

## RESPONSES OF SOME PLANT PARASITIC FUNGI TO CERTAIN AMINO ACIDS AND ANTIBIOTICS

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Growth responses of 15 plant parasitic fungi to 14 amino acids and amides were studied *in vitro*. One group of four fungi preferred glutamic acid while another group of four favoured phenylalanine. Sensitivities of these 8 fungi to 6 antibiotics were also tested using mycelia as well as spores. Almost all the test fungi were highly sensitive to aureofungin. Oxytetracycline was more or less equally growth inhibitory to test fungi except *Alternaria alternata* and *Curvularia* sp. Different concentrations of the said two antibiotics ranging from 10 µg/ml to 150 µg/ml were also tested on mycelial growth of fungi. Oxytetracycline (500 µg/ml) was effective in reducing the percentage production of lesions on leaf discs caused by fungi which preferentially utilized glutamic acid.

### INTRODUCTION

The mechanism of action of certain antifungal antibiotics and their selective toxicities towards microorganisms are not unknown (Zaumeyer, 1957; Misato *et al.*, 1959), but little work has been reported so far on amino acid utilization in relation to antibiotic sensitivity of pathogenic fungi. Since several antibiotics are used for plant disease control it is worthwhile to investigate whether pathogenic fungi preferring any particular amino acid are equally sensitive to the specific antibiotic. There is evidence that amino acids in some cases are associated with the pathogenicity of a species (Buxton, 1956; Dutta *et al.*, 1959; Purkayastha and Ray, 1974; Tuveson and Garber, 1959) and amino acid deficient mutants differ significantly in their responses to antibiotics (Purkayastha and Chattopadhyay, 1975; Purkayastha and Das, 1972). It suggests that a correlation might exist between specific amino acid utilization and antibiotic sensitivity of an organism. The purpose of this study is to investigate this possibility.

### MATERIALS AND METHODS

The fungal cultures were obtained from the IARI, New Delhi; JARI, Barrackpore, West Bengal and also from the Departmental stock. The seeds were collected from Suttons & Sons (India) Pvt. Ltd., Calcutta and the State Rice Research Station, Chinsurah, West Bengal.

For growth experiments, sterilized basal medium (1% glucose, 0.2% asparagine, 0.1%  $\text{KH}_2\text{PO}_4$ , 0.05%  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , Mn, Fe, and Zn, 2 p.p.m each) was taken in

Erlenmeyer flasks (30 ml/250 ml flask), each of which was inoculated with an agar block (3 mm diam) containing 4-days-old mycelia of the test fungus and incubated for 10 days at  $25 \pm 1^\circ\text{C}$ . Finally mycelia were collected, dried at  $60^\circ\text{C}$  for 96 hr. and weighed.

Spore germination test was carried out following the method described by Purkayastha and Mukhopadhyay (1974).

In order to study the protectant activity of antibiotics the method of Fawcett and Spencer (1970) was followed with modifications. The leaves of the desired host plants (2 months old) were washed, dried and detached. The leaf discs (1 cm diam) were cut with the help of a cork borer and floated in Petri dishes containing 20 ml of antibiotic solution and incubated for 6 hr. prior to inoculation. Similarly, a control set was maintained in sterile distilled water. Each leaf disc floating either on antibiotic solution or on sterile distilled water was inoculated with a drop (0.01 ml) of spore suspension ( $4 \times 10^5$  spores/ml) of the test fungus and incubated at  $25 \pm 1^\circ\text{C}$  under diffused light. Uninoculated leaf discs were also kept as a control in order to study the phytotoxic effect of the antibiotic, if any. The number of lesions developed on leaf discs were counted after 48 hr. following inoculation and the percentage lesion production was calculated on the basis of total number of the inoculum drops used.

#### RESULTS

##### *Growth responses of some pathogenic fungi to different amino acids and amides*

Basal medium was supplemented separately with fourteen amino acids and amides and 0.2% asparagine was used as standard. An equivalent amount of nitrogen was provided by the desired amino acid. Inoculation and incubation techniques were as described. The results in Table-1 reveal that glutamic acid was best utilized by *Alternaria tenuis*, *Helminthosporium oryzae*, *Fusarium oxysporum* and *Corynespora cassicola* while phenylalanine was favoured by *Curvularia* sp., *Pestalotia* sp., *Macrophomina phaseolina* and *Alternaria alternata*. The remaining fungi, however, showed preference for various other amino acids. Since some of the test fungi exhibited preferences for specific amino acids it was decided to test whether this group of fungi is equally sensitive to antifungal antibiotics which could be used for plant disease control.

