

INCIDENCE OF ANTIPROTOZOAL AND ANTIVERMAL ANTIBIOTICS IN FUNGI. II

CLASS OOMYCETES

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By our method of screening for antiprotozoal antibiotics and for substances with antivermal activity¹⁾ 38 species of Oomycetes were tested. A very high incidence of species with antiprotozoal activity (92%), a high incidence of species with antivermal activity (68%) and a substantially lower incidence of species with antifungal and/or antibacterial activity (29%) were found. The results indicate that the species tested as well as the class Oomycetes in general are rich sources of antiprotozoal and antivermal antibiotics.

In our search for antiprotozoal antibiotics and for substances with antivermal activity, we tested the spectra of 38 species of Oomycetes obtained from the culture collection of the Centralbureau voor Schimmelcultures in Baarn, Holland. These microorganisms are representatives of a class of fungi the natural habitat of which is water. Extracts of the cultures were tested against protozoa (*Trypanosoma cruzi*, *Leishmania brasiliensis*, *Strigomonas culicidarum*, *Euglena gracilis*, *Euglena gracilis* depigmented, *Astasia chattoni*), a nematode (*Anguillula aceti*), bacteria (*Bacillus subtilis*, *Escherichia coli*) and fungi (*Candida pseudotropicalis*, *Aspergillus fumigatus*).

Materials and Methods

All materials and methods used in this work are identical with those described in another paper¹⁾.

Results

The list of Oomycetes tested and their activities are presented in Table 1.

Fig. 1 gives the percentual incidence of various antagonisms as observed in the studied species of Oomycetes. It was found that 95% of the cultures of Oomycetes tested

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

| | |
|---|------------------------------------|
| A: <i>Trypanosoma cruzi</i> | G: <i>Leishmania brasiliensis</i> |
| B: <i>Strigomonas culicidarum</i> | H: <i>Anguillula aceti</i> |
| C: <i>Euglena gracilis</i> | I: <i>Bacillus subtilis</i> |
| D: <i>Euglena gracilis</i> depigmented | J: <i>Escherichia coli</i> |
| E: <i>Astasia chattoni</i> | K: <i>Candida pseudotropicalis</i> |
| F: <i>Tetrahymena piriformis</i> | L: <i>Aspergillus fumigatus</i> |

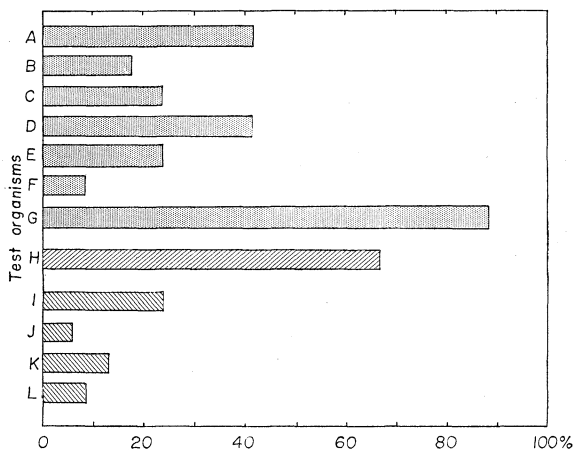


Table 1. Antibiotic spectrum of 38 Oomycetes.
(For explanation of abbreviations see footnote)

