AN ERYTHROMYCIN-LIKE ANTIBIOTIC PRODUCED BY STREPTOMYCES GRISEOPLANUS

Isolation and characterization of an antibiotic, tentatively designated as C-6, from a species of Streptomyces was reported by Rangaswami et al. This antibiotic has been found to be highly inhibitory to several bacterial plant pathogens including Pseudomonas solanacearum, Xanthomonas malvacearum, X. citri and X. oryzae. In this paper the identity of the antagonistic Streptomyces end of the antibiotic are reported.

The morphological, cultural and physiological characters of the antagonistic organism were studied following the standard methods. When the organism was grown on agar slides incubated at 37°C in moist chambers, following the technique of Gordon and Smith mostly hyaline vegetative mycelium was produced in all media and the aerial mycelium was produced mostly along the periphery of the colonies, except in the inorganic salts-starch agar wherein profuse sporulation was observed all over the colony. The sporophores in the aerial mycelium were either loosely spiralled or wavy and the spores were globose to cylindrical. When grown on nutrient glucose agar it produced scanty, white aerial mycelium, with no soluble pigment in the substratum. On yeast extract agar the vegetative mycelium was colourless to light-brown, aerial mycelium sparse and whitish and no soluble pigment produced. On Czapek’s agar the growth was poor and no aerial mycelium was produced. On glucose asparagine agar the aerial mycelium was scanty. On inorganic salts-starch agar the aerial mycelium was white to whitish-grey, but no soluble pigment produced in the medium. On potato plug the vegetative mycelium was hyaline to light brown, aerial mycelium white to gray and the apex of the plug turned slightly brown in ten days. The organism hydrolysed starch to a moderate extent, liquefied gelatin only poorly, and did not produce melanin pigment in organic media. It did not produce H₂S and failed to reduce nitrate. When grown on media containing different carbon sources there was good growth in glucose, xylose, galactose and starch, fair to moderate growth in lactose, maltose, raffinose and glyceral and poor growth in fructose, sucrose, dextrin and mannitol. Based on these characters the organism is identified as Streptomyces griseoplanus Backus et al.®

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Developmental Studies.—The stalks arise laterally on the conidiophore in acropetal succession as it grows. The stalks arise as small protuberances from the upper portion of the conidiophore. They show a characteristically drooping aspect from the beginning. Each stalk ends in a terminal vesicle. The lateral vesicles arise as slight protuberances, 1 to 3 in number. As the vesicles enlarge in size, the terminal vesicle is cut off by a septum from the rest of the stalk and assumes its characteristic upwardly bent aspect. Subsequently, the lateral vesicles are also similarly cut off by septa.

Even before the vesicles attain their full size, spore initials appear on them. They are budded out simultaneously from all over the surface of the vesicle.

At this stage, when stained with lactofuchsin, the stalks and the vesicles take up stain uniformly: As the spores attain their final size, the contents of the vesicles gradually disappear and are then poorly stained. With the shedding of the spores, the vesicles lose all their contents, take no stain, and gradually shrivel up; the stalks also shrivel up in turn.

I am very grateful to Prof. C. V. Subramanian for guidance, to Rev. Fr. T. N. Siquiera for the Latin diagnosis and to the University Grants Commission for the award of a Research Fellowship.

The antagonistic Streptomyces was grown in nutrient medium in shake cultures at a constant temperature of 30°C, following the procedure of Rangaswami et al. The culture broth was filtered through Whatman No. 1 filter-paper, the filtrate extracted three times with half the volume of ethyl ether and then with n-butanol. The two extracts were bulked and evaporated to dryness in vacuo at 45°C in a flash evaporator. The residue was dissolved in ether and the insoluble residue separated by centrifugation. The ether fraction was taken up for chromatographic assay. Thin-layer chromatograms of the antibiotic were developed on silica-gel-G coated plates, using different solvent systems. The active compound on the chromatograms was detected by pouring moulten nutrient agar seeded with *P. solanacearum* and triphenyl tetrazolium chloride at 45°C, and incubating the plates overnight at 30°C. The inhibition zone in the seeded agar, if any, was marked and the Rf value determined. Several other known antibiotics were compared with C-6 on the chromatogram. On the suggestion of Dr. F. M. Strong of the University of Wisconsin, Madison, Wisconsin, U.S.A., who examined the chemical properties of the antibiotic, it was compared with erythromycin, on identical chromatograms and was found to be similar (Fig. 1). While the erythromycin base contained only one fraction as seen in both the solvent systems, C-6 separated into three fractions in the ethanol, water and ammonium hydroxide (80:20:1) system and into one in the n-butanol, acetic acid, water (4:1:1) system. A comparison of antimicrobial spectrum of the two antibiotics revealed that C-6 is similar to the erythromycin base, both inhibiting several Gram-negative plant pathogenic bacteria but less inhibitory to *Escherichia* coli and *Erwinia carotovora*. The physical and chemical properties of the antibiotic from *S. griseoplumus* are also similar to those of erythromycin.