##### *Responses of fungi (which prefer either glutamic acid or phenylalanine) to some antibiotics*

(a) *Mycelial growth.* The tests were carried out on both mycelial growth and spore germination of fungi. Several stock solutions of antibiotics (viz. streptomycin, tetracycline, oxytetracycline, aureofungin, griseofulvin and streptocycline) were prepared in sterile distilled water and added separately to the sterilized

Table 1. Growth of parasitic fungi in presence of different amino acids and amides

| Sl. No. | Fungi                                 | Dry weight of mycelia (mg) |                 |                |                 |                |                |                |
|---------|---------------------------------------|----------------------------|-----------------|----------------|-----------------|----------------|----------------|----------------|
|         |                                       | Aspartic Acid              | Arginine        | Asparagine     | Glutamic acid   | Glycine        | Glutamine      | Histidine      |
| 1.      | * <i>Alternaria tenuis</i>            | 72.6<br>±1.45              | 70.6<br>±0.65   | 62.6<br>±0.65  | *172.0<br>±1.00 | 68.6<br>±2.39  | 83.3<br>±2.39  | 126.3<br>±2.67 |
| 2.      | * <i>Helminthosporium oryzae</i>      | 57.0<br>±2.51              | 40.2<br>±1.76   | 81.0<br>±1.15  | *190.6<br>±3.12 | 122.3<br>±1.79 | 91.0<br>±1.15  | 50.6<br>±1.45  |
| 3.      | * <i>Fusarium oxysporum</i>           | 109.6<br>±0.87             | 97.3<br>±1.45   | 274.5<br>±3.47 | *373.0<br>±4.04 | 180.0<br>±2.08 | 116.5<br>±1.45 | 103.3<br>±1.66 |
| 4.      | * <i>Corynespora cassicola</i>        | 55.3<br>±4.04              | 83.0<br>±3.27   | 120.0<br>±3.00 | *221.6<br>±4.41 | 136.0<br>±3.00 | 102.6<br>±1.45 | 69.3<br>±2.18  |
| 5.      | ** <i>Curvularia</i> sp.              | 63.0<br>±2.00              | 63.3<br>±0.87   | 63.6<br>±2.06  | 78.0<br>±1.52   | 59.0<br>±2.08  | 68.0<br>±2.52  | 39.0<br>±1.52  |
| 6.      | ** <i>Pestalotia</i> sp.              | 78.0<br>±3.61              | 130.0<br>±2.08  | 121.6<br>±1.45 | 78.6<br>±1.97   | 111.3<br>±1.21 | 11.5<br>±1.66  | 65.0<br>±14.58 |
| 7.      | ** <i>Macrophomina phaseoli</i>       | 57.0<br>±2.64              | 71.3<br>±4.07   | 83.0<br>±3.79  | 54.3<br>±2.81   | 137.0<br>±2.52 | 71.3<br>±2.19  | 37.0<br>±2.08  |
| 8.      | ** <i>Alternaria alternata</i>        | 137.0<br>±2.64             | 135.3<br>±1.97  | 124.0<br>±1.71 | 151.6<br>±4.72  | 134.6<br>±2.31 | 65.0<br>±2.08  | 49.7<br>±0.66  |
| 9.      | <i>Botrytis fabae</i>                 | 180.6<br>±2.57             | 74.6<br>±2.18   | 128.0<br>±1.52 | 161.3<br>±3.71  | 52.0<br>±1.13  | 253.3<br>±2.18 | 101.0<br>±100  |
| 10.     | <i>Botrytis alli</i>                  | 70.3<br>±0.32              | 114.0<br>±3.79  | 132.6<br>±1.45 | 127.6<br>±1.45  | 61.3<br>±2.32  | 118.0<br>±2.52 | 117.3<br>±1.45 |
| 11.     | <i>Fusarium solani</i>                | 107.6<br>±1.45             | 98.3<br>±1.08   | 10.6<br>±0.66  | 129.3<br>±3.47  | 110.3<br>±3.12 | 112.3<br>±1.08 | 90.6<br>±0.33  |
| 12.     | <i>Fusarium udum</i>                  | 110.0<br>±3.46             | 142.31<br>±2.18 | 13.0<br>±2.08  | 121.6<br>±1.97  | 91.6<br>±3.71  | 105.6<br>±1.19 | 127.3<br>±3.21 |
| 13.     | <i>Pythium debaryanum</i>             | 3.6<br>±3.27               | 97.6<br>±1.51   | 63.3<br>±2.18  | 93.0<br>±1.15   | 83.0<br>±1.51  | 47.6<br>±1.79  | 33.3<br>±1.19  |
| 14.     | <i>Colletotrichum corchorum</i>       | 100.3<br>±3.12             | 58.0<br>±1.52   | 102.0<br>±2.64 | 105.6<br>±2.18  | 98.0<br>±4.04  | 107.0<br>±2.08 | 62.0<br>±2.64  |
| 15.     | <i>Colletotrichum gloeosporioides</i> | 105.0<br>±81.52            | 85.3<br>±3.48   | 75.0<br>±2.52  | 80.0<br>±2.08   | 85.0<br>±3.21  | 84.3<br>±2.67  | 82.6<br>±3.84  |

