6. SUMMARY
6.1. A survey on the occurrence of antifungal actinomycetes in the soils of West Bengal was conducted in which 50 soil samples collected mainly from nine districts were analysed. During the course of the survey, 138 actinomycete cultures were isolated and screened for production of antifungal substances. Among them 35 cultures were found to be antifungal and 8 of them strongly so. These 8 isolates were selected for further study.

6.2. The selected actinomycete cultures were briefly characterized. They were found to belong to the gray or white series of the genus, *Streptomyces* and to have spiral or retinaculum-apertum type of sporophore. All the isolates had activity against both filamentous fungi as well as yeasts and they elaborated the antifungal substances extracellularly in the culture medium. On the basis of the quantitative production of antifungal principle in the liquid medium, the isolate $SP_5$ ME-13 was judged as the most promising culture of the lot and it was selected for detailed study.

6.3. The selected isolate, $SP_5$ ME-13 was characterised fully in respect of its morphological, cultural and physiological properties, following the methods of International Streptomyces Project (ISP). The organism possessed
non-fragmented substrate mycelium, well developed gray aerial mycelium bearing long chains of smooth spores on retinaculum-apertum type sporophores and produced melanoid pigments. It was both antifungal and antibacterial. The pattern of carbon source utilization indicated close relation with the species, *Streptomyces galbus*, *S. pulveraceous* and *S. cirratus*. On the basis of maximum similarity, the isolate was tentatively identified as *Streptomyces galbus*.

6.4. In a mineral salts glucose medium the organism accumulated maximum quantity of extracellular antibiotic after 8 days in still culture. The pH value of the medium slowly turned acidic. Antibiotic excretion continued at least for 72 hr after cessation of active growth. The organism underwent rapid lysis after attainment of maximum growth.

6.5. Among the 14 media of different types tested for growth of the selected actinomycete and its ability to produce antibiotic in them, the mineral salts media with a carbon source were found, in general, to be almost as good as rich organic media. The best antibiotic production was noticed in straw-infusion broth.
6.6. Ability to grow and to produce antibiotic at the cost of different organic compounds as substrate was tested. It was observed that fructose was the best carbon source for growth, and for antibiotic production glucose and starch were almost equally good. The optimum concentrations were 4% glucose and 2% starch.

6.7. As nitrogen source nitrate was found to be superior to ammonium salts and amino acids. Sodium nitrate or potassium nitrate was found to be the best source. Urea was also utilized.

6.8. The optimum temperature and pH were 28-30°C and 6.8 respectively.

6.9. Aeration of the culture (by mechanical shaking) accelerated growth rate and antibiotic yield. Shaking also reduced the time of maximum antibiotic production. Production of antibiotic also increased due to shaking.

6.10. For antibiotic production an inoculum size of 20% (v/v) was found to be optimal. The maximum growth and antibiotic titre were reached after 6 days.

6.11. The effects of trace elements on growth and antibiotic yield of the selected isolate were variable.
Copper was highly toxic, but manganese, zinc and iron were much less toxic. Manganese influenced more favourably growth than antibiotic yield. Iron was found to have an opposite effect. The organism was more or less indifferent to zinc.

6.12. Addition to small quantity of casein hydrolysate to the inorganic salts medium resulted in stimulation of growth and antibiotic production. But addition of small quantity of yeast extract inhibited antibiotic yield, although growth was stimulated significantly.

6.13. By growing the producing strain under the best conditions determined by the optimization experiments, it was possible to achieve nearly two-fold increase in antibiotic production in comparison to the initial observation.

6.14. Isolation of the antibiotic substance from the culture filtrate by the solvent-extraction method, using a variety of organic solvents failed. The active compound could be quantitatively removed by adsorption on activated charcoal and from the latter the major portion of the antibiotic could be reextracted in methanol. The methanolic solution of the crude antibiotic was purified by precipitation with chloroform (precipitate was antibiotically inactive) and column chromatography on neutral alumina.
The eluant, n-butanol : methanol : water (4:1:2) containing the active substance was concentrated and paper chromatographed. The active band was eluted from the chromatogram to obtain the pure product.

6.15. The purified antibiotic is a colourless, odourless, amorphous, hygroscopic, slightly acidic substance. It produced single active spots on paper and thin-layer chromatograms developed with a large number of solvent systems. The elution profile from Sephadex G-25, however, exhibited a single peak with a shoulder.

6.16. The antibiotic substance was highly soluble in water, methanol, ethanol, n-propanol, acetone and dimethylformamide, but insoluble in n-butanol, chloroform, diethyl ether, benzene ethyl acetate, methyl acetate and amyl acetate.

6.17. The methanolic solution of this antibiotic showed a single sharp peak at 210 nm.

6.18. The antibiotic substance was thermostable and insensitive to photo-oxidation. In aqueous solution it was stable at pH 6.0, but was inactivated at acidic and alkaline pH.

6.19. The active spot on chromatograms gave positive response to dinitrophenyl hydrazine, Ehrlich's reagent, acidic permanganate solution and ninhydrine solution.
6.20. The producing organism showed a broad-spectrum of antibiotic activity. It was both antifungal and antibacterial. Filamentous fungi including plant and human pathogenic types were more susceptible than yeasts and dimorphic fungi.

6.21. Minimum concentration of the antibiotic necessary for complete inhibition of various fungi and bacteria varied mostly between 15 to 50 µg/ml. The antifungal action was of fungistatic nature. Growth of both gram positive and gram negative bacteria was inhibited.

6.22. The antibiotic inhibited germination of conidia of four plant pathogenic fungi and caused irregular swelling of germ tubes at concentration allowing spore germination.

6.23. The antibiotic when incorporated in agar medium partly inhibited radial growth of fungal colonies.

6.24. Toxic effect of the antibiotic on plants was tested by several methods. Germinability of seeds of some plants, but not of all was adversely affected, when the seeds were soaked in aqueous solution of the antibiotic for varying lengths of time. Leaching of betacyanine pigment from beet root slices immersed in the
antibiotic solution was not noticed till 4 hr. On the other hand, seedlings with intact root system dipped in the antibiotic solution showed symptom of wilting within 2 to $2\frac{1}{2}$ hr. Application of the antibiotic as foliar spray also caused yellowing of leaves within two days.

6.25. The antibiotic was relatively non-toxic to experimental animals (albino rats). There was no mortality within 24 hr after intravenous administration of the antibiotic at the rate of 150 mg/kg body weight.

6.26. The literature pertaining to selected aspects of antifungal antibiotics has been reviewed till 1980. Results obtained during the present investigation have been discussed in the context of the previous observations made by earlier workers.