trapping fungi this difference is easily understood.

3. The incidence of antibacterial and/or antifungal substances is about the same in both groups and makes 39% and 42% in non-predacious and predacious imperfect fungi, respectively.

Our results indicate that not only predacious, but also non-predacious *Moniliales* are abundant sources of antiprotozoal antibiotics. On the other hand, in looking for sources of antinematodal substances, it seems to be advisable to screen in nematode-trapping *Moniliales* where the inidence of antinematodal activities is about three times higher than that found in non-predacious *Moniliales*.

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