A POLYENE ANTIBIOTIC PRODUCED BY SOIL *NOCARDIA*

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ISOLATION of actinomycetes from soils was made to study their antagonistic nature on phytopathogenic fungi and the dermatophytes causing skin diseases. Actinomycetes were isolated from soil samples at Jabalpur and tested for antifungal activity by the agar cross streak method. Of these, one culture, *Nocardia salmonicolor* showed wide antifungal activity against the phytopathogenic fungi tested, *viz. Sclerotium rolfsii, Rhizoctonia bataticola* and *Fusarium oxysporum* and against three dermatophytes, *Trichophyton rubrum, Epidermophyton floccosum* and *Microsporum gypseum.*

There are numerous reports regarding isolation of *Nocardia* from human beings. Our literature search has shown that *Nocardia salmonicolor* as an antagonist against phytopathogenic fungi and dermatophytes is not yet reported.

Direct microscopic observations on the morphological features of the present isolate were made on oat meal medium[1,2]. The two biochemical tests, (1) Production of nitrite from nitrate and (2) ammonia production gave positive results, thereby indicating that the present isolate is *Nocardia.* The colour of the isolate in different media varied from yellow to orange indicating it to be *salmonicolor.*

The organism was grown on glucose peptone agar slants at 28° C for 10 days and spores were harvested in 25 ml sterile distilled water. A standardized suspension of 10 spores per ml was used to inoculate 30 ml fermentation medium in the flask. The medium contained glucose (10 g), K_2HPO_4 (0.5 g), $MgSO_4 7H_2O$ (0.025 g) and asparagine (0.5 g) (pH 6.5). Incubation at 28° C for 10 days resulted in optimum production of the antibiotic.

The active principle was extracted from the culture broth with *n*-butanol. A dark brown yellow hygroscopic substance was obtained. This was further mixed with a minimum quantity of methanol and evaporated to dryness.

The stability of the antibiotic thus obtained was studied at 0, 7, 20, 30 and 35° C. The antibiotic activity was stable at 0, 7 and 20° C. The maximum stability was found at 0° C, for a period of 35 days. The best activity was found at 0° to 7° C at pH 6.5.

The antibiotic was more soluble in butanol than in methanol. It has low solubility in water. It gave maximum absorption at 290 nm. It was inactivated around 80° C. Maximum activity was found at 30° C, and the crude extract was active up to a dilution of 1:30. These results indicate that probably the antifungal substance produced by *N. salmonicolor* is *tetraene* type of polyene antibiotic[3,4] (absorption peak at 290 nm).

The tetranes have maximum absorption at 290, 305 or 318 nm.

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REACTION OF HETEROBASIDIOMYCETOUS YEAST GENERA *BULLERA* AND *SPOROBOLOMYCES* TO DIAZONIUM BLUE B

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THE distinction between ascomycetous and heterobasidiomycetous yeasts is often not possible until sporulation has ensued. The formation of ballistospores in these forms is known to be influenced not only by the environmental conditions but also by the age of the culture; this has particularly been evident in *Bullera tsugae*[1]. During a study of the Canadian strains of *Bullera* and *Sporobolomyces* problems of identification were encountered. It was then decided to investigate the utility of diazonium blue B reaction to rapidly assess the heterobasidiomycetous nature of the collected strains. Possible use of this dye for differentiating ascomycetous and hemibasidiomycetous yeasts has earlier been reported[2,3].

The strains of *Bullera* and *Sporobolomyces* were provided by the courtesy of Prof. R. J. Bandoni, University of British Columbia, Canada and were maintained on malt yeast extract agar[4]. For testing the colour reaction, all strains (table 1) were grown in disposable plastic plates using the same medium at 20° C for 7 and 21 days. The diazonium blue B (Sigma, St. Louis, U.S.A.) reagent was prepared immediately prior to use by dissolving the dye (1 mg/ml) in 0.1 M tris-HCl buffer (pH 7) which had been chilled to 4° C. The freshly prepared and chilled dye was applied

TABLE 1

Reaction of Bullera and Sporobolomyces to diazonium blue B

Organism	UBC strain	Colour reaction
Bullera alba	983	+ +
B. alba	8014	+ + + +
B. alba	8041	+ + +
B. alba	8165	+ +
B. aurantiaca[1]	8088	+ +
B. globospora[2]	8079	+
B. globospora[2]	8082	+ +
B. grandispora	8076	+ w.
B. salicina[1]	8081	+ +
B. tsugae	8016	-
Bullera sp.	961	+ +
Bullera sp.	8080	+ +
Bullera sp.	8084	+ + +
Sporobolomyces alborubescens	8066	+ w.
S. coralliformis	8028	+ + +
S. mucilaginosa	873	+ v.w.
S. odorus	947	+
S. odorus	8053	+ + +
S. roseus	901	+
S. ruber	8021	+
S. salmonicolor	8022	+
Sporobolomyces sp.	40	+ w.
Sporobolomyces sp.	461	+
Sporobolomyces sp.	463	+
Cryptococcus laurentii	899	-
C. terreus	8157	-
Leucosporidium pectii	770	-
L. scottii	670	-
Rhodotorula glutinus	940	-

[1] Pigmented *Bullera*. [2] *Bullera* with globose ballistospores. + , relative intensity of dye reaction from red to brown within 1–2 min. +w, weak reaction. +v.w., very weak but positive reaction.

directly to the culture plates; care was taken to see that the colonies had proper contact with the reagent. The appearance of a red to brown-red colouration within 1–2 min at room temperature (20 to 22° C) was recorded as a positive reaction; yellowish colouration was treated as negative. The experiment was repeated on 7th and 21st days to check the influence of the age of culture to the dye reaction.

Except *B. tsugae*, all other strains of *Bullera* and *Sporobolomyces* gave a positive reaction towards diazonium blue B, but the intensity of colour development was different (table 1). Thus, strains of *B. alba* gave a much stronger colour reaction than other species of this genus; *B. grandispora* gave a weak but positive reaction. *Bullera tsugae* gave a negative reaction both after 7 and 21 days; this strain did not produce ballistospores under the normal culture conditions and appeared to have lost this ability[3]. The strains 8081 and 8088 are pigmented Bulleras and new (Johri, unpublished observations), while 8079 and 8082 produce globose ballistospores and are distinctly different from other known species of this genus but all were positive for diazonium blue B reaction.

Sporobolomyces strains were comparatively slow in reacting and the intensity of colour was also not very strong for most species (table 1). One strain of *S. coralliformis* and *S. odorus* did not produce dark red colouration immediately after the addition of the dye; some difficulty in colour reaction with this genus was expected because the cells in most species are pinkish orange but a positive reaction towards the dye was not difficult to discern.

In order to check that heterobasidiomycetous yeasts alone react to this reagent, strains of *Cryptococcus laurenti* 899, *C. terreus* 8157 and *Rhodotorula glutinus* 940 were also tested at 7 and 21 day intervals but all were found to be negative. In contrast to the observations made by van der Walt and Hopsu-Havu[3], the UBC strain of *Leucosporiduim scottii* 670 (heterobasidiomycetous) did not produce a dark red colouration with this dye. While no definite chemical basis for this colour reaction is known, it is believed that diazonium salts react with tryptophan metabolites which accumulate in the cells during growth[5]. Based on our results, we conclude that positive diazonim blue reaction can be used as a presumptive test for differentiating the heterobasidiomycetous yeasts from the ascomycetous ones.

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