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Studies on the antagonistic behaviour of Indian Aspergilli·I

SHASHI CHAUHAN AND R. K. S. CHAUHAN

School of Studies in Botany, Jiwaji University,
Gwalior-474011, India

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A total of 63 Aspergilli have been tested for their antagonistic efficacy against three bacteria i.e., *Bacilus subtilis*, *Stachylococcus aureus* and *Escherichia coli* and a fungus *Mucor ramannianus*. It was observed that certain species of *Aspergillus* showed various degrees of antagonism to all the test organisms mentioned above.

INTRODUCTION

The *Aspergilli* constitute one of the dominant groups of fungi in tropical climate. They play an important role in microbiological ecosystem. The present investigation was carried out to find out the antagonistic behaviour of Indian *Aspergilli* isolated from varying environments.

All 63 strains of *Aspergilli* were tested for their antagonistic activity against three bacteria, i.e. *Bacillus subtilis* (Gram-positive), *Stachycoccus aureus* (Gram--positive), *Escherichia coli* (Gram-negative) and a sensitive fungus *Mucor ramannianus* Moeller. This fungus has many advantages over other fungi as an assay organism. It is apparently more sensitive to most of the antifungal antibiotics than many other test fungi; inhibition zones are usually more sharply defined; it grows rapidly and requires only overnight incubation; it produces a homogenous growth in assay plates than do most fungi; and spores suspensions for inocula are easily prepared and are stable for long periods (Lindenfelser et al. 1964).

In these studies, the microorganism being tested for antibiotic activity is referred to as the "antagonist" and at which the activity is directed as the "test organism".

MATERIALS AND METHODS

The screening was done by placing both a potential antagonist and test organism on the surface of a agar medium at a distance from each other following the methods given by Chauhan and Saksena (1979), Johnson and Curl (1972), and Pridham et al. (1956). For this 20 ml of sterilized molten Emerson's medium (Waksman et al., 1942) was poured into each petri dish and allowed to solidify. The antagonist was streaked on the agar medium at the petri dish. Four days later the test organism was streaked at right angles to the original streak of the antagonist. Three replicates for each test organism were kept and the petri dishes were then incubated at 30 ±1°C for 72 hrs. for bacteria and 28 ± 1°C for *Mucor ramannianus*. The following criteria were recorded for the antagonism.

– Mutual intermingling of the two organisms without any effect on the growth of the test organism – non, antagonistic (–).

– Inhibition of test organism at contact, the antagonist continues to grow unchanged or at reduced rate through the colony of inhibited organism – mildly antagonistic (+).

– Mutual inhibition at contact, the space left between the two colonies is small but marked clearly – weak antagonistic (+).

– Inhibition of test organism at a distance, antagonistic continues to grow through the resulting clear zone at an unchanged on reduced rate – strong antagonistic (+ +).

– Mutual inhibition at distance – strong antagonistic (+ +).

– Mutual inhibition zone well marked having broad distance between the two organisms – highly antagonistic (+ + +).

RESULTS AND DISCUSSION

In the primary screening 63 strains of *Aspergillus* (53 species) were tested for their antagonistic activities (Table 1). The results indicate that the majority of the

Table 1
Antagonistic activity of Aspergillus towards species
B.subtilis /1/, S. aureus /2/, E. coli /3/, M. ramannianus /4/

Species	1	2	3	4
Aspergillus aculeatus	+	-	-	-
A. allahabadi	-	+	-	-
A. amsteldomi	-	-	-	-
A. awamori	-	-	-	+
A. candidus	+	++	-	-
A. carbonarius	-	-	-	+

A. castaneus	+	+	-	-
A. chevalieri	-	-	-	-
A. clavatus	++	+++	++	+++
A. deflectus	+	+	-	-
A. dimorphicus	+	+	+	-
A. ficuum	-	-	-	-
A. fischeri	+	+	+	-
A. fischeri var. glaber and var. spinosus	-	-	-	+
A. flavipes	++	+++	++	+++
A. flavus	++	+++	-	+++
A. flavus	+	+	-	+
A. flavus var. columnaris	++	++	-	+
A. foetidus	-	-	-	-
A. fumigatus	++	+++	+	+++
A. giganteus	++	+++	++	+++
A. glaucus	+	+	-	-
A. humicola	+	++	-	+++
A. japonicus	-	-	-	+
A. luchuensis	-	+	-	-
A. lutescens	++	+++	-	++
A. melleus	-	+	-	-
A. nannus	-	-	-	-
A. nidulans	++	+++	+++	+++
A. nidulans var. dentatus	-	+	-	+
var. echinulatus	+	+	+	+
A. niger	++	++	++	+++
A. niveus	-	-	-	+
A. niveus	-	-	-	-
A. ochraceus	+	+	+	-
A. oryzae	++	++	-	-
A. oryzae var. effusus	+	+	-	-
A. parasiticus	+++	+++	++	++-
A. penicilloides	+	-	-	-
A. phoenicis	++	++	++	+++
A. proliferans	+	+	-	-
A. pulverulentus	+	+	-	+
A. quercinus	-	-	-	+
A. repens	-	-	-	-
A. restrictus	+	+	-	+
A. ruber	+	-	-	-
A. regulosus	+	+++	-	++
A. sclerotiorum	-	-	-	-
A. stellatus	-	-	-	+
A. striatus	-	-	-	-
A. sulphureus	+	+	-	++
A. sydowi	+	-	-	-
A. tamarii	++	++	++	+++
A. terreus	++	+++	+	+++
A. terreus var. floccosus	+	+	-	+
var. globosus	-	+	-	+
A. terricola	+	-	-	-
A. unguis	-	-	-	-
A. ustus	++	+++	+	+++
A. versicolor	+	+++	-	++
A. violaceo-fuscus	-	-	+	-
A. wentii	+	++	-	+++

species possessed rather broad antibacterial and antifungal spectra although some were predominantly active against bacteria and others against *M. ramannianus*, *A. clavatus*, *A. flavipes*, *A. flavus*, *A. flavus* var. *columnaris*, *A. fumigatus*, *A. giganteus*, *A. humicola*, *A. lutescens*, *A. nidulans*, *A. niger*, *A. parasiticus*, *A. phoenicis*, *A. rugulosus*, *A. tamarii*, *A. terreus*, *A. ustus*, *A. versicolor* and *A. wentii* showed both antibacterial as well as antifungal activity. Among these *A. clavatus*, *A. flavipes*, *A. fumigatus*, *A. giganteus*, *A. nidulans*, *A. niger*, *A. parasiticus*, *A. phoenicis*, *A. tamarii*, *A. terreus*, *A. ustus* and *A. wentii* were highly antagonistic and active against all the three groups of organisms i.e., Gram-positive and Gram-negative bacteria and the fungus. Wilkins and Harris (1945) have studied the production of bacteriostatic substances by various *Aspergilli* with special references to strain variation among them. There are a number of reports concerning the production of antibacteria and antifungal substances by various species of *Aspergillus* (Bush, Goth 1943; Cook, Lacey 1944; Dulaney 1947a, b; McKee, MacPhillamy 1943; Shimoda 1951). Waksman and this co-workers (1942) have given the list to bacterial species inhibited by clavacin, an antibiotic produced by *A. clavatus*. Krasilnikov and Korenyako (1945) reported that *A. niger* was active against both Gram-positive and Gram-negative bacteria. The present studies also find support from the work of Stansly and Ananenko (1949), who isolated a crystalline antibiotic substance having marked in vitro activity against acid-fast bacteria from *A. candidus*.

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