## A NEW ANTIFUNGAL ANTIBIOTIC FROM ASPERGILLUS VERSICOLOR

Sir :

Extensive screening of natural sources including fruits, vegetables, ripe and rotten, and soils resulted in the isolation of an antagonist *Bacillus subtilis*, which produces an antifungal antibiotic named mycobacillin<sup>1)</sup>. It is a cyclic polypeptide whose amino acid sequence has been worked out<sup>2)</sup>. However, it did not prove to be clinically useful<sup>3)</sup>. As a result of a fresh screening, a new antagonist later on identified as *Aspergillus versicolor*<sup>4)</sup> has been isolated which produces an antibiotic highly specific against *Trichophyton rubrum* which causes 90 % of skin infections in Eastern India.

Species belonging to Aspergillus versicolor group (as per THOM and RAPER) are known to produce one antifungal antibiotic, viz., humicolin<sup>5,6</sup>) by A. humicola and two antibacterial antibiotics<sup>7</sup>, namely versicolorin and sterigmaupsin from A. versicolor. This communication reports the isolation of a new antifungal antibiotic from the said species.

Fermentation was carried out in simple glucose-peptone medium. The antibiotic was extracted from the fermented broth by butanol or amyl acetate. After removal of the solvent under reduced pressure the crude material was purified by chromatography on acid alumina column followed by ethyl acetate elution. The eluted material was recovered by evaporation and further purified by sublimation at 110~120°C and 0.01 mm of Hg. The antibiotic was obtained as a white sublimate which was further purified by crystallising from chloroform-petroleum ether mixture (1:4) to give white needleshaped crystals, m. p. 125±1°C. The homogeneity of the antibiotic was proved by paper chromatography, bioautography and thin-layer chromatography on silica gel.

The antibiotic is highly soluble in water and the solution is acidic. It is also soluble in alcohols, esters, chloroform, and carbon tetrachloride, but insoluble in benzene and petroleum ether. It is stable in solution at acid pH and is inactivated above pH 7.0. Electrometric titration showed no sharp equivalent point. Elemental analyses show that it possesses the formula  $C_7H_8O_3$  (Mol. wt. 140). And the molecular weight determined by the RAST method is 200. The ultraviolet absorption spectrum in absolute ethanol shows maxima at 288 m $\mu$  ( $E_{1cm}^{1\%}$  280) and 206 m $\mu$  ( $E_{1cm}^{1\%}$  1760). The infrared absorption spectrum in Nujol mull shows strong peaks at 3345 cm<sup>-1</sup>, 1600 cm<sup>-1</sup> and 1250 cm<sup>-1</sup>.

The antibiotic gives positive 2,4-dinitrophenylhydrazine, TOLLENS, and ferric chloride tests. It reduces FEHLING's solution at room temperature. It gives a purple coloration with nickel and copper acetate solution. It decolorises neutral permanganate and bromine in carbon tetrachloride. It gives a permanganate color with NaHCO<sub>3</sub> and ammonia solution. It does not form a picrate. It gives negative tests for steroids.

The antibiotic is active against only a few species of pathogenic fungi, viz., T. rubrum, T. tonsurans, T. mentagrophytes, Epidermophyton floccosum, Microsporum adouini, etc., and has no activity against Candida albicans, Aspergillus niger (G<sub>8</sub>Br), Bacillus subtilis, yeast and other yeast-like fungi and other gram-positive and gramnegative bacteria. The minimum inhibitory concentrations (mcg/ml) as determined by agar streak dilution method are as follows: T. rubrum 2.0, T. tonsurans 2.0, M. adouini 2.5, E. floccosum 4.5 etc. Intravenous injection of 150 mg/kg caused no ill effect to mice.

The physical chemical evidence and the antimicrobial spectrum of the antibiotic show that it is new and altogether different from the known antibiotics from *Aspergillus* species.

## Acknowledgement

Grateful acknowledgement is made to the Commonwealth Mycological Institute for supplying the strains of *A. versicolor* group. Acknowledgement is also due to Council of Scientific and Industrial Research for the grant of scholarship to one of the authors (A.K.D.).

## A. K. Dhar S. K. Bose

Department of Biochemistry, University College of Science, 35, Ballygunge Circular Road, Calcutta-19, India

(Received November 13, 1967)

## References

- MAJUMDAR, S.K. & S.K. BOSE : Mycobacillin, a new antifungal antibiotic produced by B. subtilis. Nature 181 : 134~135, 1958.
- MAJUMDAR, S.K. & S.K. Bose: Amino acid sequence in mycobacillin. Biochem. J. 74:596~599, 1960.
- 3) BANERJEE, N.; S.K. MAJUMDAR & S.K.

BOSE: Some preliminary studies on the evaluation of mycobacillin for therapeutic use. Symp. Fungus. Diseases in India. Bull. Sch. Trop. Med. 7:35, 1959.

- THOM, C. & K. B. RAFER : A manual of the Aspergilli. Baltimore, The William & Wilkins Co., 1945.
- 5) CURTIS, P. J.; H. G. HEMMING & E. G. JEFFEREYS: Humicolin, an antifungal substance produced by Aspergillus humicola. Brit. Mycol. Soc. Trans. 35: 263~267, 1952.
- GILL-CAREY, D.: Antibiotics from Aspergilli. Brit. J. Exp. Path. 30: 114~118, 1949.
- 7) HATSUDA, Y. & S. KUYAMA: Studies on the metabolic products of Aspergillus versicolor. IV. The antibiotic properties of versicolorin and hydroxyxanthones. J. Agr. Chem. Soc. Jap. 29: 14~20, 1955.