basal medium to give a 100 µg/ml concentration. Inoculation and incubation conditions were as in the previous experiment. Percentage reduction in mycelial growth in relation to control was calculated and summarised in Table 2.

Oxytetracycline was more or less equally growth inhibitory to test fungi except *Alternaria alternata* and *Curvularia* sp. while all the test fungi were highly sensitive to aureofungin.

(b) *Spore germination and germ tube growth.* Antibiotics (2 µg/ml) were also tested on spore germination and germ tube growth of test fungi. The results given in Table 3 substantiate the previous findings.

Table—1 ( Contd. )

| Sl. No. | Dry weight of mycelia (mg) |                |                  |                |                |                |                 | Control ( Distilled water) |
|---------|----------------------------|----------------|------------------|----------------|----------------|----------------|-----------------|----------------------------|
|         | Methionine                 | Leucine        | Phenylalanine    | Serine         | Tryptophan     | Threonine      | Valine          |                            |
| 1.      | 42.0<br>±1.52              | 56.0<br>±3.51  | 91.0<br>±4.73    | 67.3<br>±2.18  | 54.6<br>±2.34  | 94.0<br>±1.52  | 114.6<br>±3.28  | 11.6<br>±0.32              |
| 2.      | 126.3<br>±2.67             | 35.6<br>±0.87  | 46.0<br>±2.08    | 80.3<br>±0.32  | 71.0<br>±1.52  | 26.0<br>±1.52  | 51.0<br>±1.00   | 14.0<br>±2.08              |
| 3.      | 76.3<br>±2.39              | 148.0<br>±1.52 | 55.5<br>±3.38    | 137.3<br>±2.33 | 144.6<br>±0.87 | 119.5<br>±0.87 | 136.5<br>±2.39  | 16.0<br>±1.52              |
| 4.      | 210.6<br>±2.15             | 53.0<br>±4.58  | 170.6<br>±1.15   | 71.0<br>±1.52  | 92.6<br>±3.84  | 67.3<br>±2.91  | 140.0<br>±4.41  | 2.0<br>±0.00               |
| 5.      | 44.6<br>±1.33              | 47.6<br>±2.67  | 91.6**<br>±0.87  | 61.0<br>±2.08  | 33.0<br>±1.52  | 31.3<br>±2.91  | 74.0<br>±1.52   | 1.0<br>±1.52               |
| 6.      | 75.3<br>±2.41              | 77.0<br>±3.21  | 188.3**<br>±1.76 | 124.0<br>±3.05 | 123.0<br>±2.64 | 150.0<br>±1.00 | 160.6<br>±1.45  | 12.3<br>±1.45              |
| 7.      | 33.0<br>±1.52              | 49.0<br>±3.05  | 141.3**<br>±2.32 | 86.0<br>±2.00  | 36.6<br>±3.27  | 53.6<br>±2.54  | 32.6<br>±1.45   | 13.3<br>±0.66              |
| 8.      | 68.0<br>±5.77              | 101.6<br>±3.38 | 240.0**<br>±5.56 | 149.6<br>±1.45 | 83.3<br>±1.66  | 72.0<br>±1.52  | 111.33<br>±3.78 | 5.3<br>±0.66               |
| 9.      | 176.0<br>±2.31             | 110.0<br>±2.64 | 64.6<br>±2.51    | 161.0<br>±1.52 | 59.6<br>±1.81  | 135.6<br>±1.81 | 51.0<br>±1.52   | 14.6<br>±1.45              |
| 10.     | 68.0<br>±1.52              | 125.6<br>±2.91 | 86.6<br>±1.75    | 69.3<br>±2.18  | 128.0<br>±1.73 | 107.6<br>±1.19 | 87.0<br>±1.51   | 10.6<br>±0.66              |
| 11.     | 73.0<br>±1.52              | 151.0<br>±1.51 | 101.0<br>±2.08   | 142.3<br>±2.18 | 105.6<br>±1.79 | 128.0<br>±1.13 | 125.0<br>±3.79  | 12.3<br>±1.46              |
| 12.     | 67.6<br>±3.87              | 112.0<br>±1.51 | 60.0<br>±2.52    | 115.3<br>±3.71 | 113.0<br>±1.51 | 110.0<br>±1.51 | 96.6<br>±2.41   | 14.0<br>±1.13              |
| 13.     | 10.6<br>±1.19              | 17.0<br>±1.00  | 5.0<br>±0.0      | 95.6<br>±5.29  | 8.6<br>±0.66   | 5.3<br>±0.33   | 5.3<br>±0.87    | 4.0<br>±0.0                |
| 14.     | 52.0<br>±1.52              | 27.0<br>±2.52  | 82.6<br>±3.28    | 112.6<br>±2.67 | 44.3<br>±3.47  | 132.6<br>1.79  | 73.3<br>±3.84   | 13.3<br>±1.37              |
| 15.     | 75.6<br>±3.33              | 74.0<br>±2.64  | 103.0<br>±1.52   | 69.0<br>±3.00  | 102.0<br>±3.51 | 59.0<br>±2.64  | 100.0<br>±4.04  | 1.6<br>±0.32               |

