23 July 1984

1. Mourya, D. T., Padbidri, V. S. and Dhanda, V., *Indian J. Med. Res.*, 1983, **78**, 201.
2. Dhanda, V., Padbidri, V. S. and Mourya, D. T., *Proc. All India Sym. Vectors and Vector Borne Diseases*, Pratibha Printers, Trivandrum, India, 1982, 69.
3. Padbidri, V. S., Mourya, D. T. and Dhanda, V., *Indian J. Med. Res.*, 1982, **76**, 185.
4. Gimenez, D. F., *Stain Technol.*, 1964, **29**, 134.
5. Mourya, D. T., Dhanda, V. and Padbidri, V. S., *Indian J. Parasitol.*, 1983, **7**, 55.

ANTIBIOTICS PRODUCED BY A STREPTOMYCETE STRAIN

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In the course of screening for antibiotic producing microorganisms an actinomycete strain was isolated from a soil sample. The strain designated as Ac(E)-6 produced antibiotics mainly active against gram-positive bacteria.

The strain was identified by the conventional methods of taxonomical studies of streptomyces. The sporophores are long, monopodially branched, twisting into long closed spirals. The spores are greyish white, produced in chains containing more than 25 spores per chain, ovoid, $0.55–0.62\,\mu \times 0.78–1.02\,\mu$ in size, with smooth surface; at early stage the spore chain forms a hook-like structure (figure 1) as observed in electron microscope (Philips PSEM 500). Colour of aerial mycelium is grey; the reverse side varies from yellow to orange to brown. Experiments according to the International Streptomyces Project (ISP)[1], Waksman[2] and cell wall analysis by the method of Boone and Pine[3] suggest the strain Ac(E)-6 closely related, although not identical, to *Streptomyces parvullus* Waksman and Gregory. Mycelial characters, mode of growth in specialised media, proteolytic activity, negative H_2S production and negative melanin formation resemble similarities with *S. parvullus*. The strain Ac(E)-6 exhibits some differences in the carbon utilisation pattern, solubility and colour of the pigment and in cellulose decomposition. However, the nature of the antibiotic

