Universal Journal of Environmental Research and Technology
 **eISSN 2249 0256**
Available Online at: www.environmentaljournal.org
Volume 2, Issue 6: 625-629

**Open Access** **Short Communication**

# Antibiotics Susceptibility Pattern of Actinomycetes Isolated from Soil under Cultivation of *Curcuma longa* L.

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## Abstract:
**Actinomycetes are promising tool to control plant disease and to promote plant growth. In ayurved system of medicine rhizome portion of *Curcuma longa* L. was used as an antiseptic. Antimicrobial substances released from these plants diffuse into the surrounding soil area of the plant. Actinomycetes which grow in these areas are resistant to these substances. Different types of pesticide and chemicals are also used to control plant pest. The residual component of these substances diffuses into the soil and causes soil pollution. The present study was carried out to search actinomycetes which can be used to degrade the residual components of pesticide, chemicals and antibiotics. Total four soil samples were collected from the villages around Barshi, Dist. Solapur. Total six isolates were tested for their ability to grow in presence of antibiotics such as penicillin, streptomycin, chloramphenicol gentamycin and tetracycline on Glycerol aspargine agar containing these antibiotics separately by spot inoculation technique after incubation at an ambient temperature for 5 to 7 days. Out of six actinomycetes isolates 83.33% were resistant to chloramphenicol, 66.66% were resistant to penicillin and 50% were resistant to tetracycline, while all the actinomycetes isolates were sensitive to gentamycin and streptomycin. The actinomycetes resistant to penicillin, chloramphenicol and tetracycline may have potential to produce antibiotics of this category and they will be studied further for the production of this type of antibiotics which will be used to prevent human and plant bacterial and fungal diseases. They may have ability to degrade chemical or antibiotics and may be used to control soil pollution to some extent.**

**Keywords:** Actinomycetes, Antibiotics, *Curcuma longa* L., Soil pollution.

## 1.0 Introduction:
*Curcuma longa* L. (Turmeric) is one of the important spice crops in India and plays a vital role in national economy. India is the largest producer and exporters of turmeric in the world and accounts for more than 50 percent of the world trade (Philip, 1983). Turmeric is categorised under the Zingiberaceae family, extensively used as therapeutic agent for over 6000 years in Ayurveda, Siddha and Unani medicinal systems (Eigner and Scholz, 1999). In ayurved system of medicine rhizome portion of *Curcuma longa* L. was used as an antiseptic. Antimicrobial substances released from these plants diffuse into the surrounding soil area of the plant. Actinomycetes which grow in these areas are resistant to these substances. As agriculture is concerned it is necessary to focus on plant growth, rhizospheric microorganisms especially actinomycetes and soil health. Though wide genetic variability exist in this crop with regard to the yield and yield attributes, however not much work has been done on an antibiotics susceptibility pattern of actinomycetes from soil under cultivation of *Curcuma longa* L. So there is need to study the tolerance pattern of actionomycetes to various antibiotics from soil under cultivation of *Curcuma longa* L. The actinomycetes are Gram positive bacteria having high G+C (>55%) content in their DNA. Actinomycetes are prolific producer of antibiotics and majority of the antibiotics in clinical use today are produced by them. Among the various microorganisms, the actinomycetes have yielded over two- third of the naturally occurring antibiotics and continued to a major source of novel and useful antibiotics.