\* Maximum growth in presence of glutamic acid.

\*\* Maximum growth in presence of phenylalanine.

3 replicates/treatment  $\pm$  = Standard error.*Effect of different concentrations of oxytetracycline and aureofungin on growth of fungi*

The responses of fungi to different concentrations of aureofungin and oxytetracycline were tested under identical conditions. Different concentrations (10, 25, 125, 50, 125 and 150  $\mu\text{g/ml}$ ) of the aforesaid antibiotics were used on both groups of fungi. The results in Table 4 show that the percentage reduction in mycelial growth of fungi (which preferred glutamic acid) increased proportionately with a rise in concentration of oxytetracycline but no such consistency was observed in case of fungi which preferred phenylalanine. The percentage reduction in growth of the phenylalanine group increased in a similar pattern with increased concentration of aureofungin although mycelial growth of all the test fungi irrespective of their

amino acid utilization capacities decreased at higher concentrations of both aureofungin and oxytetracycline.

*Protectant activity of oxytetracycline*

Since the glutamic acid group of fungi equally sensitive to oxytetracycline it was thought worthwhile to test the pathogenicities of these fungi on their respective host species in the presence of oxytetracycline. This was done to determine whether diseases caused by this group of pathogens could be controlled by the said antibiotic. The protectant activity of oxytetracycline has been tested following the method described earlier. For this experiment two hosts (viz. *Solanum melongena* L. and

