we get

$$\frac{1}{k_{(\text{apparent})}} \cdot \frac{(D - D_1 - D_0)}{a} = \frac{T_M \cdot \epsilon_{ML}}{(1 + C)} = (D - D_1 - D_0) \quad (8)$$

when all the metal is complexed, T_M is the sum of the concentrations of ML and MHL. Therefore, $T_M = [ML]_{max.} (1 + C)$.

Defining

$$\frac{T_M \cdot \epsilon_{ML}}{(1 + C)} = D_1^*$$

where D_1^* represents the optical density when all the metal is complexed as (ML + MHL), we obtain an expression

$$(D - D_L - D_0) = D_1^* - \frac{1}{k_{(\text{apparent})}} \times (D - D_L - D_0) \cdot \frac{1}{a} \quad (9)$$

This expression is similar to the generalised expression (4) of Newton and Arcand. As discussed earlier, $\bar{D}$ can be plotted against $\bar{D}/a$ to get the value of $k_{(\text{apparent})}$. Incidentally the intercept of this straight line on $\bar{D}$ axis is now

$$D_1^* \left\{\epsilon_{ML} - \frac{(1 + C)(\epsilon_L + \epsilon_0)}{\epsilon_{ML}}\right\}.$$

McConnell and Davidson's expression can also be used to analyse such data. Expression (5) is still the correct expression for getting $k_{(\text{apparent})}$; however, $\Delta\epsilon$, under these circumstances, is equal to

$$\epsilon_{ML} - \frac{(1 - C)(\epsilon_L - \epsilon_0)}{(1 - C)}.$$

From the value of $k_{(\text{apparent})}$, it is possible to calculate k in reaction (2) if the concentration of ML can be ascertained by a suitable method.

This type of analysis can be applied in circumstances in which one of the two species ML and MHL or both the species have absorption at some suitable wavelength.

Our thanks are due to Shri S. Fareeduddin, Head, Chemical Engineering Division, Atomic Energy Establishment, Trombay, for his keen interest and encouragement.

Chemical Engineering Division, Atomic Energy Establishment, Trombay, Bombay-74, and Tata Institute of Fundamental Research, Bombay-5, *November* 10, 1965.

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ANTIBIOTIC ACTIVITY OF *SCLEROTIUM ROLFSII* SACC.

IN an earlier communication some aspects of the study of oxalic acid production by *Sclerotium rolfsii* have been described.[1]

So far, the studies on *S. rolfsii*, the causative organism of the root rot disease of many host plants, have been mainly directed towards its phytotoxicity and the considerable amount of oxalic acid produced has been incriminated as the toxic agent.[2] The inhibition of growth of a soil micro-organism of bacillus species by the culture filtrates of *S. rolfsii* has recently been reported.[3]

Since many plant pathogens are known to produce substances which besides phytotoxicity also exhibit inhibitory action on many soil micro-organisms and on human pathogens,[4–8] antibiotic production by *S. rolfsii* has been studied and reported in this communication.

MATERIALS AND METHODS

The strain studied was isolated from diseased bean plant and maintained on potato dextrose agar. For antibiotic screening, the fungus was grown on nutrient peptone broth (Peptone 5 g., Beef extract 3 g., and distilled water 1000 ml.) at pH 6·0. Flasks containing this medium were inoculated with a single sclerotium and incubated at room temperature. Culture filtrate after 30 days incubation was used for the preliminary screening test for antibiotic action.

