Bull. Org. mond. Santé
Bull. World Hlth Org. 1952, 6, 163-172

ANTIFUNGAL ANTIBIOTICS *

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The great majority of antibiotics that have been isolated in the numerous screening programmes concerned with the search for new chemotherapeutic agents have been tested primarily for their activity against different bacteria. Only limited consideration has been given to those antibiotics which possess largely antifungal properties. With the growing importance of various antibiotics in clinical medicine, however, especially in the treatment of diseases caused by bacteria and some of the larger viruses, the need for substances with antifungal properties, especially substances which are not too toxic and which offer promise in human and animal therapy, has become of great importance.

Antifungal antibiotics are needed for three chief purposes :

(*a*) to control—if not completely to eradicate—various surface and deep-seated infections caused by fungi;

(*b*) to control the fungus infections (notably those caused by *Candida albicans* [1]) which frequently follow extensive use of antibiotics in the treatment of respiratory and gastro-intestinal diseases caused by bacteria;

(*c*) to control plant diseases ; the discovery of antibiotics highly effective against human and animal diseases of bacterial origin has raised hopes of finding similar agents active against fungi pathogenic to plants.

The various antibiotics so far isolated from cultures of different microorganisms may be divided into certain broad groups on the basis of their respective antimicrobial spectra :

(*a*) Antibiotics that have the capacity to inhibit the growth of both bacteria and fungi. The variations in activity against these organisms are both quantitative and qualitative in nature, corresponding to the specific

* Journal Series Paper, New Jersey Agricultural Experiment Station, Rutgers University — The State University of New Jersey, Department of Microbiology.

spectra of the various substances. Here belong a number of compounds produced by fungi, actinomycetes, and bacteria—notably, gliotoxin, clavacin, actinomycin, streptothricin, and tyrothricin. They vary greatly in their chemical nature, antibiotic spectra, and toxicity to animals.

(*b*) Antibiotics that are active upon bacteria and actinomycetes, but not at all, or only to a very limited extent, upon fungi. This group includes most of the substances that have found extensive application as chemotherapeutic agents—notably, penicillin, streptomycin, chloramphenicol, aureomycin, terramycin, and neomycin.

(*c*) Antibiotics that are active upon fungi, but not at all, or only to a very limited extent, upon bacteria and actinomycetes. This group of substances appears to be most significant from the point of view of their utilization in the treatment of fungus diseases. It is sufficient to mention actidione, antimycin, fradicin, and fungicidin. These substances, too, vary greatly in their chemical nature, antifungal spectra, and toxicity to animals.

Sources

Although antifungal agents are produced by several different groups of micro-organisms, it is the actinomycetes which offer the greatest promise from the chemotherapeutic point of view. Alexopoulos [1] was the first

TABLE I. ACTIVITY OF 197 CULTURES OF STREPTOMYCES AGAINST CERATOSTOMELLA ULMI *

Width of inhibition zone (mm)	Cultures	
	number	%
0	103	52.0
1-5	22	11.0
6-10	26	13.0
11-15	24	12.0
16-20	16	8.0
21-25	5	2.5
25-30	1	0.5

* The cross-streak method of inoculation on a potato-glucose or peptone-glucose medium was used.

to show that as many as 56% of all the cultures of actinomycetes isolated from the soil possessed some antifungal properties; nearly a third of these, or 17.5% of the cultures, were strong inhibitors of fungal growth.

This wide distribution of antifungal agents among the actinomycetes was confirmed by Cooper & Chilton [3] and by a number of other investigators. This is well illustrated in table I, where the width of the inhibition zone may be considered as indicating the capacity for the production of antifungal agents.

