Indian Journal of Microbiology Vol 42, September 2002, pp. 241-243

Antimycotic Activity of a Fungal Pigment Against Pathogenic Fungi

S. Nazareth* and B. Koshy
Department of Microbiology, Goa University, Taleigao Plateau, Goa 403 206, India

Fusarium solani (Mart.) Sacc produces an extracellular diffusible red pigment. It is shown to be a quinone in nature with antimycotic property and effective against human pathogenic fungi *Candida albicans* and an onychomycotic fungus, in low concentration of 2.5 - 3.0 μg mL^{-1}. The pigment is separable into two components, both of which have antimycotic activity.

Key words: Antimycotic, *Candida, Fusarium,* onychomycotic, pigment.

Fungi have been known for production of antibiotics, such as *Penicillium* spp. for the synthesis of penicillin and griseofulvin and *Cephalosporium* for cephalosporins (1). Fungi are also known for the synthesis of pigments which are important in dying of textiles, leather, paper and other items (2), and also for their antimicrobial properties (3-5). *Fusarium* spp. are reported to produce antimetabolites, as well as mycotoxins (6) and are also beneficial to control nematodes which cause root rot of *Phaseolus vulgaris* (7).

The isolate of *Fusarium solani* under study, produces a pigment. Earlier work has indicated its potential as an antimicrobial, displaying activity against Gram positive bacteria, as well as against fungi of the genus *Penicillium* (8). Through this study, we show its effectiveness against two pathogenic molds, *Candida albicans*, and an onychomycotic culture, that was isolated from a human infected toe nail.

Materials & Methods

Organisms and culture conditions: The culture of *Fusarium solani* (9) was grown in mineral salts medium, incubated for 2 d at 150-200rpm and room temperature (RT) of 30°C, as described earlier (8). *Candida albicans* was obtained from the Department of Microbiology, Goa Medical College, Goa, and cultured in Wickerham's medium (10). The onychomycotic fungus was isolated from an infected toe nail after swabbing the nail exteriorly with spirit, scraping the inner infected part using a sterile scalpel and streaking on potato dextrose agar (PDA, Hi Media). The pure isolate was identified on the basis of cultural and morphological characteristics (11).

*Corresponding author; E-mail: sarita@unigoa.ernet.in
Tel: 91(832)451375, 451345-48; Fax: 91(832)451184

Pigment isolation: The pigment from the 2d grown culture filtrate of *F. solani* was extracted in chloroform and concentrated to dryness aseptically. To the residue, a drop of acetone was added to aid solubilisation, followed by sterile distilled water to make a stock solution. The concentration of the pigment was measured in terms of absorbance at its λmax and the corresponding dry weight. The pigment was also separated by column chromatography on a silica gel column prepared in chloroform and eluted with a gradient of 0 - 10% ether in chloroform.

Chemical analysis of the pigment: The pigment extract was separated by thin layer chromatography (TLC) in a solvent system of chloroform. The individual components obtained by chromatography were scanned between 400 - 700nm. The pigment solution was also treated with 1N NaOH to increase pH upto 10; an absorbance scan was obtained at different pH values, and the change(s) in colour were noted.

Test for antimycotic activity of the pigment: *Candida albicans* in Wickerham's broth, and the onychomycotic isolate in PDB (Hi Media) were grown each with 2μg of the pigment, at RT and 150rpm for 3d. The growth was observed visually.

MIC testing: The test cultures were grown in the respective media containing the pigment and its individual component in concentrations as indicated in Tables 1 and 2. Growth of *Candida* was monitored in terms of absorbance at λ650 nm and of the onychomyotic isolate in terms of d wt after 3d of incubation.

Results & Discussion

The pigment was produced as an extracellular, diffusible red compound, after 2 d of growth in liquid as well as solid

medium and was designated as R. The TLC pattern showed two spots, a less polar, light purple spot, and a more polar, orange spot, designated P and O respectively (Fig 1A). The individual components separated by column chromatography, and the original extract showed absorbance maximum between 497 to 500nm (Fig 1B). The pigment on alkalisation showed change in colour from red to purple, and a shift in absorbance maxima to a higher wavelength (Fig 2). This 'alkali shift' was typical of quinones (10), and confirmed the chemical nature of the pigment.