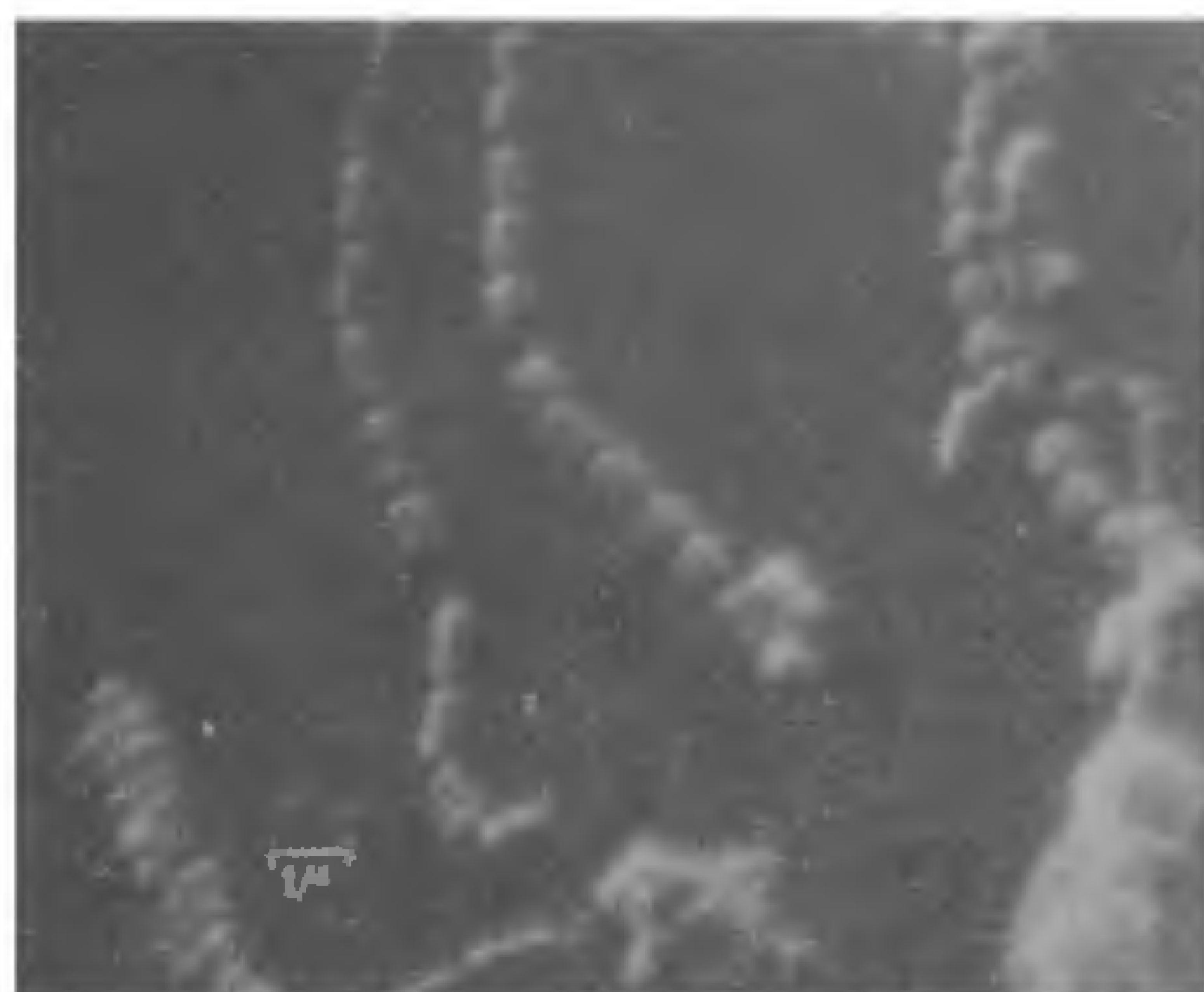

Figure 1. Electron micrograph of *Streptomyces parvullus* strain No. Ac(E)-6 (× 6,400).

produced by both the organisms is almost similar. Hence the strain is designated as *S. parvullus* strain No. Ac(E)-6.

Antibiotic production was carried out in Pridham and Gottlieb's medium[4], maintaining the other conditions[5] at optimum. Antibiotic substances were extracted from the culture broth by benzene which were later dried by a flash evaporator. The crude extract was purified by silica gel (200 mesh) column chromatography using different organic solvents. Two antibiotically active fractions, A and B were obtained which were further purified and crystallised. The R_f values for fractions A and B were 0.44 and 0.21 respectively in the solvent system of chloroform: methanol :: 95:5 (v/v). The fraction A, designated as AB(E)-6 was obtained in considerable amount and hence further work was carried out with this fraction only. That the antibiotic AB(E)-6 is homogeneous was confirmed by 2-dimentional thinlayer chromatography in the solvent systems (i) chloroform: methanol (95:5) and (ii) ethyl acetate: chloroform: water (60:40:1).

The antibiotic AB(E)-6 is orange coloured, pyramid-shaped, non-hygroscopic, water insoluble compound but highly soluble in organic solvents, *viz.* benzene, ether, chloroform, acetone etc. It is stable throughout a range of pH 3 ~ 10 and temperature 4 ~ 100°C and is decomposed at 240°-242°C. The compound is optically active with sp. rotation $[\alpha]^{20,20'}_{578\,nm} = -191.66$ (c 0.66 in chloroform) but has no fluorescent property in UV light exposure. The UV-Vis spectrum bears three characteristic peaks at 445,

430 (shoulder) and 245 cm^{-1} (figure 2) which are shifted in acidic and alkaline conditions. IR spectrum (figure 3) has characteristic absorption at 3400, 3320, 1745, 1650, 1625, 1585, 1510, 1190 and 1095 cm^{-1}. From elemental analysis the molecular formula as $C_{62}H_{86}O_{17}N_{12}$ and molecular weight as 1270 (calc.) have been suggested. The antibiotic gives positive results to neutral $KMnO_4$, biuret, diazo-reaction and 2:4 DNP but negative results to neutral $FeCl_3$, ninhydrin, Elson-Morgan, anthrone, Sakaguchi, Ehrlich, Fehling's and Schiff's tests. The acidic hydrolysate of the antibiotic shows six ninhydrin positive spots on paper chromatogram of which four were identified as L-threonine, D-valine, L-proline and sarcosine. The IR

