INCIDENCE OF ANTIPROTOZOAL AND ANTIVERMAL ANTIBIOTICS IN FUNGI V

CLASS *FUNGI IMPERFECTI*, COLLECTED IN THE VIETNAMESE DEMOCRATIC REPUBLIC

L. KRIŽKOVÁ, L. MARVANOVÁ*, P. NEMEC, J. BALANOVÁ and J. DOBIAS

Department of Biological Properties of Low Molecular Weight Substances,
Institute of Molecular Biology, Slovak Academy of Sciences,
885 34 Bratislava, Jánska 1, Czechoslovakia
*Czechoslovak Collection of Microorganisms,
University of J. E. Purkyně, Brno, Czechoslovakia

(Received for publication May 17, 1976)

From soil samples collected in the jungles of Ninh Binh Province of the Vietnamese Democratic Republic, 97 isolates belonging to 54 species of *Fungi imperfecti* were obtained and screened for the production of antibiotics. The isolates were identified as belonging to the orders *Melanconiales*, *Moniliales*, *Sphaeropsidales* and *Mycelia sterilia*. In the species studied a very high incidence of antiprotozoal activities were observed.

In continuation of our search for new antiprotozoal and antivermal antibiotics[1,2,3,4] we have collected soil samples in the vicinity of the Cúc Phúóng Biological Station in the jungles of Ninh Binh Province of the Vietnamese Democratic Republic (altitude, about 400 m). From these samples 97 isolates identified as 54 species of *Fungi imperfecti* belonging to the orders *Melanconiales*, *Moniliales*, *Sphaeropsidales* and *Mycelia sterilia* were obtained and screened for antiprotozoal, antivermal, antibacterial and antifungal activities.

Materials and Methods

The following test organisms were used:
Protozoa: *Trypanosoma cruzi, Leishmania brasiliensis, Tetrahymena pyriformis, Euglena gracilis, Astasia chattoni*;
Nematodes: *Turbatrix (Anguillula) aceti*;
Bacteria: *Bacillus subtilis, Escherichia coli*;
Fungi: *Candida pseudotropicalis, Aspergillus fumigatus.*

The screening procedure was as previously described[1]: the dry residue of butanolic extracts was dissolved in 1.0 ml of solvent and strips of Whatman No. 3 chromatographic paper were

Fig. 1. Percentual incidence of various antagonisms in *Fungi imperfecti* (Percentages calculated without regard to degree of activity).
Protozoa:
A – *Trypanosoma cruzi*
B – *Leishmania brasiliensis*
C – *Tetrahymena pyriformis*
D – *Euglena gracilis*
E - *Astasia chattoni*
Nematode:
F – *Turbatrix (Anguillula) aceti*
Bacteria and Fungi:
G – *Bacillus subtilis*
H – *Escherichia coli*
I – *Candida pseudotropicalis*
J – *Aspergillus fumigatus*

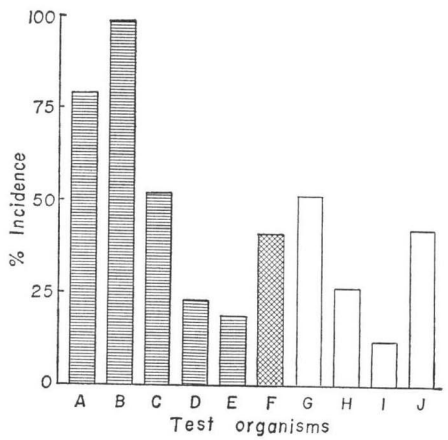

Table 1. Antibiotic spectrum of 97 isolates of *Fungi imperfecti* from The Vietnamese Democratic Republic