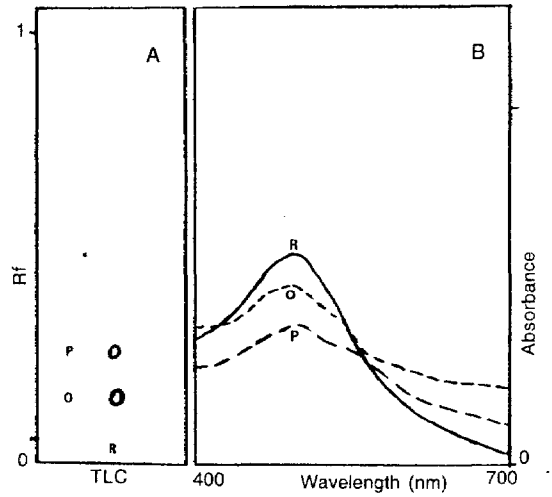

Fig 1. TLC pattern [A] and absorption scan [B] of the pigment (R) and its components (O) and (P).

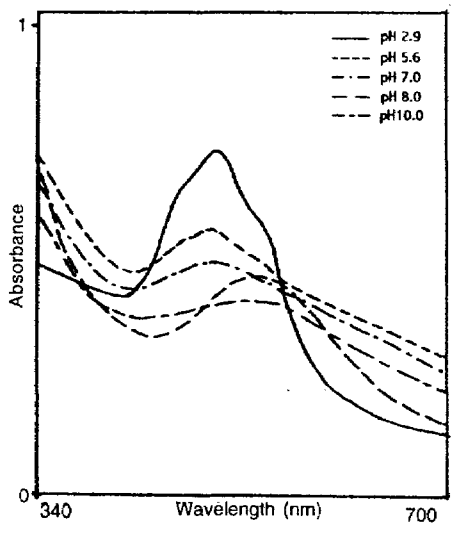

Fig 2. Absorption scan of the pigment at increasing pH values.

The onychomycotic fungal isolate was identified as a *Fusarium* sp., based on its cultural and morphological characteristics, particularly the septate mycelium with sickle shaped microconidia. Antimycotic activity studies showed that 2 μg of the pigment was inhibitory to the growth of the pathogens tested. As seen in Table 1, the growth of *Candida* was adversely affected by the pigment R at as low a concentration of 1 μg mL^{-1}; however at concentrations upto 2 μg mL^{-1}, the culture demonstrated some measure of recovery and slow growth after 24 h. At concentrations of 3 μg mL^{-1} and more, the growth was completely inhibited. A similar pattern was seen in the culture's response to the fraction O at a concentration of 1 μg mL^{-1}, the growth of the culture was initially inhibited, but then recovered after 48h. At 2 μg mL^{-1}, the culture grew a little, but the growth pattern remained stationary. Concentrations of 2.5 - 3.0 μg mL^{-1} permited a very slight to negligible growth and then a decline in the growth pattern. The fraction P was obtainable in small amounts, and was therefore insufficient for the tests. The culture grew in the first 24h at concentrations of 0.3 - 0.6 μg mL^{-1} but then showed a sharp decline. Approximate ten-fold decrease in growth at 48 h was obtained at 3 μg mL^{-1} R, 2 μg mL^{-1} O, and 0.3 μg mL^{-1} P.

The growth of the onychomycotic *Fusarium*-isolate decreased with an increase in concentration of the pigment R and its individual components, O and P (Table 2), being

Table 1. Growth of *Candida* in presence of the pigment R and its isolated components O and P

Pigment (μg mL^{-1})	Growth (A_{650}) after incubation period (h)			
	0	24	48	72
Pigment R				
0	0.205	0.587	1.176	0.729
1	0.203	0.254	0.456	0.505
2	0.228	0.263	0.395	0.487
3	0.208	0.265	0.332	0.305
4	0.218	0.253	0.317	0.298
5	0.219	0.247	0.316	0.256
Pigment O				
0	0.201	0.555	1.105	0.724
1	0.126	0.398	0.409	0.973
2	0.143	0.315	0.290	0.226
2.5	0.125	0.223	0.156	0.150
3	0.145	0.225	0.098	0.037
Pigment P				
0	0.038	0.84	1.31	
0.3	0.059	0.46	0.19	
0.6	0.075	0.59	0.14	

Table 2. Growth of the onychomycotic isolate of *Fusarium* sp. in presence of pigment R and its isolated components O and P

Pigment R	Growth*	Pigment O	Growth*	Pigment P	Growth*
0.0	2.05	0.0	2.15	0.0	2.05
0.5	1.22	1.2	1.02	0.3	1.8
1.0	1.49	1.8	0.40	0.6	1.7
1.5	0.60	2.4	0.21	0.9	1.64
2.0	0.20	3.0	0.10	1.2	1.24
2.5	0.19	3.6	0.10	1.5	0.42
3.0	0.18				

* μg d wt in 10 mL medium; pigment R, O and P in μg mL^{-1}

reduced ten-fold at a concentration of 2 μg mL^{-1} R, 2.4 μg mL^{-1} O and just over 1.5 μg mL^{-1} P.