| Number of culture | Order, family, genus, species (variety) | Hour of testing | Activity against | | | | | | | | | | | |
|-------------------|---|-----------------|--------------------------------|--------------------------------|-------------------------|---------------------------------------|-------------------------|-------------------------------|-----------------------------------|--------------------------|-------------------------|---------------------------------|-------------------------|------------------------------|
| | | | Protozoa after 24 and 48 hours | | | | | | Bacteria and fungi after 24 hours | | | | Nematode after 72 hours | |
| | | | <i>Trypanosoma cruzi</i> | <i>Strigomonas culicidarum</i> | <i>Englena gracilis</i> | <i>Englena gracilis</i> (depigmented) | <i>Astasia chattoni</i> | <i>Tetrahymena piriformis</i> | <i>Leishmania brasiliensis</i> | <i>Bacillus subtilis</i> | <i>Escherichia coli</i> | <i>Candida pseudotropicalis</i> | | <i>Aspergillus fumigatus</i> |
| | Order : Saprolegniales Family : Saprolegniaceae | | | | | | | | | | | | | |
| 1 | <i>Achlya ambisexualis</i> RAPER | 24 48 | 0 0 | 0 0 | 0 0 | 0 0 | D D | 0 0 | L L | 0 0 | 0 0 | 0 0 | 0 0 | + |
| 2 | <i>Achlya americana</i> HUMPHREY | 24 48 | ++ D | + | D D | D D | D D | D D | D D | +++ 0 | 0 0 | +++ 0 | +++ 0 | ++ |
| 3 | <i>Achlya bisexualis</i> COKER | 24 48 | (+++) D | 0 0 | 0 0 | ++ ++ | ++ ++ | 0 0 | D D | 0 0 | 0 0 | 0 0 | 0 0 | 0 |
| 4 | <i>Achlya caroliniana</i> COKER | 24 48 | 0 0 | 0 0 | 0 0 | 0 0 | D D | 0 0 | L L | 0 0 | 0 0 | 0 0 | 0 0 | ++ |
| 5 | <i>Achlya conspicua</i> COKER | 24 48 | + | 0 | 0 | 0 | 0 | 0 | D | + | 0 | +++ | 0 | 0 |
| 6 | <i>Achlya crenulata</i> ZIEGLER | 24 48 | 0 0 | 0 0 | 0 0 | 0 0 | 0 0 | 0 0 | D D | 0 0 | 0 0 | 0 0 | 0 0 | 0 |
| 7 | <i>Achlya dubia</i> COKER | 24 48 | 0 0 | 0 0 | 0 0 | 0 0 | 0 0 | 0 0 | D D | 0 0 | 0 0 | 0 0 | 0 0 | 0 |
| 8 | <i>Achlya flagellata</i> COKER | 24 48 | 0 0 | 0 0 | 0 0 | 0 0 | 0 0 | 0 0 | +++ D | ++ 0 | 0 0 | 0 0 | 0 0 | 0 |
| 9 | <i>Achlya glomerata</i> COKER | 24 48 | 0 (+++) | 0 0 | 0 0 | 0 0 | 0 0 | 0 0 | D D | 0 0 | 0 0 | 0 0 | 0 0 | 0 |
| 10 | <i>Achlya intricata</i> BENECKE | 24 48 | 0 +++ | 0 0 | 0 0 | ++ ++ | 0 0 | 0 0 | D D | 0 0 | 0 0 | 0 0 | 0 0 | 0 |
| 11 | <i>Achlya klebsiana</i> PIETERS | 24 48 | D D | 0 0 | 0 0 | 0 0 | 0 0 | 0 0 | D D | +++ 0 | 0 0 | 0 0 | 0 0 | + |
| 12 | <i>Achlya proliferoides</i> COKER | 24 48 | 0 0 | 0 0 | 0 0 | 0 0 | 0 0 | 0 0 | D D | 0 0 | 0 0 | 0 0 | 0 0 | 0 |
| 13 | <i>Achlya radiosa</i> MAURIZIO | 24 48 | 0 0 | 0 0 | ++ ++ | 0 ++ | 0 0 | 0 0 | D D | 0 0 | 0 0 | 0 0 | 0 0 | ++ |
| 14 | <i>Achlya sparrowii</i> REISCHER | 24 48 | 0 0 | 0 0 | 0 0 | 0 0 | 0 0 | 0 0 | 0 ++ | 0 0 | 0 0 | 0 0 | 0 0 | + |
| 15 | <i>Aphanomyces cladogamus</i> DRECHSLER | 24 48 | 0 +++ | 0 0 | 0 0 | 0 0 | 0 0 | 0 0 | D D | ± 0 | 0 0 | 0 0 | 0 0 | + |
| 16 | <i>Aphanomyces cochlioides</i> DRECHSLER | 24 48 | +++ D | D D | 0 0 | + | 0 | 0 | D D | 0 0 | 0 0 | 0 0 | 0 0 | ++ |
| 17 | <i>Aphanomyces euteiches</i> DRECHSLER | 24 48 | ++ D | 0 0 | ++ +++ | ++ +++ | 0 0 | 0 0 | D D | 0 0 | 0 0 | 0 0 | 0 0 | + |
| 18 | <i>Aphanomyces laevis</i> DEBARY | 24 48 | 0 0 | 0 0 | 0 0 | 0 0 | 0 0 | 0 0 | 0 0 | 0 0 | 0 0 | 0 0 | 0 0 | ++ |
| 19 | <i>Aphanomyces treleaseanus</i> (HUMPHREY) COKER | 24 48 | D D | D D | 0 0 | 0 0 | 0 0 | 0 0 | D D | 0 0 | 0 +++ | + | + | ++ |
| 20 | <i>Brevilegnia gracilis</i> v. EEK | 24 48 | 0 0 | 0 0 | 0 0 | 0 0 | 0 0 | 0 0 | ++ +++ | ++ 0 | 0 0 | 0 0 | 0 0 | +++ |
| 21 | <i>Brevilegnia macrospora</i> v. EEK | 24 48 | 0 0 | 0 0 | 0 0 | 0 0 | 0 0 | 0 0 | +++ (+++) | 0 0 | 0 0 | 0 0 | 0 0 | + |

