# BREVIMYCINS, A NEW GROUP OF ANTIBIOTIC SUBSTANCES FROM A *BREVIBACTERIUM* SP. ISOLATE

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ANTIBIOTICS have now been isolated from a variety of fungi, streptomycetes, algæ, bacteria and lichens.[1-3] They vary widely in their spectrum of activity and chemical nature. Although nearly 1,100 antibiotic substances are discovered, only 66 have attained the level of commercial application.[4] Certain antibiotics, especially those of bacterial origin, are now gaining importance due to lack of resistance to them by the susceptible organisms.

Of the bacterial species, the organisms belonging to the genera *Bacillus, Clostridium* and *Pseudomonas* are of added interest due to the wide variety of antibiotics produced by them, as well as, the variety of antibiotics produced by the same species. Quite a few organisms belonging to other genera have also been attributed with the capacity for antibiotic production.[1]

While studying soil samples from oil-bearing areas, by using the dilution plate technique, a few isolates showing characteristic antagonism to others in their vicinity were encountered.[5] One of these isolates, which showed promising activity, was taken up for detailed study. This isolate, a gram-positive bacterium, was identified as *Brevibacterium ammoniagenes* species according to the methods detailed in Skerman's *Guide to the Identification of the Genera of Bacteria*,[6] and *Bergey's Manual of Determinative Bacteriology*.[7]

The antibiotic production by this isolate when grown in a liquid medium containing glucose (2%), yeast extract (0·25%) and peptone (1%), at pH 7·2, was maximal at the end of 72 hours when incubated on a rotary shaker (200 rpm) at room temperature (*ca.* 30° C.). This was associated with a drop in the pH upto 4·5. Other liquid media like nutrient broth, cornsteep-liquor-peptone-yeast extract broth supported good growth of the organism, but failed to induce antibiotic production.

The antibiotic activity was not extractable in hexane, benzene and other non-polar solvents; but could be quantitatively extracted in diethyl ether, and chloroform. These solvents were then used to extract the active principle from the culture filtrate.

The crude extract on purification on preparative TLC using Silica Gel G (E. Merck), as adsorbent, in a solvent system containing 10% methanol in chloroform gave two active fractions with $R_f$ values 0·85 and 0·23, respectively. These have been tentatively designated by us as Brevimycin A and Brevimycin B to commemorate the genus responsible for their production.

Both Brevimycin A and Brevimycin B are soluble in dilute alkali but insoluble in dilute HCl (2 N). They are sparingly soluble in acetone and ethanol.

The antibiotic complex being predominant in Brevimycin A, this substance was obtained in quantities to study the antibiotic spectrum.

This fraction, Brevimycin A, was very active against a variety of bacteria as is seen from Table I which gives the minimum inhibitory concentration for the various test organisms.

TABLE I

*Minimum inhibitory concentration (MIC) of antibiotic Brevimycin A*

| Test Organism | MIC μg./ml. |
|---|---|
| *S. aureus* 3750 | 75 |
| *Strep. β hemolyticus* | 15 |
| *D. pneumoniæ* | 15 |
| *S. typhosa* $H_{901}$ | 90 |
| *Sh. dysenteriæ* | 90 |
| *E. coli* 0119 | 90 |
| *Ps. pyocyanea* (*Ps. aeruginosa*) | 95 |
| *V. choleræ* Ogawa | 95 |

On the basis of the information, on hand, of chemical characterisations, Brevimycin A seems to be an aliphatic carboxylic acid of the butyric acid series, showing a certain degree of unsaturation and the presence of a hydroxyl group.

Efforts towards the elucidation of the chemical nature and probable structure of Brevimycin A and Brevimycin B (active principle, having $R_f$ 0·23 in the methanol-chloroform system) as well as details of fermentation process are in progress.

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