

PRODUCTION OF VERNAMYCIN
BY A *MICROMONOSPORA*

Sir:

While not uncommon to find the same or a closely related antibiotic produced by members of two different taxonomic families¹⁾, it is less than common to find an antibiotic produced by three or more such taxonomic groups. A few examples of such biosynthetic diversity reported in the literature are: β -lactam production by bacteria^{2,3)}, actinomycetes (*Streptomyces* and *Nocardia*⁴⁾) and fungi; actinomycin production by streptomycetes, *Actinoplanes*⁵⁾ and *Micromonospora*⁶⁾; and erythromycin production by a bacterium⁷⁾, streptomycetes and *Micromonospora*⁸⁾. In this communication, we present evidence for the addition of *Micromonospora* to the list of diverse microorganisms capable of synthesizing vernamycins. Previously reported to synthesize members of this antibiotic group are streptomycetes⁹⁾, *Actinoplanes*^{10,11)} and *Actinomadura*¹²⁾.

The organism in our study was identified as a *Micromonospora* based on the following criteria: aerial mycelia was absent but a well developed, branched and septate vegetative mycelium was present. Spores were borne singly, either sessile or on sporophores of varying lengths. The cells were Gram-positive and non-acid fast. The vegetative mycelium was orange in color, becoming brownish-black and somewhat viscid in 7 to 10 days, when the organism was grown on a medium composed of beef extract, 3 g; tryptone, 5 g; yeast extract, 5 g; soluble starch, 24 g; dextrose, 1 g; agar, 15 g; CaCO₃, 4 g and tap water, 1 liter.

Fermentations by *Micromonospora* sp. SC 12,650 to produce the antibiotic were done in a medium consisting of 20 g each of tomato paste and baby oatmeal per liter of tap water. The medium was adjusted to pH 7.0 prior to sterilization, inoculated and incubated at 28°C on a rotary shaker at 300 r.p.m. for 5 days. The filtrate (4.5 liters), obtained after separation of the mycelial cake, was extracted with ethyl acetate. The extract was concentrated to dryness under reduced pressure and the residue put onto a silicic acid-cellulose column (2:1, w/w). Elution was done with a linear gradient of methanol in chloroform. The active fractions, detected by assay vs. *S. aureus*, were combined and further purified by column chromatography on Sephadex LH-20 with methanol as the developing solvent. Two

bio-active components were obtained. Final purification was achieved by preparative TLC on silica gel, with a solvent consisting of 8% methanol in chloroform.

A variety of techniques were used to compare the two active fractions with vernamycins A and B α . The major component that we isolated proved to be vernamycin A. The identification was made on the basis of TLC, UV, IR and ¹H NMR spectroscopy and by mass spectroscopy in which the molecular ion and major fragmentation patterns were identical to those of vernamycin A. The minor component from the fermentation was identified as a member of the B group of vernamycin, though its exact relationship to other members of the vernamycin B group has not been ascertained.

Biological support for these identifications was obtained by demonstrating synergy between the major component (identified as vernamycin A) and a known sample of vernamycin B α ; similarly, synergy was observed with the minor component and a sample of vernamycin A. The major and minor components were synergistic with each other, in keeping with the identifications.

To our knowledge, this constitutes the first report of synthesis of vernamycins by *Micromonospora*.

WEN-CHIH LIU
VERA SEINER
LORETTA D. DEAN
WILLIAM H. TREJO
PACIFICO A. PRINCIPE
EDWARD MEYERS
RICHARD B. SYKES

The Squibb Institute for Medical Research
P.O. Box 4000
Princeton, New Jersey 08540, U.S.A.

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