

## INCIDENCE OF ANTIPROTOZOAL AND ANTIVERMAL ANTIBIOTICS IN FUNGI. III

### GENUS *PENICILLIUM*

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The results of screening for antiprotozoal and antivermal antibiotics in fungi of the genus *Penicillium* isolated from soil samples from Indonesia are presented. The incidence of cultures with antiprotozoal activity was found to be rather high (60%), substances with antivermal activity were detected in 38% of the cultures studied and antibacterial and/or antifungal activities were present in 53% of the cultures. The results indicate that the *Penicillia* could be a rich source of antiprotozoal and antivermal substances.

In our search for antiprotozoal and antivermal antibiotics we have tested 47 *Penicillia* isolated from soil samples collected in Indonesia. Extracts of the cultures were tested against protozoa (*Trypanosoma cruzi*, *Strigomonas culicidarum*, *Euglena gracilis*, *Euglena gracilis* depigmented, *Astasia chattoni*, *Tetrahymena piriformis*), a nematode (*Anguillula aceti*), bacteria (*Staphylococcus aureus*, *Escherichia coli*), and fungi (*Candida albicans*, *Aspergillus fumigatus*).

#### Materials and Methods

The methods used in this work are identical with those described in another paper<sup>1)</sup>, but the antiprotozoal activities were established only after 24 hours and *Leishmania brasiliensis* was excluded from the test. For the cultivation of the *Penicillia* CZAPEK-Dox medium was used.

#### Results

The individual activities of the cultures tested are presented in Table 1; the incidence of various antagonisms expressed in percentages is given in Fig. 1. Sixty percent of the *Penicillia* tested were active against protozoa, 38% were active against the nematodal test

Fig. 1. Percentual incidence of various antagonisms in *Penicillia*

- |   |                                  |
|---|----------------------------------|
| A: <i>Trypanosoma cruzi</i>               | F: <i>Tetrahymena piriformis</i> |
| B: <i>Strigomonas culicidarum</i>         | G: <i>Anguillula aceti</i>       |
| C: <i>Euglena gracilis</i>                | H: <i>Staphylococcus aureus</i>  |
| D: <i>Euglena gracilis</i><br>depigmented | I: <i>Escherichia coli</i>       |
| E: <i>Astasia chattoni</i>                | J: <i>Candida albicans</i>       |
|   | K: <i>Aspergillus fumigatus</i>  |

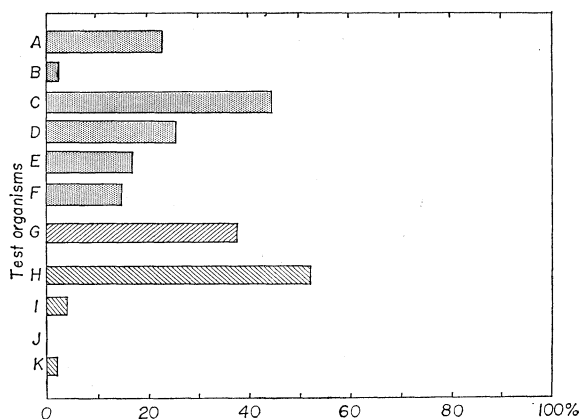


Table 1. Antibiotic spectrum of 47 *Penicillia*  
(For explanation of abbreviations see footnote)