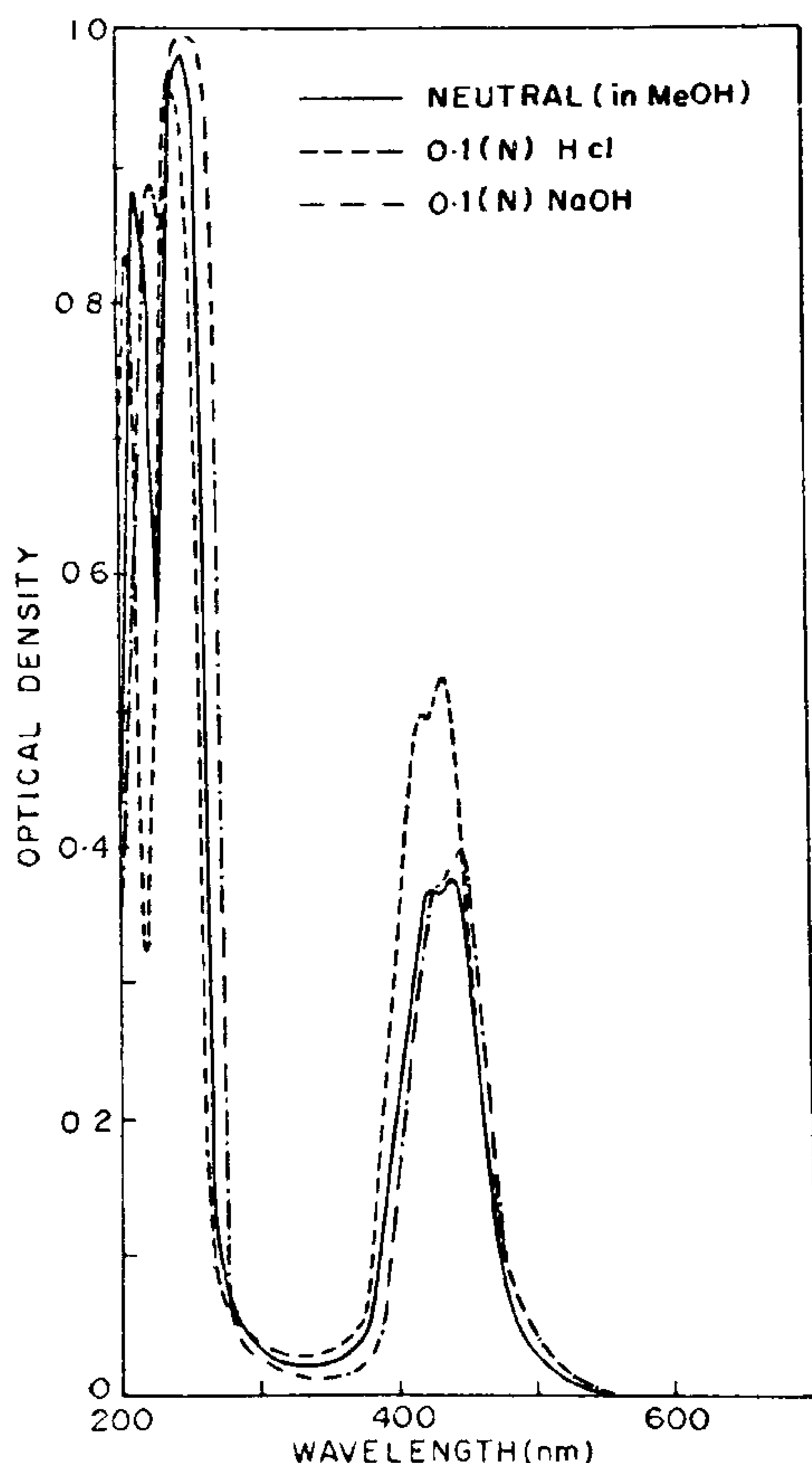

Figure 2. UV-Vis spectrum of the antibiotic AB(E)-6 in methanol.