(To be continued)

Table 1 (continued)

| Number of culture | Order, family, genus, species (variety) | Hour of testing | Activity against | | | | | | | | | | | |
|-------------------|--|-----------------|--------------------------------|--------------------------------|-------------------------|---------------------------------------|-------------------------|-------------------------------|-----------------------------------|--------------------------|-------------------------|---------------------------------|-------------------------|------------------------------|
| | | | Protozoa after 24 and 48 hours | | | | | | Bacteria and fungi after 24 hours | | | | Nematode after 72 hours | |
| | | | <i>Trypanosoma cruzi</i> | <i>Strigomonas culicidarum</i> | <i>Euglena gracilis</i> | <i>Euglena gracilis</i> (depigmented) | <i>Astasia chattoni</i> | <i>Tetrahymena pyriformis</i> | <i>Leishmania brasiliensis</i> | <i>Bacillus subtilis</i> | <i>Escherichia coli</i> | <i>Candida pseudotropicalis</i> | | <i>Aspergillus fumigatus</i> |
| 22 | <i>Brevilegnia unisperra</i> COKER et BRAXTON var. <i>delica</i> COKER | 24 48 | 0 0 | 0 0 | ++ ++ | ++ ++ | 0 0 | 0 0 | D D | 0 0 | 0 0 | 0 0 | 0 0 | ++ ++ |
| 23 | <i>Isoachlya eccentrica</i> COKER | 24 48 | 0 0 | 0 0 | 0 0 | ++ ++ | 0 0 | 0 0 | 0 0 | 0 0 | 0 0 | 0 0 | 0 0 | ++ ++ |
| 24 | <i>Isoachlya intermedia</i> (COKER et HARY) COKER | 24 48 | D D | D D | 0 0 | ++ ++ | 0 0 | 0 0 | D D | 0 0 | 0 0 | +++ 0 | 0 0 | ++ ++ |
| 25 | <i>Isoachlya unisperra</i> COKER et COUCH | 24 48 | 0 0 | 0 0 | 0 0 | 0 0 | 0 0 | 0 0 | 0 0 | 0 0 | 0 0 | 0 0 | 0 0 | 0 0 |
| 26 | <i>Saprolegnia crustosa</i> MAURIZIO | 24 48 | 0 +++ | +++ D | 0 0 | 0 0 | 0 0 | 0 0 | 0 D | 0 0 | 0 0 | 0 0 | 0 0 | ++ ++ |
| 27 | <i>Saprolegnia delica</i> COKER | 24 48 | +++ D | +++ +++ | +++ +++ | +++ +++ | +++ +++ | 0 0 | D D | 0 0 | 0 0 | 0 0 | 0 0 | +++ +++ |
| 28 | <i>Saprolegnia dictina</i> HUMPHREY | 24 48 | 0 0 | 0 0 | 0 0 | 0 0 | 0 0 | 0 0 | D D | 0 0 | 0 0 | 0 0 | 0 0 | ++ ++ |
| 29 | <i>Saprolegnia ferax</i> (GRUTH) THURET | 24 48 | D D | 0 0 | 0 0 | +++ +++ | 0 0 | 0 0 | D D | 0 0 | 0 0 | 0 0 | 0 0 | 0 0 |
| 30 | <i>Saprolegnia laponica</i> GÄUMANN | 24 48 | D D | 0 0 | D D | D D | D D | D D | D D | + | ++ | + | 0 | 0 |
| 31 | <i>Saprolegnia litoralis</i> COKER | 24 48 | D D | +++ D | D D | D D | D D | D D | D D | ± | ± | ± | ± | +++ +++ |
| 32 | <i>Saprolegnia mixta</i> DEBARY | 24 28 | 0 0 | 0 0 | 0 0 | 0 0 | 0 0 | 0 0 | ++ +++ | ++ | 0 | 0 | 0 | +++ +++ |