| Serial number | Culture collection number | Activity against         |                                |                         |                                       |                         |                               |                                   |                         |                         |                              |                         |
|---------------|---------------------------|--------------------------|--------------------------------|-------------------------|---------------------------------------|-------------------------|-------------------------------|-----------------------------------|-------------------------|-------------------------|------------------------------|-------------------------|
|               |                           | Protozoa after 24 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>Staphylococcus aureus</i>      | <i>Escherichia coli</i> | <i>Candida albicans</i> | <i>Aspergillus fumigatus</i> | <i>Anguillula aceti</i> |
| 1             | I-386                     | 0                        | 0                              | 0                       | 0                                     | 0                       | 0                             | ++                                | 0                       | 0                       | 0                            | +                       |
| 2             | I-390                     | 0                        | 0                              | ++                      | +++                                   | 0                       | ++                            | ±                                 | 0                       | 0                       | 0                            | 0                       |
| 3             | I-394                     | +                        | 0                              | 0                       | 0                                     | 0                       | +++                           | 0                                 | 0                       | 0                       | 0                            | 0                       |
| 4             | I-396                     | 0                        | 0                              | 0                       | 0                                     | 0                       | 0                             | 0                                 | 0                       | 0                       | 0                            | ++                      |
| 5             | I-398                     | ++                       | 0                              | 0                       | 0                                     | 0                       | 0                             | ±                                 | 0                       | 0                       | 0                            | +                       |
| 6             | I-406                     | 0                        | 0                              | 0                       | 0                                     | 0                       | 0                             | 0                                 | 0                       | 0                       | 0                            | +                       |
| 7             | I-414                     | 0                        | 0                              | 0                       | 0                                     | 0                       | 0                             | 0                                 | 0                       | 0                       | 0                            | +                       |
| 8             | I-419                     | 0                        | 0                              | 0                       | 0                                     | 0                       | 0                             | +++                               | 0                       | 0                       | 0                            | +                       |
| 9             | I-422                     | 0                        | 0                              | 0                       | 0                                     | 0                       | 0                             | ±                                 | 0                       | 0                       | 0                            | 0                       |
| 10            | I-423                     | 0                        | 0                              | D                       | 0                                     | ++                      | 0                             | 0                                 | 0                       | 0                       | 0                            | 0                       |
| 11            | I-433                     | 0                        | 0                              | +++                     | +++                                   | 0                       | 0                             | 0                                 | 0                       | 0                       | 0                            | +                       |
| 12            | I-434                     | 0                        | 0                              | ++                      | 0                                     | 0                       | 0                             | ±                                 | 0                       | 0                       | ++                           | ++                      |
| 13            | I-440                     | 0                        | 0                              | ++                      | ++                                    | +++                     | 0                             | 0                                 | 0                       | 0                       | 0                            | ++                      |
| 14            | I-441                     | ++                       | 0                              | +++                     | 0                                     | ++                      | 0                             | 0                                 | 0                       | 0                       | 0                            | 0                       |
| 15            | I-444                     | 0                        | 0                              | 0                       | 0                                     | 0                       | 0                             | ++                                | 0                       | 0                       | 0                            | 0                       |
| 16            | I-449                     | 0                        | 0                              | +                       | +                                     | 0                       | 0                             | ++                                | 0                       | 0                       | 0                            | ++                      |
| 17            | I-452                     | 0                        | 0                              | 0                       | 0                                     | D                       | 0                             | ±                                 | 0                       | 0                       | 0                            | 0                       |
| 18            | I-453                     | 0                        | 0                              | +++                     | 0                                     | 0                       | 0                             | 0                                 | 0                       | 0                       | 0                            | 0                       |
| 19            | I-458                     | D                        | 0                              | +++                     | 0                                     | 0                       | 0                             | 0                                 | 0                       | 0                       | 0                            | 0                       |
| 20            | I-461                     | D                        | 0                              | ++                      | 0                                     | 0                       | 0                             | 0                                 | 0                       | 0                       | 0                            | 0                       |
| 21            | I-465                     | 0                        | 0                              | 0                       | 0                                     | 0                       | 0                             | 0                                 | 0                       | 0                       | 0                            | 0                       |
| 22            | I-467                     | 0                        | 0                              | D                       | +                                     | (+++)                   | D                             | 0                                 | 0                       | 0                       | 0                            | ++                      |
| 23            | I-475                     | 0                        | 0                              | D                       | ++                                    | 0                       | 0                             | 0                                 | 0                       | 0                       | 0                            | +                       |
| 24            | I-476                     | 0                        | 0                              | 0                       | ++                                    | ++                      | 0                             | 0                                 | 0                       | 0                       | 0                            | ++                      |
| 25            | I-488                     | 0                        | 0                              | D                       | +++                                   | 0                       | D                             | ±                                 | 0                       | 0                       | 0                            | 0                       |
| 26            | I-494                     | 0                        | 0                              | ++                      | 0                                     | 0                       | 0                             | 0                                 | 0                       | 0                       | 0                            | +                       |
| 27            | I-497                     | 0                        | 0                              | ++                      | +                                     | +                       | ++                            | ±                                 | 0                       | 0                       | 0                            | 0                       |
| 28            | I-499                     | 0                        | 0                              | +++                     | D                                     | 0                       | 0                             | ±                                 | 0                       | 0                       | 0                            | +                       |
| 29            | I-511                     | +++                      | 0                              | 0                       | 0                                     | 0                       | 0                             | +                                 | 0                       | 0                       | 0                            | 0                       |
| 30            | I-519                     | 0                        | 0                              | 0                       | 0                                     | 0                       | 0                             | 0                                 | 0                       | 0                       | 0                            | 0                       |
| 31            | I-524                     | 0                        | 0                              | 0                       | 0                                     | 0                       | 0                             | 0                                 | 0                       | 0                       | 0                            | +                       |
| 32            | I-525                     | D                        | 0                              | 0                       | 0                                     | 0                       | 0                             | ++                                | 0                       | 0                       | 0                            | 0                       |
| 33            | I-531                     | D                        | 0                              | +++                     | ++                                    | 0                       | D                             | +++                               | ++                      | 0                       | 0                            | 0                       |
| 34            | I-534                     | D                        | 0                              | 0                       | 0                                     | 0                       | 0                             | ++                                | ±                       | 0                       | 0                            | +                       |
| 35            | I-537                     | 0                        | 0                              | 0                       | 0                                     | 0                       | 0                             | 0                                 | 0                       | 0                       | 0                            | 0                       |
| 36            | I-538                     | 0                        | 0                              | 0                       | 0                                     | 0                       | 0                             | 0                                 | 0                       | 0                       | 0                            | 0                       |
| 37            | I-539                     | 0                        | 0                              | ++                      | 0                                     | 0                       | 0                             | 0                                 | 0                       | 0                       | 0                            | 0                       |
| 38            | I-540                     | +++                      | +                              | ++                      | ++                                    | 0                       | 0                             | ±                                 | 0                       | 0                       | 0                            | 0                       |