The above results on the chemical nature and antimycotic activity of the pigment, indicated that it was a quinone which was active against fungal pathogens at low concentration, being effective even in its crude form, (without separation into its constituent components). While each component had antimycotic activity, the component P appears to be a little more toxic.

Both antibacterial and antifungal properties of quinones have been reported (4,5), including the action of 1, 4-napthoquinones on *C. albicans* (5,13,14). The pigment produced by *Fusarium solani* (Mart) Sacc. showed an inhibitory effect on the test cultures, in very low concentrations, which makes it a highly potential candidate for use in chemotherapeutic topical applications to dermatophytic and superficial infections. *Fusarium* spp. have been shown to be resistant to standard MIC of imidazoles (15) used for topical treatment of dermatophyte infections.

The cure of nail infections by dermatophytes is much higher than non dermatophytic onychomycosis. Antimycotics have not proved ideal because of adverse side-effects and narrow range of activity (16). Little attention has been paid to the problem of onychomycosis in India, where its incidence is fairly high (16). This certainly calls for greater efforts to find suitable remedies.

References

1. Pape H & Rehm HJ (1986) Microbial Products II. In: Biotechnology 4 (Rehm HJ & Reed G ed), VCH, Weinheim, Germany, pp 251, 262, 483-484.
2. Mounter J (1997) Dyeing with fungi. Mycologist 11:175-176.
3. Miller MW (1961) Quinone and related compounds. In: The Pfizer Handbook of Microbial Metabolites. McGraw Hill Book Co Inc, New York, pp. 231-272.
4. Brewer D, Maass WSG & Taylor A (1977) The effect on fungal growth of some 2,5-dihydroxy - 1,4-benzoquinones. Can J Microbiol 23: 845-857.
5. Brewer D, Jen WC, Jones CA & Taylor A (1984) The antibacterial activity of some naturally occuring 2,5-dihydroxy-1,4-benzoquinones. Can J Microbiol 30: 1068-1070.
6. Mall OP & Chauhan SK (1990) *Fusarium* mycotoxins: An overview. In: Perspectives in mycological research II (Hasija SK & Bilgrami KS ed). Today and Tomorrows printers and publishers, New Delhi, pp. 247-260.
7. Vadhera J, Shukla BN & Bhatt J (1995) Interaction between reniform nematode *Rotylenchulus reniformis* and *Fusarium solani* causing root rot of french bean *Phaseolus vulgaris*. Indian J Agric Sc 65: 774-777.
8. Nazareth S & D'Sousa W (2001) Antimicrobial spectrum of a fungal pigment. Kavaka 28-29.
9. Nazareth S & Mavinkurve S (1987) Isolation of potential ligninolytic organisms. International Biodeterioration 23:271-280.
10. Thomson RH (1976) Isolation and identification of quinones. In: Chemistry and Biochemistry of Plant Pigments 2. (Goodwin TW ed), Academic Press, London, pp. 207-231.
11. Beach FW & Davenport RR (1971) Isolation, purification and maintenance of yeasts. In: Methods in Microbiology 4 (Norris JR & Ribbons DW eds), Academic Press, London, pp. 158.
12. Gilman JC (1959) A Manual of Soil Fungi. Iowa State Press, Iowa. pp. 354-355.
13. Gershon H & Shanks L (1975) Fungitoxicity of 1,4-napthoquinones to *Candida albicans* and *Trichophyton mentagrophytes*. Can J Microbiol 21:1317-1321.
14. Gupta HP, Singh R, Srivasta OP, Khanna JM, Mathur IS, Gupta SK (1981) Antifungal property of some substituted napthoquinones, anthraquinones and imidazole quinones. Indian J Microbiol 21: 57-59.
15. Weinberg ED (1989) Antifungal Agents. In: Principles of Medicinal Chemistry. (Foye EO ed.), Varghese Publishing House, Bombay, pp. 731-738.
16. Singh SM (1990) Non-dermatophytic onychomycosis: a review. In: Perspectives in Mycological Research II (Hasija SK & Belgrami KS eds). Today and Tomorrows Printers and Publishers, New Delhi, pp. 293-310.

Received 11 December 2001, final revision 11 September 2002 and accepted 17 September 2002.