Order, family, genus, species (variety)	Activity against									
	Protozoa after 48 hours					Bacteria and fungi after 24 hours				Nematode after 72 hours
	Trypanosoma cruzi	*Leishmania brasiliensis*	*Tetrahymena pyriformis*	*Euglena gracilis*	*Astasia chattoni*	*Bacillus subtilis*	*Escherichia coli*	*Candida pseudotropicalis*	*Aspergillus fumigatus*	*Turbatrix aceti*
Order: Melanconiales										
Family: Melanconiaceae										
Pestalotia sp. (2 isolates)	0	D	0	0	0	0	0	0	0	0
	0	D	0	0	0	0	0	+	0	0
Order: Moniliales										
Family: Dematiaceae										
Humicola fuscoatra TRAAEN	D	D	D	0	0	±	++	0	0	0
Nodulisporium gregrarium/BERKELEY *et* CURTIS (MEYER) 2 isolates	0	0	0	0	0	++	++	+	++	0
	D	D	0	0	0	0	0	0	0	+
Phialophora cf. *cyclaminis* VAN BEYMA (2 isolates)	0	D	0	0	0	±	+	0	±	+
	++	D	++	0	0	0	0	0	0	0
Scytalidium sp.	0	D	++	0	0	0	±	0	0	0
Family: Moniliaceae										
Aspergillus aculeatus IIZUKA (5 isolate)	0	D	++	0	0	++	0	0	0	0
	D	D	0	++	0	0	0	0	±	0
	D	D	0	0	0	+	0	0	++	++
	D	D	0	0	0	+	0	0	++	++
	D	D	0	0	0	++	0	0	+	0
Aspergillus awamori NAKAZAWA	++	D	0	0	0	0	0	0	0	0
Aspergillus flavipes BAINIER *et* SARTORY	D	D	D	++	++	++	0	0	0	0
Aspergillus japonicus SAITO (2 isolates)	++	D	0	0	0	0	0	0	0	0
	D	D	0	0	++	++	++	+	++	0
Aspergillus niger VAN TIEGHEM (2 isolates)	D	D	D	D	D	0	0	0	0	0
	D	D	0	0	0	0	0	0	+	0
Aspergillus terreus THOM (4 isolates)	D	D	D	0	0	++	0	0	++	+
	D	D	D	0	0	++	±	0	++	0
	D	D	0	0	0	++	0	0	0	0
	D	D	D	0	0	++	0	0	++	++
Geotrichum sp. (2 isolates)	D	D	D	D	D	±	0	+	0	++
	D	D	0	0	0	+	0	0	0	++
Gliocladium roseum (LINK) BAINIER (2 isolates)	D	D	D	0	0	0	±	0	0	+
	++	D	0	0	0	+	±	0	±	++
Gliocladium cf. *roseum* (LINK) BAINIER (2 isolates)	D	D	++	0	0	0	0	0	±	0
	D	D	0	0	0	0	0	0	0	0
Myrothecium verrucaria (ALBERTINI *et* SCHWEINITZ) DITMAR ex FR. (2 isolates)	D	D	D	0	0	0	±	0	0	+
	D	D	D	0	D	0	0	++	++	++
Paecilomyces varioti BAINIER	0	D	0	0	0	0	0	0	0	0
Penicillium brevi-compactum DIERCKX	D	D	D	0	0	0	0	0	0	++
Penicillium citrinum THOM	D	D	D	0	0	++	0	0	+	0
Penicillium citrinum group	0	D	0	0	0	0	0	0	0	+
Penicillium claviforme BAINIER	D	D	D	0	0	±	++	0	+	0
Penicillium verrucosum DIERCKX var. *cyclopium* (WESTLING) SAMSON, STOLK *et* HADLOK	D	D	D	0	0	++	0	±	0	++
Penicillium funiculosum THOM (2 isolates)	0	D	0	0	0	0	0	0	0	0
	D	D	D	++	0	0	0	0	0	+
Penicillium gladioli MACHACEK	0	D	D	0	D	0	0	0	0	0
Penicillium expansum LINK ex S. F. GRAY (7 isolates)	D	D	D	D	++	++	++	++	++	0
	D	D	D	D	D	++	++	±	++	+
	0	D	0	0	0	0	0	0	0	0
	D	D	D	D	D	++	++	+	0	0
	D	D	D	D	D	++	0	0	++	0
	D	D	D	0	0	+	++	0	+	++
	D	D	D	D	0	++	++	++	++	++
Penicillium herquei BAIN *et* SARTORY	D	D	D	++	++	+	±	±	+	0
Penicillium chrysogenum THOM	D	D	D	++	++	+	+	0	+	0

(to be continued)

Table 1. (continued)