spectrum indicates the presence of unsaturation, peptide bond, $-NH_2$ and $>C=O$ groups which were confirmed from the chemical test results. These properties along with other characteristics suggested the antibiotic AB(E)-6 as one of the actinomycin compounds. The different properties were therefore compared with actinomycin D (table 1) as standard and also with other actinomycins from the literature. The

Table 1 *Comparison of AB(E)-6 with actinomycin D*

	AB(E)-6	Act. D
State & Shape	Crystalline, pyramid	Crystalline, prism
Colour	Orange-red	Red
Melting point	240°–242°C (dec.)	246°–247°C (dec.)
Sp. rotation, $[\alpha]^t\lambda$	−191.66° (c 0.66, $CHCl_3$)	−262° (c 0 2, $CHCl_3$)
UV-Vis spectrum, max (in MeOH)	445 nm, 430 nm 245 nm	443 nm, 240 nm
R_D value*	1 07	1 00
LD_{50} (mg/Kg body wt.)	1.25 (ip)	1 0 (Sc) 0.7 (iv)

*R_D = The ratio of distance of AB(E)-6 from origin, to that of Act. D in a solvent system of $CHCl_3$: MeOH..95:5.

Table 2 *MIC value of AB(E)-6 against bacteria and fungi*

Organisms	MIC values (μg/ml)
Bacteria	
1. *Sarcina lutea*	< 1
2. *Staphylococcus aureus*	< 1
3. *Bacillus subtilis*	< 1
4. *Bacillus cereus*	< 1
5. *Klebsiella pneumoniae*	< 2
6. *Streptococcus faecalis*	2
7. *Brucella bronchiseptica*	2
8. *Neisseria flavescens*	2
9. *Escherichia coli*	> 100
10. *Salmonella typhimurium*	> 100
11. *Pseudomonas aeruginosa*	> 100
12. *Proteus vulgaris*	> 100
Fungi	
1. *Helminthosporium oryzae*	< 40
2. *Alternaria solani*	< 50
3. *Curvularia lunata*	< 50
4. *Fusarium udum*	< 70
5. *Torulopsis sp.*	< 50
6. *Saccharomyces cerevisiae*	> 100
7. *Trichophyton mentagrophytes*	> 100