(To be continued)

Table 1 (continued)

| Serial number | Culture collection number | Activity against         |                                |                         |                                       |                         |                               |                                   |                         |                         |                              |                         |
|---------------|---------------------------|--------------------------|--------------------------------|-------------------------|---------------------------------------|-------------------------|-------------------------------|-----------------------------------|-------------------------|-------------------------|------------------------------|-------------------------|
|               |                           | Protozoa after 24 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>Staphylococcus aureus</i>      | <i>Escherichia coli</i> | <i>Candida albicans</i> | <i>Aspergillus fumigatus</i> |                         |
| 39            | I-544                     | +                        | 0                              | ++                      | 0                                     | 0                       | ++                            | +++                               | 0                       | 0                       | 0                            | 0                       |
| 40            | I-548                     | 0                        | 0                              | 0                       | 0                                     | 0                       | 0                             | ±                                 | 0                       | 0                       | 0                            | 0                       |
| 41            | I-549                     | 0                        | 0                              | 0                       | 0                                     | 0                       | 0                             | ±                                 | 0                       | 0                       | 0                            | 0                       |
| 42            | I-555                     | 0                        | 0                              | 0                       | 0                                     | 0                       | 0                             | ++                                | 0                       | 0                       | 0                            | 0                       |
| 43            | I-561                     | 0                        | 0                              | 0                       | 0                                     | 0                       | 0                             | ++                                | 0                       | 0                       | 0                            | 0                       |
| 44            | I-562                     | 0                        | 0                              | 0                       | 0                                     | 0                       | 0                             | ±                                 | 0                       | 0                       | 0                            | 0                       |
| 45            | I-564                     | 0                        | 0                              | D                       | 0                                     | +                       | 0                             | ++                                | 0                       | 0                       | 0                            | 0                       |
| 46            | I-569                     | 0                        | 0                              | 0                       | 0                                     | 0                       | 0                             | ++                                | 0                       | 0                       | 0                            | 0                       |
| 47            | I-572                     | 0                        | 0                              | 0                       | 0                                     | 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  
 (+++) only single organisms living  
 D all organisms dead  
 L pronounced lysis

## Bacteria, fungi :

0 No activity  
 ± diffuse zone, not more than 2 mm from edge of disc  
 + zone up to 15 mm diameter  
 ++ 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

organism, and 53 % were active against bacteria and/or fungi.

### Discussion

Although a relatively high incidence of antiprotozoal and antivermal substances was found in the *Penicillia* tested, it is evident that Fungi imperfecti and Oomycetes are richer sources of antibiotics of this type<sup>1,2</sup>. Not only the incidence but under our conditions of testing also the degree of activity of the substances produced by *Penicillia* seems to be lower. Nevertheless we are of the opinion that even under these circumstances it would be advisable to include protozoa as test organisms into the screening program for new antibiotics in *Penicillia* as substances with a specific antiprotozoal or antivermal activity cannot be detected by the usual agar-plate screening methods. Our results clearly indicate that substances of this type are produced by this genus of fungi and compounds of eventual interest certainly passed unnoticed through routine screening programmes using only bacteria and fungi as test organisms.

### References

- 1) NEMEC, P.; L. KRÍŽKOVÁ, J. BALAN, J. BALANOVÁ & M. KUTKOVÁ : Incidence of antiprotozoal and antivermal antibiotics in fungi. I. Class Fungi imperfecti. J. Antibiotics 22 : 345~350, 1969
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