Test organisms.—These included pathogenic bacteria and fungi. *Staphylococcus aureus, Escherichia coli, Shigella dysenteriæ, Salmonella paratyphi, Candida albicans, Cryptococcus neoformans, Trichophyton interdigitale, Aspergillus flavus, Erwinia carotovora, Pseudomonas fluorescens, Alternaria brassicæ, Fusarium orthoceras, Helminthosporium arecæ* and *Pestatotia* sp. These organisms were seeded in suitable culture media in petri dishes. The standard agar cup plate method was used for the evaluation. As the rates of growth of the organisms differ, the zone of inhibition was noted when fair amount of growth of the organisms was observed in the control areas. Hence the time of recording varied from 18 hours in the case of *S. aureus* to 6 days with *T. interdigitale.*

The optimum period for maximum antibiotic activity was determined by testing the culture filtrate at varying intervals of incubation against *S. aureus.*

For the isolation of the active principles, solvent extraction procedure adopted was as follows ;

The culture filtrate after 20 days incubation, at which period the crude filtrate showed the maximum antibiosis, was extracted exhaustively with ether. The residue was further extracted with benzene and chloroform. The solvent extracts and the crude filtrate were evaporated to dryness under reduced pressure. Varying concentrations of the dried material were taken in alcohol and screened for antibiotic activity on *S. aureus* by the cup plate method.

The minimum inhibitory concentration of the extracts on *S. aureus* was determined in the liquid nutrient broth medium by the serial dilution technique. The extent of inhibition after 24 hours incubation was estimated by turbidimetric measurements.

Considering the possibility that oxalic acid which is one of the main metabolic products of this organism might itself be the antibiotic principle, the amount of oxalic acid present in the culture at the time of antibiotic assay of the culture filtrate was determined by permanganate titration method.[9] Oxalic acid at this level and also three times this concentration were taken in nutrient broth and tested for antibiotic activity. The culture filtrate which favoured the maximum oxalic acid synthesis was also tested for antibiotic activity. The ether extract showing potent antistaphylococcal activity was chromatographically analysed for the presence of oxalic acid using Whatman No. 1 filter-paper, butanol : acetic acid : water (4 : 1 : 5) as the solvent and 0·04% bromophenol blue in 95% alcohol as the spraying agent.

Results

The crude culture filtrate exhibited inhibition of growth on only *Staphylococcus aureus*. The extent of inhibition during incubation is shown in Table I.

Table I

Anti-staphylococcal activity of the culture filtrate

Days of incubation ..	4	8	12	16	20	24	28
Zone of inhibition (in mm).	20	21	26	30	30	28	20

Table II

Inhibitory activity of the ether extract of S. rolfsii

Concentration/ml. ..	5 mg.	1 mg.	100 μg.	50 μg.
Zone of inhibition (in mm.)	48·5	41	37·5	25·5

Maximum antibiotic activity appears to have been reached by about sixteen days.

The ether extract of the culture, tested in various concentrations on *S. aureus*, gave the following inhibition (Table II).

The serial dilution study showed the ether extract to be bacteriostatic at 1 μg./ml. level and bactericidal at 100 μg./ml.

Benzene and chloroform extracts obtained from the residue were inactive.

That oxalic acid was not the inhibitory agent was proved by the absence of this acid in the ether extract by chromatographic analysis as also by the non-inhibition of staphylococcal growth in concentrations three times than that present in the cultures. Also, the culture filtrate from glucose peptone broth medium inducing maximum oxalic acid was non-inhibitory to *S. aureus*.

It is thus seen that *S. rolfsii* produces, besides oxalic acid, antibiotic substances specifically active against *S. aureus*. Glucose peptone medium, the most favoured one for oxalic acid synthesis, exhibits the least antibiotic activity while nutrient peptone medium, a poor supporter for oxalic acid production is a good medium for antibiotic production. The antibiotic is easily extractable with ether and is bacteriostatic to *Staphylococcus aureus* even at 1/1 million dilution.

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Bangalore-12, *December* 15, 1965.

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FISH REMAINS FROM THE MIDDLE PALAEOZOIC OF THE KASHMIR HIMALAYAS

While engaged on investigations in the Palaeozoic rocks of the Kashmir Himalayas the author found some specimens, *in situ*, of fish remains near Margan Pass (33° 44' : 75° 32') from the black calcareous shales immediately underlying the white Muth Quartzite exposed on the talus covered slopes, S.W. of Margan Pass.