Erythromycin was first isolated from *Streptomyces erythreus* Waksman and Henrici by McGuire et al. and since then at least three derivatives of the antibiotic, viz., erythromycin A, B and C, have so far been differentiated. In the TLC developed with ether extracts of the antibiotic obtained from *S. griseoplumus*, three different active fractions were obtained (Fig. 1). It is possible that this streptomycete isolate produced more than one derivative of erythromycin or other closely related antibiotics and further detailed studies are required to isolate and identify these fractions.

![Fig. 1](image.jpg)


The inhibitory effect of erythromycin against some of the virulent plant pathogenic bacteria is of considerable interest. In another paper from this laboratory the effective control of brown rot of potato caused by *P. solanacearum* with the antibiotic has been reported.

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Hebbal, Bangalore-24, October 15, 1969.

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NODULATION UNDER UNFAVOURABLE SOIL CONDITIONS

Pelleting inoculated legume seed is not in vogue in this country although this method is being practised extensively in U.S.A., New Zealand and Australia. The pelleted seed enhances the survival of Rhizobium on the seed and some of the hazards associated with problem soils like absence of nodulation, delayed germination and desiccation are removed. By this method Murguia and Date could obtain 67-87% nodulation in soil where rain failed for 45 days after sowing. Under low pH conditions the bacteria are also poisoned by the soluble metallic ion like Mn (Dovereiner et al.). Further, toxicity due to excess of soluble Al or Fe may also affect the survival. The difficulty could be overcome by liming or more economically by lime pelleting the inoculated seed. Various powders such as lime (carbonate), bentonite clay, clay, lime mixtures, charcoal and rock phosphate have been used as pelleting or coating materials.

Failure of inoculation of soybean with imported and indigenous ones in soil at Suratgarh has been reported. In acid soils like that at Palampur, a similar situation is likely to occur. In order to overcome this, soybean seeds (var. Bragg) pelleted with lime or rock phosphate using Indian peat culture containing Rhizobium japonicum S.B. 16 isolated locally and maintained in this Division were sown in the experiment, described below, following the method of Hastings with the modification that sugar was included among the constituents.

Soil core from Suratgarh (pH 8.1) and well-mixed soil sample from Palampur (pH 4.9) were used. Soybean inoculated with peat culture constituted the control while the other two treatments consisted of seeds pelleted with lime and rock phosphate. In the case of Palampur soil only lime-pelleted treatment was used because of high acidity. Both the soils were treated with a basal dressing of P₂O₅, 100:0; ZnSO₄, 1:0; MnSO₄, 1:0; Borax, 1:0; Ammonium molybdate, 0:5 Kg/H. Duplicate sets were maintained in each treatment, each pot having four plants in the case of Suratgarh soil and three plants in Palampur soil. At the time of sowing the initial Rhizobium count was 16 × 10⁵/seed.

Lime and rock phosphate pelleted seeds germinated within 4 days of sowing while the germination was delayed by 8 and 13 days in the peat culture treated seeds in Suratgarh and Palampur respectively. The overall growth of the plants in the pelleted ones was good. The plants were uprooted after 8 weeks. The nodulation pattern and dry yields of the crop are given in Table I.

**Table I**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Suratgarh</th>
<th>Palampur</th>
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<tbody>
<tr>
<td></td>
<td>Nodulation</td>
<td>Average yield</td>
</tr>
<tr>
<td>Inoculated and non-pelleted</td>
<td>+</td>
<td>1.80</td>
</tr>
<tr>
<td>Inoculated and lime-pelleted</td>
<td>++</td>
<td>3.45</td>
</tr>
<tr>
<td>Inoculated and rock phosphate pelleted</td>
<td>++</td>
<td>3.23</td>
</tr>
</tbody>
</table>

+ Fair nodulation; ++ Good nodulation; + + Excellent nodulation.

The results show the beneficial effects of pelleting the seeds and using them in such soils. The seed pelleting treatments reacted best with inoculation. The nodulation and dry weight per plant increased with both lime and rock phosphate pelleting. There are some unknown factors related to soil conditions which restrict the development of nodules in legumes. Pelleting in all possibility has a protective influence against these factors particularly pH as can be seen in the present study. Pelleting materials also help in suppressing the effect of toxic substances present in the soil and thereby might help the organisms to infect the