Table 2. *Effect on some antibiotics on the mycelial growth of fungi*

| Fungi                          | % reduction in mycelial growth in relation to control* |                   |                      |                   |                  |
|--------------------------------|--------------------------------------------------------|-------------------|----------------------|-------------------|------------------|
|                                | Strepto-<br>mycin**                                    | Tetra-<br>cycline | Oxytetra-<br>cycline | Griseo-<br>fulvil | Aureo-<br>fungin |
| <i>Alternaria tenuis</i>       | 40.5                                                   | 1.4               | 26.3                 | 3.4               | 88.3             |
| <i>Helminthosporium oryzae</i> | 10.8                                                   | 55.8              | 27.5                 | 97.5              | 95.0             |
| <i>Fusarium oxysporum</i>      | 10.5                                                   | 40.1              | 24.1                 | 16.1              | 30.8             |
| <i>Corynespora cassicola</i>   | 35.7                                                   | 64.2              | 28.6                 | 9.4               | 0.0              |
| <i>Curvularia</i> sp.          | 6.4                                                    | 8.9               | 0.0                  | 82.4              | 33.3             |
| <i>Pestalotia</i> sp.          | 30.5                                                   | 7.6               | 17.8                 | 6.3               | 0.0              |
| <i>Macrophomina phaseoli</i>   | 2.8                                                    | 64.1              | 31.6                 | 89.4              | 38.1             |
| <i>Alternaria alternata</i>    | 10.2                                                   | 43.5              | 0.0                  | 16.6              | 20.1             |

\*Basal medium was used as control.  
Temperature 25 ± 1°C

Initial pH was adjusted to 5.4 in each case.  
\*\*Concentration of each antibiotic is 100 µg/ml

Table 3. *Effect of different antibiotics on the germination and germ tube growth of fungi*

| Fungi                          | % reduction in germ tube growth** |                   |                      |                   |                  |
|--------------------------------|-----------------------------------|-------------------|----------------------|-------------------|------------------|
|                                | Strepto-<br>mycine***             | Tetra-<br>cycline | Oxytetra-<br>cycline | Griseo-<br>fulvin | Aureo-<br>fungin |
| <i>Alternaria alternata</i>    | 24.3<br>(100)                     | 0<br>(100)        | 53.4<br>(100)        | 0<br>(100)        | 48.7<br>(100)    |
| <i>Helminthosporium oryzae</i> | 63.6<br>(87.9)                    | 33.3<br>(78.2)    | 50.5<br>(76.5)       | 80.6<br>(86.4)    | 17.4<br>(81.0)   |
| <i>Fusarium oxysporum</i>      | 43.9<br>(100)                     | 74.5<br>(100)     | 69.6<br>(100)        | 54.9<br>(100)     | 54.2<br>(100)    |
| <i>Corynespora cassicola</i>   | 25.7<br>(95.0)                    | 77.8<br>(96)      | 58.5<br>(85.0)       | 0<br>(100)        | 0<br>(100)       |
| <i>Pestalotia</i> sp.          | 48.8<br>(100)                     | 0<br>(100)        | 7.9<br>(100)         | 0<br>(96.0)       | 0<br>(100)       |
| <i>Alternaria alternata</i>    | 23.6<br>(100)                     | 69.2<br>(100)     | 14.5<br>(100)        | 22.0<br>(100)     | 50.1<br>(100)    |

\*Number given in the parenthesis denotes the percentage of spore germination (400-500 spores counted/treatment).

\*\*In relation to distilled water control.

\*\*\*Concentration of antibiotic is 25 µg/ml in all cases.

*Solanum nigrum* L.) of *Corynespora cassicola*, two hosts (viz. *Rosa* sp. and *Lycopersicum esculentum* L.) of *Alternaria alternata* and one host (viz. *Oryza sativa* L.) of *Helminthosporium oryzae* were selected. The leaf discs floating on antibiotic solution were inoculated and incubated for 48 hr. The results are summarised in Table 5.

It is evident from the data that percentage lesion production decreased in all cases. The diseases caused by *C. cassicola*, *A. alternata* and *H. oryzae* can be partially controlled by oxytetracycline.

Table 4. Effect of different concentration of oxytetracycline and aureofungin on the mycelial growth of fungi