TABLE II. ORIGIN, CHEMICAL NATURE, AND ACTIVITY OF ANTIFUNGAL ANTIBIOTICS

Antibiotic	Producing organism	Chemical nature	Active upon
	Actinomycetes		
Actidione	*Streptomyces griseus*	Diketone	Yeasts and fungi
Actinomycin	*Streptomyces antibioticus*	Nitrogen-containing aromatic pigment	Bacteria and fungi
Antimycin A	*Streptomyces* sp.	Nitrogenous phenol	Yeasts and fungi
Fradicin	*Streptomyces fradiae*	Nitrogenous weak base	Yeasts and fungi
Musarin	*Streptomyces* sp.	Organic acid	Fungi and bacteria
Streptothricin	*Streptomyces lavendulae*	Organic base	Fungi and bacteria
Fungicidin	*Streptomyces* sp.	—	Yeasts and fungi
Rimocidin	*Streptomyces rimosus*	Amphoteric substance	Yeasts and fungi
C381*	*Streptomyces* sp. (WC 3569)†	—	Yeasts and fungi
C135*	*Streptomyces* sp. (WC 3570)†	—	Yeasts and fungi
	Fungi		
Clavicin	*Aspergillus clavatus*	Unsaturated ketone	Bacteria and fungi
Gliotoxin	*Trichoderma*	Contains sulfur and nitrogen	Bacteria and fungi
Trichothecin	*Trichothecium roseum*	Unsaturated ketone	Fungi
Viridin	*Trichoderma viride*	Contains carbon, hydrogen, oxygen	Fungi
	Bacteria		
Eumycin	*Bacillus subtilis*	Alcohol-soluble	Bacteria and fungi
Pyocyanin	*Pseudomonas aeruginosa*	α-ketophenazine	Bacteria and fungi
Hemipyocyanin	*Pseudomonas aeruginosa*	α oxyphenazine	Bacteria and fungi
Tyrothricin	*Bacillus brevis*	Polypeptide	Bacteria and fungi

*** These substances have recently been isolated in crude form in our laboratories.**
† WC = Waksman Collection

Table II lists some of the antibiotics now known to be active against fungi. As seen from this list, antifungal substances are produced by the various groups of micro-organisms. The source of the substance is no indication, however, of its relative potency, its antimicrobial activity, or its potential toxicity.

TABLE III. ANTIBIOTIC SPECTRUM OF FRADICIN [6,10]

Test organism	Minimum inhibitory concentration (μg/ml)
Staphylococcus aureus	>1,000
Bacillus mycoides	>1,000
Bacillus subtilis	>1,000
Escherichia coli	>1,000
Streptomyces griseus	>1,000
Trichophyton mentagrophytes	2.4
Trichoderma sp.	1.5
Aspergillus niger	2.4
Fusarium sp.	1.5
Penicillium notatum	0.13
Ceratostomella ulmi	0.12
Candida albicans	1.5
Histoplasma capsulatum	1.0-3.0
Coccidioides immitis	1.25
Endamoeba histolytica	1.0

TABLE IV. ANTIFUNGAL SPECTRUM OF FUNGICIDIN [5]

Test organism	Minimum inhibitory concentration (μg/ml)
Cryptococcus glutinis	1.6
Saccharomyces cerevisiae	3.1
Geotrichum lactis	6.3
Aspergillus fumigatus	6.3
Penicillium notatum	3.1
Rhizopus nigricans	3.1
Ceratostomella ulmi	6.3
Histoplasma capsulatum	1.6
Blastomyces dermatitidis	1.6
Coccidioides immitis	6.3
Cryptococcus neoformans	1.6
Candida albicans	3.1
Trichophyton mentagrophytes	6.3
Trichophyton rubrum	6.3
Sporotrichum schenckii	13.0
Monosporium apiospermum	100.0
Phialophora verrucosa	13.0

Antibiotic Spectra

The characteristic spectra of three antibiotics active primarily against fungi are shown in tables III, IV, and V. Table VI gives the comparative potency of several antibiotics, determined under similar test conditions. None of these antibiotics has any antibacterial activity, and most of them have very little anti-actinomycetes activity. Some are highly active against the yeastlike fungi, e.g., *C. albicans*, whereas others are more active against the filamentous fungi.

TABLE V. ANTIBIOTIC SPECTRUM OF C381, INCUBATED FOR 7 DAYS AT 28°C

Test organism	Minimum inhibitory concentration (μg/ml)
Candida albicans	1.4-2.0
Candida tropicalis	6.0 [a]
Candida pseudotropicalis	12.0 [a]
Candida kruzii	3.0-6.0
Candida brumptii	6.0-7.0
Cryptococcus neoformans	1.4
Blastomyces dermatitidis,[b] mycelial phase	0.6
Blastomyces dermatitidis,[b] yeast phase	1.5
Sporotrichum schenckii	1.4
Phialophora verrucosa	<1.0
Hormodendron pedrosoi	<1.0
Trichophyton mentagrophytes	20.0 [c]
Trichophyton rubrum	20.0 [c]
Saccharomyces cerevisiae	3.0
Torulopsis pulcherrima	4.0
Debaryomyces kloeckleri	6.0
Penicillium notatum	1.0-5.0
Aspergillus niger	330.0
Ceratostomella ulmi	6.6
Fusarium sp.	25.0
Chaetomium sp.	330.0
Nocardia asteroides	>100.0
Streptomyces sp. 3535	>100.0
Escherichia coli	>100.0
Staphylococcus aureus	>100.0

[a] >20.0 μg/ml after incubation for 3 days
[b] incubated at 37°C
[c] 30.0 μg/ml after incubation for 1 week