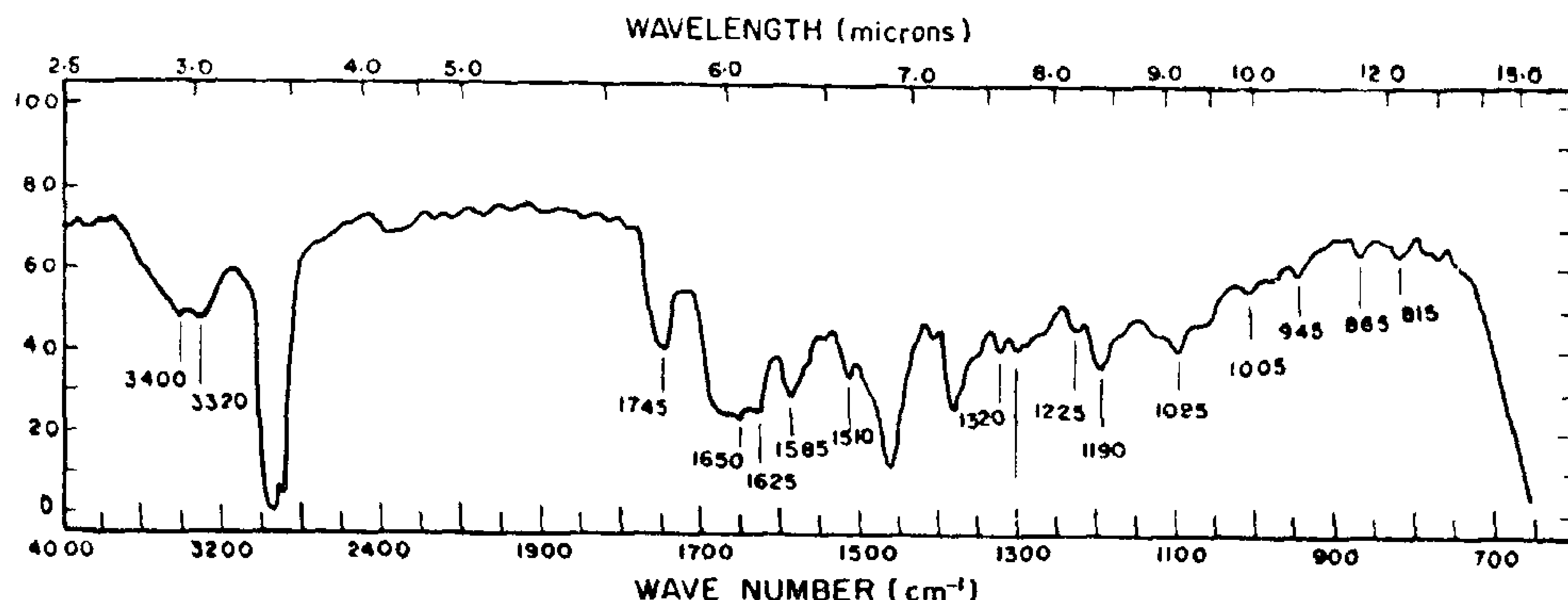

Figure 3. IR spectrum of the antibiotic AB(E)-6 in nujoll mull.

R_D values were 1.00 and 1.07 with actinomycin D and the antibiotic AB(E)-6 respectively in a solvent system of chloroform : methanol :: 95:5 (v/v).

The MIC values against different gram positive, gram negative bacteria and human and plant pathogenic fungi were determined (table 2). The antibiotic was particularly active against the gram positive bacteria. The antibiotic AB(E)-6 is a toxic compound, the LD_{50} being 1.25 mg/kg body weight in Swiss mice (ip route).

On comparison with Actinomycin D and other reported Actinomycins from the literature[6,7] the antibiotic AB(E)-6 could not be correlated with any one of them and hence assumed to be a new type of actinomycin compound.

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1. Shirling, E. B. and Gottlieb, D., *J. Syst. Bacteriol.*, 1966, **16**, 313.
2. Waksman, S. A., *The actinomycetes*, Vol. 2, Baillier, Tindall and Cox, London, 1961, p. 171.
3. Boone, C. H. and Pine, L., *Appl. Microbiol.*, 1968, **16**, 279.
4. Pridham, T. G. and Gottlieb, D., *J. Bacteriol.*, 1948, **56**, 107.
5. Ghosh, A. K. and Chakrabarty, S. L., *Indian J. Biochem. Biophys.*, 1981, **18**, 91.
6. Korzybski, T., Kowszyk-Gindifer, Z. and Kurylowiez, W., *Antibiotics*, Vol. 1, Pergamon Press, PWN-Polish Scientific Publishers, Warszawa, 1967, p. 1025.
7. Meienhoffer, J. and Atherton, E., *Adv. Appl. Microbiol.*, 1973, **16**, 203.

COMPARATIVE BOD REMOVAL EFFICIENCY OF CERTAIN MICROORGANISMS ISOLATED FROM A STABILIZATION POND

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THE stabilization pond harbours a heterogeneous community of microorganisms consisting of viruses, bacteria, algae, fungi and protozoa[1]. The biodegradative abilities of some of these groups in terms of the percent removal of biochemical oxygen demand (BOD) have been reported[2-5]. The literature concerning a comparative BOD removal efficiency of pond microorganisms is meagre. Hence the present investigation has been undertaken.

Three species of bacteria, *Pseudomonas aeruginosa, Proteus vulgaris* and *Escherichia coli*, two species of fungi, *Aspergillus niger* and an yeast sp and a species of alga, *Chlorella vulgaris* were isolated from the stabilization pond situated in the Botanical Garden on the Karnatak University, Dharwad Campus. These species were grown individually and in various combinations in flasks containing 1500 ml of sterile domestic sewage collected from the sewers of the University Boy's Hostel. The complex pond community as it occurs in the pond was inoculated into another flask and an uninoculated flask served as control. These flasks were maintained under the laboratory conditions exposed to reflected daylight near a window. Samples were collected from each flask aseptically on every alternate day and analysed for BOD[6]. The experiment was continued for 15 days and at the end of