| Fungi                 | Anti-biotic           | % reduction in mycelial growth in relation to control ** |             |             |              |              |
|-----------------------|-----------------------|----------------------------------------------------------|-------------|-------------|--------------|--------------|
|                       |                       | * 10<br>µg/ml                                            | 25<br>µg/ml | 50<br>µg/ml | 125<br>µg/ml | 150<br>µg/ml |
| <i>A. tenuis</i>      | *Oxytetra-<br>cycline | 10.3                                                     | 10.9        | 19.7        | 44.8         | 64.4         |
| <i>H. oryzae</i>      | "                     | 8.7                                                      | 14.7        | 22.5        | 54.3         | 60.1         |
| <i>F. oxysporum</i>   | "                     | 11.2                                                     | 12.5        | 17.7        | 50.0         | 63.1         |
| <i>C. cassicola</i>   | "                     | 7.1                                                      | 16.6        | 21.4        | 55.9         | 69.1         |
| <i>Curvularia</i> sp. | "                     | 0                                                        | 0           | 0           | 1.4          | 10.0         |
| <i>Pestalotia</i> sp. | "                     | 0                                                        | 0           | 4.0         | 22.4         | 29.6         |
| <i>M. phaseolina</i>  | "                     | 15.3                                                     | 18.0        | 29.3        | 51.3         | 56.0         |
| <i>A. alternata</i>   | "                     | 0                                                        | 0           | 0           | 4.3          | 6.1          |
| <i>A. tenuis</i>      | *Aureofungin          | 37.1                                                     | 56.2        | 83.1        | 94.5         | 96.7         |
| <i>H. oryzae</i>      | "                     | 79.9                                                     | 85.0        | 85.5        | 92.7         | 92.7         |
| <i>F. oxysporum</i>   | "                     | 17.8                                                     | 20.6        | 21.8        | 66.2         | 86.6         |
| <i>C. cassicola</i>   | "                     | 69.9                                                     | 71.4        | 82.1        | 89.9         | 100          |
| <i>Curvularia</i> sp. | "                     | 69.9                                                     | 81.2        | 84.9        | 100          | 100          |
| <i>Pestalotia</i> sp. | "                     | 72.0                                                     | 82.4        | 96.0        | 100          | 100          |
| <i>M. phaseolina</i>  | "                     | 70.0                                                     | 77.3        | 82.6        | 100          | 100          |
| <i>A. alternata</i>   | "                     | 70.1                                                     | 78.1        | 84.2        | 93.8         | 100          |

\*\* Basal medium

Table 5. Effect of oxytetracycline on the production of lesions on leaf discs by fungi

| Host                              | Pathogen                       | % lesion production * (48 hr.) |                                |
|-----------------------------------|--------------------------------|--------------------------------|--------------------------------|
|                                   |                                | Control                        | Oxytetracycline<br>(500 µg/ml) |
| <i>Solanum melongena</i> L.       | <i>Corynespora cassicola</i>   | 93.75                          | 62.50                          |
| <i>Solanum nigrum</i> L.          | <i>Corynespora cassicola</i>   | 100.00                         | 60.00                          |
| <i>Rosa</i> sp.                   | <i>Alternaria alternata</i>    | 50.00                          | 12.50                          |
| <i>Lycopersicum esculentum</i> L. | <i>Alternaria alternata</i>    | 68.75                          | 31.25                          |
| <i>Oryza sativa</i> L.            | <i>Helminthosporium oryzae</i> | 100.00                         | 72.50                          |

\* 80 inoculum drops/treatment.

## DISCUSSION

The results of the present investigation reveal that there are two major groups of fungi among the test organisms, one group prefers glutamic acid, while the other one favours phenylalanine. But these two groups of fungi did not show any difference in their specificity towards antibiotics. For instance, aureofungin was highly inhibitory to both groups of fungi. Again, oxytetracycline was more or less equally effective against all test fungi excepting *A. alternata* and *Curvularia* sp. Some workers (Gibson *et al*, 1955) have reported that tetracyclines inhibit several enzyme systems. Franklin (1963) found that a number of tetracyclines strongly inhibited the transfer of amino acids from aminoacyl S-RNA to ribosomes. It is likely that the mechanism of growth inhibitory action of oxytetracycline on four fungi is similar. Inhibitory action has been further confirmed when the leaves of *Solanum melongena* L., *S. nigrum* L., *Lycopersicum esculentum* L., *Rosa* sp. and *Oryza sativa* L. were inoculated with their respective parasites in the presence of oxytetracycline. In all cases the reduction in percentage lesion production was significant. This suggests that the same antibiotic could be used against the test plants parasites.

## ACKNOWLEDGEMENT

The authors are grateful to Prof. A. K. Sharma, Head of the Department of Botany, Calcutta University, for providing necessary facilities.

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