TABLE VI. COMPARATIVE SPECTRA OF VARIOUS ANTIFUNGAL ANTIBIOTICS

Test organism	Minimum inhibitory concentration (μg/ml) of					
	C381	C135	actidione[11]	anti-mycin A	fradi-cin[10]	fungi-cidin[5]
Candida albicans	1.7	0.25	>1,000	1	0.8	3.1
Cryptococcus neoformans	1.4	<2.5	0.2	—	3.0	1.0
Trichophyton mentagrophytes	20.0	10.0	>1,000	>33.0	4.0	6.3
Trichophyton rubrum	20.0	—	>1,000	—	1.6	6.3
Blastomyces dermatitidis, yeast phase	1.5	—	>1,000	—	—	1.6
Histoplasma capsulatum	—	—	—	—	2.0	1.6
Coccidioides immitis	—	—	>1,000	—	1.3	6.3
Saccharomyces cerevisiae	3.0	<2.5	10.0	—	—	3.1
Penicillium notatum	1.0-5.0	25.0	—	13.0	0.4	3.1
Aspergillus niger	—	5	20.0	>33.0	2.4	—
Nocardia asteroides	>100.0	—	>1,000	—	—	—
Bacteria	0	0	0	0	0	0

The recognition of selective activity of these antibiotics upon different groups of micro-organisms permits one to make certain very striking observations. It is of interest to note that from a taxonomic point of view the relative sensitivity and resistance to various antibiotics displayed by the actinomycetes resembles the behaviour of the bacteria. In this respect the actinomycetes are quite distinct from the fungi, and this fact adds further weight to the concept, now generally held, that these organisms should be classed with the bacteria rather than with the fungi. This has been the tendency recently in the various manuals of bacteriology, notably in that of Bergey.[2] Moreover, it should be recognized that human and animal diseases caused by actinomycetes may respond to the same antibiotics which control bacterial infections; those agents found to be highly effective against the latter should also logically prove to be effective against the former. Actually, penicillin and various other antibiotics have already found extensive application in the treatment of actinomycotic diseases.

Fungistatic and Fungicidal Properties

Tables VII and VIII present certain pertinent data concerning the fungistatic and fungicidal properties of two recently isolated antifungal antibiotics, C381 and fradicin.

C381, which is highly fungistatic to *C. albicans*, has little fungicidal effect upon this organism; on the other hand, it exhibits strong fungicidal

TABLE VII. FUNGICIDAL ACTIVITY OF C381

Concentration of antibiotic (μg/ml)	Time of contact (hours)	Test organism			
		Candida albicans	*Cryptococcus neoformans*	*Trichophyton mentagrophytes*	*Blastomyces dermatitidis* (yeast phase)
10	0.5	+	+	+	+
	1	+	+	+	±
	24	+	+	+	0
	48	+	±	+	0
50	0.5	+	+	±	±
	1	+	±	0	0
	24	+	0	0	0
	48	+	0	0	0
100	0.5	+	+	0	0
	5	+	+	0	0
	24	+	±	0	0
	48	+	0	0	0

+ = good growth ; ± = limited growth ; 0 = no growth

TABLE VIII. FUNGISTATIC AND FUNGICIDAL ACTIVITY OF FRADICIN

Period of incubation (days)	Effect	Minimum concentration of fradicin (μg/ml) active against			
		Aspergillus niger	*Penicillium notatum*	*Fusarium* sp.	*Candida albicans*
2	Fungistatic	33	0.3	8.3	8.3
	Fungicidal	> 166	1.6	33	> 166
4	Fungistatic	83	0.83	8.3	8.3
	Fungicidal	> 166	0.83	33	> 166
6	Fungistatic	83	0.83	16.6	8.3
	Fungicidal	83	0.83	16.6	166
8	Fungistatic	83	0.83	16.6	8.3
	Fungicidal	83	0.83	16.6	8.3

properties against certain dermatophytes. The fungicidal action of fradicin against *C. albicans* is also limited; it is more effective against *Penicillium notatum*. However, the reverse may be true, as in the case of C135, which produces an antifungal substance highly fungicidal upon *C. albicans*.

The fungicidal potency of fradicin was measured as follows : tubes containing 5 ml of 1% peptone plus 2% glucose broth were inoculated

with 0.5 ml of a suspension of the test organism. Fradicin was added to the tubes to give concentrations ranging from 0 to 166 μg/ml. The tubes were incubated at 28°C and were then examined daily for growth and viability by streaking on plates. Fradicin was found to kill *P. notatum* and *Fusarium* sp. within 48 hours, but more than 96 hours were required to kill *C. albicans* and *Aspergillus niger*. Fradicin may, therefore, be considered as only weakly fungicidal.

