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Antimicrobial spectrum of a fungal pigment

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ABSTRACT

Fusarium solani produces an orange-red, extracellular pigment which is shown to possess antibiotic activity against some Gram positive bacteria and fungi. Growth of the organisms is inhibited with as low a pigment concentration as 0.5 – 1.0 μg ml^{-1}.

INTRODUCTION

Fungal production of secondary metabolites has drawn attention for their importance in synthesis of antibiotics and alkaloids on the one hand and toxins on the other hand (Pape and Rehm, 1986; Mall and Chauhan, 1990). Pigments are one form of these secondary metabolites, which have found use in different ways such as in the dyeing industry (Mounter, 1997) as also for their antimicrobial properties (Miller, 1961); well known examples of antibiotics are penicillin and griseofulvin produced by *Penicillium* spp. (Pape and Rehm, 1986). A report also shows that the culture filtrate of *Fusarium solani* can cause destruction of the nematode responsible for root rot of *Phaseolus vulgaris* (Vadhera *et al.*, 1995).

Antimicrobials may be specific for eukaryotes or prokaryotes, and further, for either Gram positive or gram negative organisms. The present study was carried out firstly, to examine the antimicrobial property of the fungal pigment, and further, its spectrum of activity.

MATERIALS AND METHODS

Organisms and culture conditions:

Host culture: *Fusarium solani* (Nazareth and Mavinkurve, 1986) was grown in mineral salts medium (Fernandes and Nazareth, 1999) incubated for 2d at room temperature of 30°C and 200 rpm.

Bacterial test cultures: Laboratory standard cultures of *Staphylococcus. aureus, Micrococcus roseum, Sarcina lutea, Bacillus, Klebsiella, E. coli, Salmonella, Shigella, Vibrio, Proteus, Serratia, P. aeruginosa, Flavobacter* and *Aerobacter* were used for the study.

Fungal test cultures: Air-borne species were isolated and purified on Sabouraud's agar, and identified on the basis of morphological and cultural characteristics. These, together with laboratory cultures, *Aspergillus terreus* and *Pleurotus ostreatus,* were used to study the antimycotic effect of the pigment. Inhibition of growth of the host culture by the pigment was also examined.

Preparation of pigment stocks: The orange-red, extracellular pigment was extracted from the culture broth with chloroform and concentrated to near dryness, then redissolved in distilled water by addition of a drop of acetone to aid solubilization, and the concentration measured in terms of absorbance at λ max of the pigment at 500 nm. A proportionate amount of chloroform extract was also concentrated to dryness to obtain the corresponding dry weight of the pigment.

Antimicrobial testing: The pigment stock solution was added to 10 ml of nutrient broth or potato dextrose broth to an O.D. of 1.0 at 500 nm, for bacteria or fungi respectively; this contained an equivalent of 2 μg pigment ml^{-1} broth.

Bacterial test cultures *S. aureus, Micrococcus, Sarcina, Bacillus, Klebsiella, E. coli, Salmonella, Shigella, Vibrio, Proteus, Serratia, P. aeruginosa, Flavobacter* and *Aerobacter* were inoculated in NB with and without pigment grown at room temperature, 200 rpm and the growth monitored on 0,1,2,3d, by increase in O.D. at λ650 nm

Fungal cultures isolated, *Pleurotus* and the *Fusarium* host culture were also inoculated into PDB with or without the pigment, and incubated at room temperature, and 200 rpm. Growth was monitored visually from 0-11d. In a subsequent experiment, growth was monitored visually from 0-7d, then estimated on 7th day, in terms of dry weight.

MIC test: Three bacterial and three fungal cultures susceptible to the pigment, were inoculated into NB/PDB respectively, containing 0–4 μg pigment ml^{-1} broth, and incubated and examined for growth as given above.

RESULTS AND DISCUSSION

Pigment: *Fusarium solani* produced an orange-red, quinone type, extracellular pigment within 48h of growth under shaker conditions and RT of 30°C; the pigment absorbs maximally at a wavelength of 500 nm.

