Effect of Inhibitory Substances on Microbiospora Isolated from Soil under Cultivation of Curcuma Longa L.

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INTRODUCTION

In India plants were grown for medicinal uses. Ayurveda mentioned the importance of medicinal plant and ancient Indian literature emphasized with message that these resources should be used and conserved. Curcuma longa L. is one of important medicinal plant. Many workers worked on antibacterial activity of Curcuma longa L. but in literature there are very few reports of antibacterial activities of actinomycetes isolated from rhizosphere of Curcuma longa L.

Actinomycetes produce many secondary metabolites which are very useful in agriculture. They are well known producer of antibiotics. Actinomycetes have long been source of commercially useful enzymes and therapeutically useful bioactive molecules. Actinomycetes biodiversity is potential goldmine for the biotechnology industry because it offers countless new genes and biochemical pathway to probe for enzymes, antibiotics and other useful molecules. Actinomycetes may indirectly contribute to plant growth stimulation and their activity as biological control agent in control of fungal disease. Hence economical and bio friendly approaches are needed to find out novel bioactive compounds from actinomycetes.

Now a day’s various types of pesticides used for agriculture purpose are the main cause of soil pollution. Residual quantity of pesticide has great effect on flora and fauna of soil. Actinomycetes play major role to abate such soil pollution and sustain the flora and fauna of soil ecosystem.

The rhizome underground portion of Curcuma longa L. used as antibacterial substances. These substances diffuse into the surrounding soil area of the plant. The release of antibacterial substances inhibits growth of other microorganism. Actinomycetes which are resistant to these substances only grow in this area. Little attention has been given in studying the effect of other inhibitory substance on actinomycetes especially Microbiospora isolated from soil under cultivation of Curcuma longa L.

Very few workers worked on actinomycetes. Abraham and Herr (1964) worked on actinomycetes...
from rhizosphere and non-rhizosphere soil of corn and soybean. Studies have been reported that Microbiospora hainanensis sp.nov was isolated from rhizosphere soil of Excoecaria agallocha in a mangrove (Xu et al., 2011). Sodium azide in concentration 400 or 800 part/10^6 inhibited the bacteria and actinomycetes and drastically reduced the fungal population (Skipper and Westermann, 1973). The presence of trichloroethylene and phenol provided a selective pressure favouring the enrichment of actinomycetes over a TEC- degrading filamentous enrichment that was used to seed reactor. Phenol concentration above 15 mg L^{-1} inhibited phenol degradation. Comparative inhibition between growth substrate, phenol and trichloroethylene was observed (Lee et al., 2000).

Simultaneous degradation of P-nitrophenol and phenol by newly isolated Nocardiodes sp. was also reported by Cho et al. (1998). Therefore it is also necessary to study the effect of other inhibitory substances on actinomycetes under cultivation of Curcuma longa L.

In the present study actinomycetes were isolated from soil under cultivation of Curcuma longa L. From these Microbiospora isolates were studied for the effect of inhibitory substances like dettol, lysol, phenol, sodium azide and crystal violet.

**MATERIALS AND METHODS**

- Soil-Soil under cultivation of Curcuma longa L. from the villages around Barshi, Dist. Solapur, M.S, India
- Glycerol aspargine agar
- Inhibitory substances-Crystal violet, Sodium azide, Dettol, Phenol, Lysol

**Isolation of actinomycetes:** For the present study 10 soil samples collected from the villages around Barshi, Dist. Solapur M.S, India were used for isolation of actinomycetes. Total 15 actinomycetes were isolated by streak inoculation technique on glycerol aspargine agar (L-aspargine- 0.1g, K_{2}HPO_{4}-0.1g, glycerol- 1g, trace salt solution- 0.1mL, agar- 2.5g, distilled water-100 mL pH-7.4) after incubation at an ambient temperature for 5-7 days.

**Identification of actinomycetes:** These actinomycetes were identified by performing morphological, cultural and biochemical studies. Morphological characters were studied by cover slip culture technique (Mycelium pattern e.g., Arial, submerged and surface mycelium and structure of spore chain). Cultural characters were studied by observing growth on different media e.g., Bennet’s agar, Dextrose agar. Biochemical studies include enzymatic and sugar utilization test. These isolates were also identified by using Bergey’s Manual of Systematic Bacteriology vol-4 and Micro IS software.

**Effect of inhibitory substances on Microbiospora:**

Among all actinomycetes isolates five actinomycetes were identified as Microbiospora. The effect of inhibitory substances like Dettol, Phenol, Lysol, Sodium azide, Crystal violet were carried out by using Glycerol aspargine agar. These inhibitory substances were added in glycerol aspargine agar having concentration Dettol (0.1), Phenol (0.1), Lysol (0.1), Sodium azide (0.01), Crystal violet (0.0001) % w/v separately. The inhibitory effect were studied by spot inoculating on glycerol aspargine agar containing these inhibitory substances and incubated at an ambient temperature for 5-7 days. Results were recorded on the basis of presence or absence of growth.

**RESULTS**

The organisms were isolated on Glycerol aspargine agar and identified as Microbiospora on the basis of morphological and cultural characteristics by using Bergey’s Manual of Systematic Bacteriology vol. 4 (Bergey et al., 1989) and MIRO- IS software. Effect of inhibitory substances on Microbiospora were recorded in Table 1.

All the Microbiospora isolates were showed no growth on dettol while showed growth on crystal violet, 60% Microbiospora isolate were showed growth on lysol, 40% Microbiospora showed growth on sodium azide and 20% Microbiospora showed growth on phenol. These data were presented in Fig. 1.

Table 1: Effect of inhibitory substances on Microbiospora isolated from soil under cultivation of Curcuma longa L.

| Isolates | Dettol | Phenol | Lysol | Sodium azide | Crystal violet |
|----------|--------|--------|-------|--------------|---------------|
| 1        | -      | +      | +     | +            | +             |
| 2        | -      | -      | +     | +            | +             |
| 3        | -      | -      | -     | -            | +             |
| 4        | -      | -      | +     | -            | +             |
| 5        | -      | -      | -     | -            | +             |

Where: + = Growth; - = No growth

![Fig. 1: Percentagewise inhibition of Microbiospora by inhibitory substances](image-url)
DISCUSSION

Skipper and Westermann (1973) were found that Sodium azide in concentration 400 or 800 part/10[^6] inhibited the bacteria and actinomycetes and drastically reduced the fungal population. Cho et al. (1998) also reported that simultaneous degradation of P-nitrophenol and phenol by newly isolated Nocardioiodes sp. The presence of trichloroethylene and phenol provided a selective pressure favouring the enrichment of actinomycetes over a TEC- degrading filamentous enrichment. Phenol concentration above15mg L[^−1] inhibited phenol degradation (Lee et al., 2000). Comparative inhibition between growth substrate, phenol and trichloroethylene was also observed by Lee et al. (2000).

Comparative to these studies we found that all Microbiospora were sensitive to dettol while resistant to crystal violet, 60% Microbiospora isolates were resistant to lysol, 40% were resistant to sodium azide and 20% were found resistant to phenol.

CONCLUSION

From the result it is concluded that strain of Microbiospora resistant to phenol and other inhibitory substances can be used for degradation of residual components of pesticide which are released into the soil and abate soil pollution to some extent.

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