When the cultures were examined microscopically, the following phenomenon was observed in the case of *C. albicans* : in the tubes containing no fradicin, the growth was entirely yeastlike with no tendency to filament formation. At concentrations just under the inhibiting one, extensive filamentation was observed. At higher concentrations, very minute filaments were found. When the filamentous cells were transferred to a medium containing no fradicin, all signs of filamentous development disappeared and growth became entirely yeastlike.

Possible Mode of Action of Fradicin

These observations suggest that there is a connexion between the mechanism of the dimorphism of *C. albicans* and the mode of action of fradicin. According to Nickerson,[7, 8, 9] the formation of filaments by *C. albicans* may be caused by a deficiency of sulfhydryl (-SH) groups within the cell. The -SH groups are produced only when there is a strong reducing potential in the cell. Under normal conditions, glucose provides this reducing potential, -SH groups are formed, and the organism reproduces normally by yeastlike budding.

If this is true, the addition of cysteine to a medium should reverse the action of fradicin. This was found to be the case. Addition of cysteine in a concentration of 10^{-3} caused a reversal to the yeast form; in a solid medium containing fradicin, 0.05% cysteine completely removed the inhibition of growth of *C. albicans*.

To determine whether the effect of cysteine was due to its sulfhydryl group or to its reducing properties, other reducing agents were tried. All the reducing agents tested removed the antifungal action of fradicin, as shown in table IX.

There is apparently no chemical reaction between these reducing agents and fradicin, but rather a biological effect, since the organism is able to overcome the activity of the antibiotic when the reducing agents are present in small quantities in the medium. This was shown by the fact that no drop in potency of fradicin was observed after the reducing agents were added to fradicin solutions in test-tubes, and assays made. It seems, therefore, that the action of fradicin is concerned with the oxidation-reduction potential of the cell.

TABLE IX. INHIBITING EFFECTS OF REDUCING AGENTS UPON THE ANTIFUNGAL ACTION OF FRADICIN AGAINST CANDIDA ALBICANS

Period (hours)	Fradicin dilution for inhibition (×1000)			
	control	cysteine *	ascorbic acid*	sodium bisulfite*
48	500	<33	<33	200
96	500	<33	<33	50
120	450	<33	<33	30

* 0.05% concentration

Oxygen tension appears to have no effect on the activity of fradicin. Plates containing fradicin and inoculated with *C. albicans* and *Saccharomyces cerevisiae* were incubated at various oxygen tensions. There was no difference in the potency of fradicin under these conditions.

SUMMARY

A comparative study was made of the antifungal properties of various antibiotics. In nature these are widely distributed and are produced by various groups of microorganisms. They comprise two groups of substances : (*a*) those which are active against bacteria, actinomycetes, and fungi, and (*b*) those which are active against fungi but not against bacteria or actinomycetes.

The production of antifungal antibiotics by actinomycetes was also studied. As many as half of the freshly isolated cultures were found to have some effect upon fungi, and 20% or more had a marked effect. The antibiotics produced by these organisms have a wide spectrum, although some are highly active against yeastlike fungi and others against filamentous fungi. These antibiotics vary also in their fungicidal properties.

The mode of action of fradicin appears to have some relation to the oxidation-reduction potential of the cell, as is suggested by the fact that the inhibiting activity of this substance can be suppressed by reducing agents.

RÉSUMÉ

Les propriétés antifongiques de divers antibiotiques ont été étudiées. Ces antibiotiques, très répandus dans la nature, sont produits par différents groupes de micro-organismes. Ils comprennent des substances appartenant à deux groupes : *a*) celles qui sont actives contre des bactéries, des actinomycètes et des champignons; *b*) celles qui sont actives contre les champignons, mais sont sans action sur les bactéries et les actinomycètes.

La production d'antibiotiques antifongiques par des actinomycètes a aussi fait l'objet de recherches. La moitié des cultures fraîchement isolées exerçaient quelque activité sur les champignons, et 20% au moins avaient sur ces derniers un effet marqué. Les antibiotiques produits par ces organismes ont un spectre d'activité étendu, bien que certains d'entre eux soient particulièrement actifs contre les champignons levuriformes et d'autres contre les champignons filamenteux. Les propriétés fongicides de ces antibiotiques diffèrent.

Le mode d'action de la fradicine — dont le pouvoir inhibiteur peut être supprimé par l'addition de réducteurs au milieu de culture — semble en relation avec le potentiel d'oxydo-réduction cellulaire.

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