Antimicrobicity test: Of the fourteen bacterial genera screened for response to the pigment (Fig. 1), it was observed that the three gram positive cultures: *S. aureus, Micrococcus roseum, Sarcina lutea* were sensitive. However *Bacillus* was resistant, this is attributable to its sporulating nature and consequently increased resistance to adverse conditions. Likewise, growth of the Gram negative cultures were not inhibited by the pigment, except for *Klebsiella*, which is susceptible to some extent at 2 μg ml^{-1} concentration of the pigment. Gram negative bacteria are known to be more resistant than gram positive bacteria to microbicidal drugs. The cell wall of gram positive organisms impedes the passage of only quite large molecules (Rose and Wilkinson, 1970) and would therefore permit the entry of the pigment molecules. On the other hand, the cell envelope in particular, the outer membrane of gram negative bacteria is more elaborate (Nikaido, 1979), and thus is more resistant to the ac-

Table 1. Growth of test cultures: [C] in absence and [T] in presence of the pigment

Sr. No.	Culture	Growth									
		Od		2d		5d		7d		11d	
		C	T	C	T	C	T	C	T	C	T
1.	*Aspergillus*	–	–	+++	++	+++	++	+++	++	+++	++
2.	*Aspergillus*	–	–	+++	+	+++	++	+++	++	+++	++
3.	*Aspergillus*	–	–	++	+	++	+	+++	++	+++	+++
4.	*A. terreus*	–	–	+	+/–	++	+	++	+	++	++
5.	*Penicillium*	–	–	++	–	++	+	++	+	++	+
6.	*Penicillium*	–	–	+++	–	+++	+	+++	++	+++	++
7.	*Penicillium*	–	–	+	–	++	+	++	+	++	+
8.	*Penicillium*	–	–	+	–	+	–	+	–	+	+
9.	*Penicillium*	–	–	+	–	++	+	++	+	++	+
10.	*Pleurotus*	–	–	+/–	–	++	+	++	++	++	++
11.	*F. solani*	–	–	++	+	++	++	++	++	++	++

-, No growth; +/– to +++, increasing intensity of growth as observed visually.

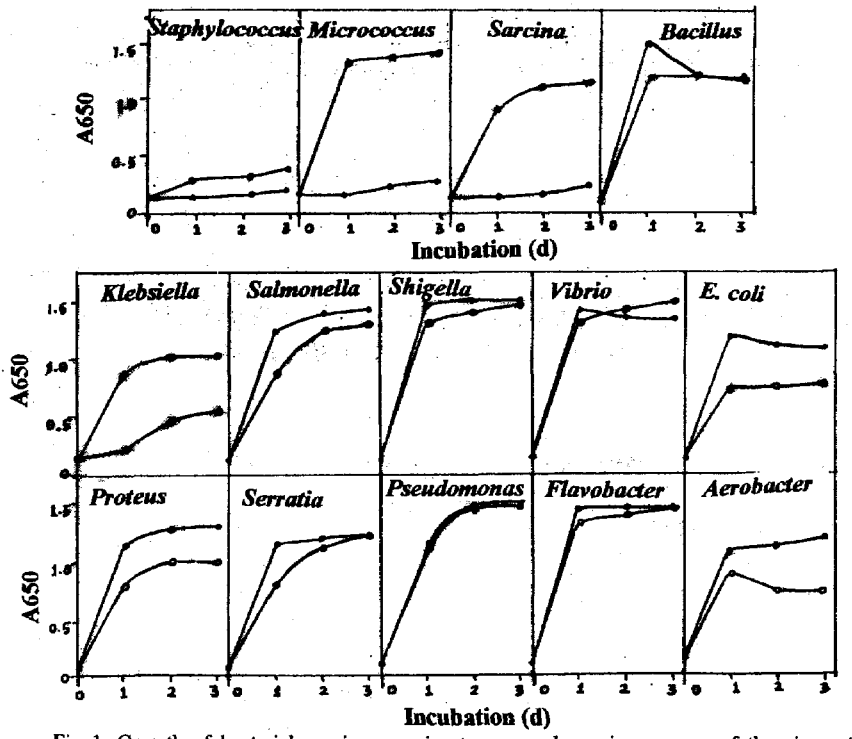

Fig 1. Growth of bacterial species: o-o, in absence and •-•, in presence of the pigment

