Survey of the Sensitivity of Microorganisms to Rubratoxin B

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Of the 133 microorganisms tested, Tetrahymena pyriformis and Volvox aureus were the most sensitive to rubratoxin B, being inhibited at 25 and 50 µg/ml, respectively.

Virtually no information on the antimicrobial activity of rubratoxin B, a toxic secondary metabolite of Penicillium rubrum Stoll, is available. Wilson and Wilson (4) reported inhibition upon Bacillus subtilis, B. cereus, and Staphylococcus aureus by 30 mg of an acidic fraction containing this mycotoxin. Lightly seeded Chlorella pyrenoidosa-buffered agar plates inoculated with P. rubrum NRRL A-11785 produced a zone of inhibition preceding the mycelial growth (3). This paper deals with the antimicrobial activity of rubratoxin B.

Rubratoxin B was produced by P. rubrum NRRL A-11785 by the method of Hayes and Wilson (2). All test strains were obtained from our culture collection or from the Southern Research Institute, Birmingham, Ala. The agar streak method with filter paper disc (Schleicher and Schuell, no. 740-E) containing 100 to 3,000 µg of rubratoxin B per disc was employed for determination of antimicrobial activity against bacteria, fungi, and algae. Control cultures with discs containing equivalent volumes of solvent (acetone or dimethyl sulfoxide) were run simultaneously. When a positive response with a bacterium was observed on agar plates, it then was tested turbidimetrically in appropriate liquid media. Inhibition of Tetrahymena pyriformis and Volvox aureus induced by rubratoxin added at time zero to liquid cultures was determined after 24, 48, and 72 hr by counting cells under low-field magnification.

Rubratoxin B had no effect at 100 to 1,000 µg on the algae, fungi, or gram-negative bacteria investigated. However, there was a decrease in pigment in Pseudomonas fluorescens growth adjacent to the disc containing rubratoxin (1,000 µg). There was no difference in cell density when measured turbidimetrically in concentrations as high as 3,000 µg of toxin per ml, but there was a marked decrease in pigment in rubratoxin-treated (1,000 µg per ml) liquid cultures of P. fluorescens.

Four species of Bacillus including B. subtilis, two species of Micrococcus, and an S. aureus were inhibited by 1,000 µg of rubratoxin B per disc (Table 1). Colonial characteristics of the Bacillus species were markedly changed on nutrient-agar plates (1,000 µg). The colonial growth was thin, spreading, and mycoides-like, whereas solvent-control colonies were thicker and softer in appearance. The inhibitory effect of rubratoxin on M. lysodeikticus growth was noted in broth cultures after 18 to 24 hr. This particular organism requires 48 hr to reach the stationary growth phase. No change in growth of S. aureus was observed for 6 hr when tested against 2,000 µg of toxin per ml in Trypticase Soy broth; however, by the 6th hr, the S. aureus growing in the presence of rubratoxin was very flocculant, and turbidity could not be determined.

Growth of T. pyriformis and V. aureus was inhibited by less than 25 and 50 µg of rubratoxin B per ml, respectively (Table 1).

Some mycotoxins, such as aflatoxin, appear to be toxic to a range of biological systems including animals, plants, and microbial systems, whereas others are more restricted in action. Rubratoxin B appears to be toxic to a variety of

### Table 1. Rubratoxin B-sensitive microorganisms

| Microorganism          | No. of species inhibited | Minimal inhibitory concn (µg/ml) |
|------------------------|-------------------------|----------------------------------|
| Bacillus sp............| 4                       | 1,000                            |
| Micrococcus sp.........| 2                       | 1,000                            |
| Staphylococcus sp......| 1                       | 1,000                            |
| Tetrahymena pyriformis.| 1                       | 25                               |
| Volvox aureus..........| 1                       | 50                               |

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higher animals (1, 4) but limited in its toxicity to microorganisms.

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