Order, family, genus, species (variety)	Activity against									
	Protozoa after 48 hours					Bacteria and fungi after 24 hours				Nematode after 72 hours
	Trypanosoma cruzi	*Leishmania brasiliensis*	*Tetrahymena pyriformis*	*Euglena gracilis*	*Astasia chattoni*	*Bacillus subtilis*	*Escherichia coli*	*Candida pseudotropicalis*	*Aspergillus fumigatus*	*Turbatrix aceti*
Penicillium janthinellum BIOURGE	D	D	D	0	0	0	0	0	+	++
(3 isolates)	D	D	0	0	0	±	+	0	0	0
	D	D	D	0	0	+	0	0	±	0
Penicillium martensii BIOURGE	D	D	D	0	0	0	0	0	0	+
Penicillium nigricans BAIN ex THOM	++	D	0	0	0	++	0	0	0	0
Penicillium oxalicum CURRIE *et* THOM	D	D	0	0	0	++	0	0	0	0
Penicillium cf. *paraherquei* ABE ex G. SMITH (2 isolates)	D	D	D	D	D	+++	++	++	++	++
	D	D	D	0	0	+	0	0	+	0
Penicillium rugulosum THOM	D	D	D	++	0	++	+	0	+	0
Penicillium steckii ZALESKI	D	D	D	0	0	0	0	0	±	0
Penicillium sp. (11 isolates)	D	D	D	0	0	++	++	0	++	++
	D	D	D	0	0	++	++	0	++	+
	D	D	D	++	++	0	0	0	0	+++
	D	D	D	0	0	0	0	0	0	++
	D	D	D	0	0	0	0	0	0	++
	D	D	D	0	0	+++	0	0	0	0
	D	D	0	0	0	+++	+	0	+	0
	D	D	D	0	0	++	0	0	0	0
	D	D	D	D	0	++	0	0	++	0
	0	D	0	0	0	0	0	0	0	+
	D	D	D	0	0	0	0	0	0	+
Podospora sp. (conidial stage?)	D	D	D	0	++	0	0	0	0	0
Scopulariopsis parva (BROWN *et* SMITH) SAMSON	D	D	0	0	0	+	0	0	0	0
Trichoderma harzianum RIFAI	0	D	0	0	0	0	0	0	0	0
Trichoderma koningii OUDEMANS	0	D	0	0	0	0	0	0	0	0
Trichoderma cf. *longibrachiatum* RIFAI	D	D	0	0	0	0	0	0	0	++
Trichoderma cf. *viride* PERS. ex S. F. GRAY	D	D	++	++	++	0	0	0	0	+
Verticillium niveo-stratosum LIND.	D	D	D	0	0	0	0	0	0	0
Family: Tuberculariaceae										
Dendrodochium, sp.	++	D	0	0	0	+	0	0	+	0
Fusarium acuminatum ELL. *et* EVERH.	D	D	0	0	0	0	0	0	+	0
Fusarium decemcellulare BRICK	0	D	0	0	0	0	0	0	++	++
Fusarium graminearum SCHWABE	0	++	0	0	0	+	0	0	0	D
Fusarium lateritium NEES	+	D	0	0	0	0	0	0	0	+
Fusarium oxysporum SCHLECHT. emend. SNYD. *et* HANS. (3 isolates)	D	D	0	0	0	++	0	0	++	+
	++	D	0	++	0	0	+	0	0	0
	D	D	0	0	0	0	0	0	0	0
Fusarium solani (MART.) SACC., emend. SNYD. *et* HANS. (4 isolates)	D	D	D	0	0	0	0	0	0	++
	D	D	0	++	0	0	0	0	0	++
	++	D	0	++	0	0	±	0	0	0
	0	D	0	++	++	±	0	0	±	0
Fusarium semitectum BERK. *et* RAV. in BERKELEY, var. *majus* WR. (3 isolates)	D	D	D	0	0	++	0	0	0	0
	D	D	D	0	0	0	0	0	+	++
	0	D	0	0	0	0	0	0	0	0
Order: Sphaeropsidales										
Family: Sphaeropsidaceae										
Coniothyrium sp.	0	D	0	0	0	0	0	0	0	0
Diplodia mutila (FRIES.) MONT.	D	D	D	++	D	++	0	0	++	0
Lasiodiplodia theobromae (PAT.) GRIFFON *et* MAUBL.	D	D	0	0	0	+	0	0	0	0
Robillardia sp.	0	++	0	0	0	+	0	0	0	++
Order: Mycelia sterilia										
Rhizoctonia sp.	++	D	0	0	0	+	0	0	0	+

Protozoa 0: No activity. +: about 25% of organisms dead. ++: about 50% of organisms dead. +++: about 75% of organisms dead. D: all organisms dead.

Nematode 0: No activity. +: about 25% of organisms dead. ++: about 50% of organisms dead. +++: about 75% of organisms 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

dipped into the solution. The strips were dried and cut in 1×1 cm squares which were added to 1.0 ml of the protozoa and nematode suspensions. Antibacterial and antifungal activities were tested by the usual agar-plate method employing similar squares of impregnated paper.

Results and Discussion

The *Fungi imperfecti* identified and tested as well as their antibiotic activities are presented in Table 1; the percentual incidence of the various activities observed is reported in Fig. 1.

Ninety-nine percent of the *Fungi imperfecti* tested were active against protozoa, 41% showed antinematodal activity, 57% of the cultures studied were active against bacteria, and antifungal activity was detected in 45% of the fungi screened.

The high incidence of antiprotozoal activity (99%) is in good agreement with the results we published previously[1,2,4] and further indicates that the *Fungi imperfecti* are a rich source of antiprotozoal substances.

References

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