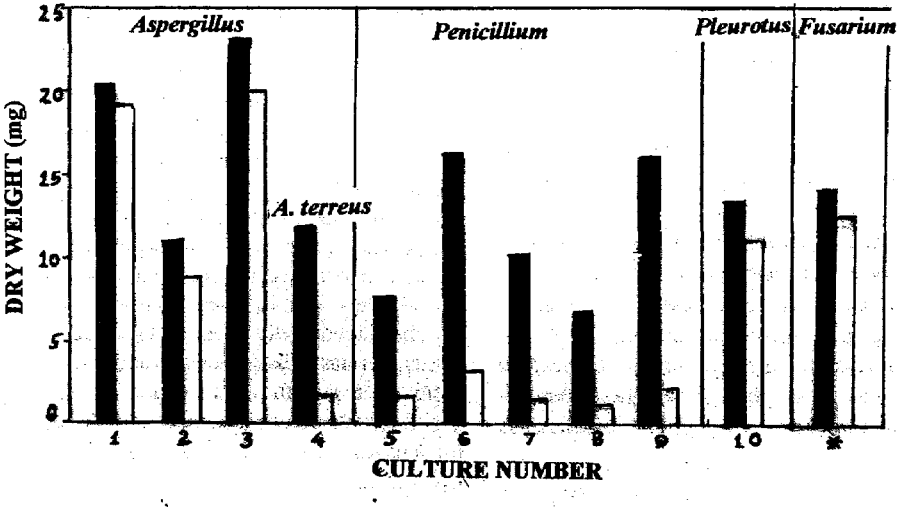

Fig 2. Growth of fungal species: □, in absence, and ■, in presence of the pigment

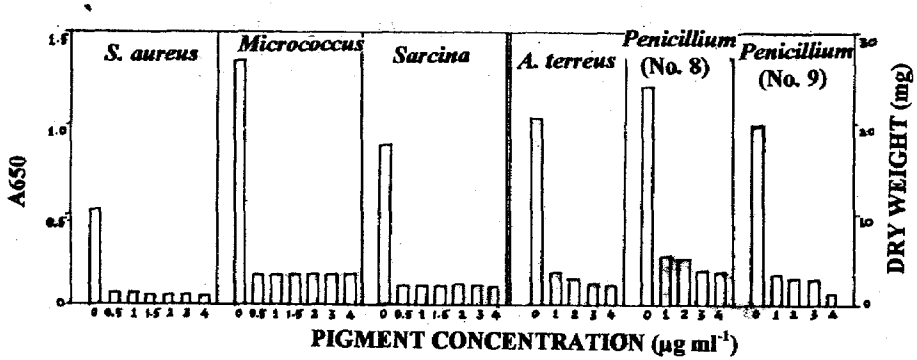

Fig. 3. MIC for bacterial and fungal species

tion of dyes, chemicals, enzymes or antibiotics.

Of the fungal cultures isolated, three were identified as *Aspergillus*, and five as *Penicillium* spp. Amongst the fungi tested (Fig. 2), the three air-borne isolates of *Aspergillus* were resistant, exhibiting marginal decrease in growth in presence of the pigment, while *A. terreus* and all the isolates of *Penicillium* were sensitive. It is seen thus that the affectivity of an antimicrobial may differ with respect to different genera of fungi, and further, may even be species-specific. Growth of *Pleurotus ostreatus* was not appreciably affected by the pigment. It was in fact observed that the culture absorbed the pigment, the mycelial inoculum plug having turned reddish, leaving a clear culture broth.

The pigment was not self-inhibitory to the host *Fusarium solani* culture.

MIC test: The MIC testing of the sensitive gram positive bacterial cultures, *A. terreus* (No 4), and two of the *Penicillium* spp. (No 8, 9), showed that these were inhibited by the lowest pigment concentration tested: 0.5 $\mu g\ ml^{-1}$ and 1.0 $\mu g\ ml^{-1}$ for bacteria and fungi respectively (Fig. 2). This level is comparable with/even lower than concentrations of standard antibiotics used such as kanamycin, streptomycin, tetracycline.

The results presented above hold promise for use of the extracellular pigment as an antimicrobial agent in laboratory experiments for elimination of the undesired, susceptible organisms such as gram positive bacteria, and consequent isolation through selection of organisms with natural resistance to the pigment, namely, the gram negative bacteria; or similarly, the selection of certain genera/species of fungi over others.

Furthermore, with the increasing incidence of antibiotic-resistant organisms and nosocomial infections involving *Staphylococci, Streptococci, E. coli, Serratia marcescens, Proteus, Pseudomonas aeruginosa, Flavobacterium* (McKane and Kandel, 1996) it is of vital importance to investigate the occurrence of newer antimetabolic agents. While the pigment may not gain importance for oral chemotherapy, it could very well be considered as a topical chemotherapeutic agent in localised infections. It has indeed shown promise in inhibition of growth of *S. aureus* as seen above, as well as of a dermatophytic fungus (unpublished data), and study is being carried out in this regard.

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