| 33 | <i>Saprolegnia monoica</i> PRINGSHEIM | 24 48 | 0 0 | 0 0 | 0 0 | +++ +++ | +++ +++ | 0 0 | 0 0 | 0 | 0 | 0 | 0 | ++ ++ |
| 34 | <i>Saprolegnia parasitica</i> COKER | 24 48 | 0 0 | 0 0 | 0 0 | 0 0 | 0 0 | 0 0 | 0 + | 0 | 0 | 0 | 0 | +++ +++ |
| 35 | <i>Thraustotheca clavata</i> (DEBARY) HUMPHREY | 24 48 | 0 0 | 0 0 | 0 0 | 0 0 | D D | 0 0 | (+++) D | 0 | 0 | 0 | 0 | +++ +++ |
| | Order : Perenosporales Family : Pythiaceae | | | | | | | | | | | | | |
| 36 | <i>Phytophthora cryptogen</i> PETHYBR. | 24 48 | 0 0 | 0 0 | ++ ++ | ++ +++ | ++ +++ | 0 0 | D D | 0 | 0 | 0 | 0 | +++ +++ |
| 37 | <i>Phytophthora megasperma</i> DRECHSLER | 84 42 | 0 0 | 0 0 | 0 0 | 0 0 | 0 0 | 0 0 | D D | 0 | 0 | 0 | 0 | 0 0 |
| | Order : Leptomitales Family : Rhipidiaceae | | | | | | | | | | | | | |
| 38 | <i>Sapromyces elongatus</i> (CORNU) THAXTER | 24 48 | 0 0 | 0 0 | ++ ++ | ++ ++ | 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
(+++)
D only single organisms living
L all organisms dead
L pronounced lysis

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

Nematode :

0 No activity
+ about 25% of nematodes dead
++ about 50% of nematodes dead
+++ about 75% of nematodes dead
D all nematodes dead

are active against protozoa, 68 % against the nematodal test organisms, and 29 % against bacteria and/or fungi.

Discussion

The high incidence of cultures with antiprotozoal as well as of antivermal activities in the species of Oomycetes studied indicates that these organisms are a rich source of antibiotics of this type. Chromatographic studies of the cultures tested indicate that the antiprotozoal or antivermal activities are mostly due to the production of specifically active antiprotozoal or antivermal substances, whereas the antibacterial and/or antifungi activities are due to the production of different antibiotics.

The production of specifically active antiprotozoal or antivermal antibiotics can not be detected by the usual screening methods, and it is fairly certain, that in spite of their relatively high incidence in various microorganisms, substances of this type passed unnoticed through many agar-plate screening programs. In other papers we have published data on the incidence of antiprotozoal and antivermal antibiotics in Fungi imperfecti¹⁾ and in *Penicillia*²⁾. The incidence of antiprotozoal substances in Aspergillaceae was published in Reference³⁾.

References

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