Based on several studies among bacteria, the actinomycetes are noteworthy as antibiotics producer, making three quarters of all known product; the *Streptomyces* are especially prolific (Lacey, 1973; Lechevalier, 1989; Locci, 1989; Saadoun and Gharaibeh, 2003; Waksman, 1961). A search of the recent literature revealed that at least 4,607 patent have been issued on actinomycetes related product and processes (Williams and Vickers, 1988). *Streptomyces* covers around 80% of total antibiotic product, with other genera trailing numerically; *Micromonospora* is the runner up with less than one-tenth as many as *Streptomyces*. If we include secondary metabolites with biological activities other than antimicrobial, actinomycetes are still being out in front (Hopwood *et al*., 2000). Microbes are gaining resistance to existing antibiotics. Still there is a desperate need of screening antibiotics for antimicrobial compound. It is suggested that the *Streptomyces* isolated from sea sediment from west coast of India, possessing potent antimicrobial activity and assigned to *Streptomyces* is worth investigating in details in future for deriving the antibiotics for human welfare (Gulve and Deshmukh, 2011). Yasunaka *et al.,* (2005) reported antimicrobial activity of crude ethanol extracts from Mexican of medicinal plants and purified coumarins and xanthones. Mahesh and Satish (2008) also studied antimicrobial activity of some important medicinal plant against plant and human pathogens.

The present research was carried out to find out antibiotics resistant actinomycetes and they will be studied further for the production of antibiotics used to prevent human and plant bacterial and fungal diseases. They may have ability to degrade chemical or antibiotics and may be used to control soil pollution to some extent.

## 2.0 Materials and Methods:

### *2.1 Materials-*

Soil-Four Soil samples under cultivation of *Curcuma longa* L. from the villages around Barshi, Dist. Solapur, M.S., India. Glycerol aspargine agar.

Antibiotics- penicillin, streptomycin, chloramphenicol gentamycin and tetracycline

### *2.2 Methods:*

### *2.2.1 Isolation of actinomycetes:*

For the present study four soil samples collected from the villages around Barshi, Dist. Solapur M.S., India and were used for isolation of actinomycetes. The site of collected soil samples were shown in figure 1. Actinomycetes were isolated by streak inoculation technique on glycerol aspargine agar ( L-aspargine- 0.1g, $K_2HPO_4$-0.1g, glycerol- 1g, trace salt solution- 0.1ml, agar- 2.5g, distilled water-100ml pH-7.4) after incubation at an ambient temperature for 5 to7 days.

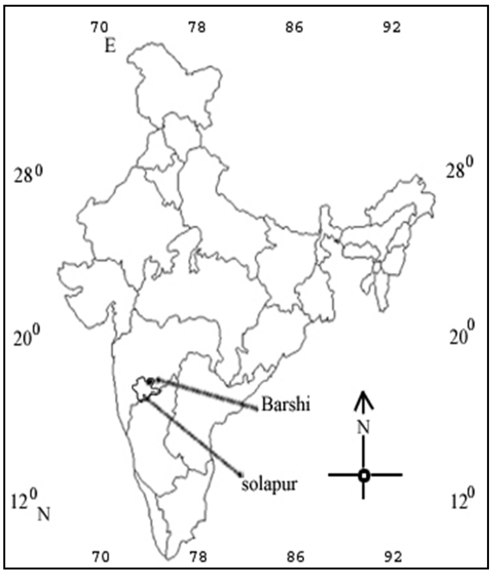


**Figure 1:** Map of soil samples collection site

### *2.2.2 Identification of actinomycetes:*

Tentatively on morphological basis they were identified as actinomycetes.

### *Antibiotics susceptibility pattern of actinomycetes*

Antibiotics- penicillin ($10IUml^{-1}$), streptomycin ($100mg\ ml^{-1}$), chloramphenicol ($100mg\ ml^{-1}$) gentamycin and tetracycline ($100mg\ ml^{-1)}$ were added in glycerol aspargine agar. Antibiotics susceptibility pattern of actinomycetes were studied by spot inoculating isolates on glycerol aspargine agar containing these antibiotics concentration and incubated at an ambient temperature for 5 to 7 days. Results were recorded on the basis of presence or absence of growth.

## 3.0 Results and Discussion:

The organisms were isolated on Glycerol aspargine agar and tentatively on morphological basis they were identified as actinomycetes. Total six actinomycetes were isolated. Kulkarni and Deshmukh (2001) were studied tolerance of soil actinomycetes to inhibitory substances. Actinomycetes were tested for their ability to grow in presence of antibiotics such as penicillin (10IUml$^{-1}$), streptomycin (100 mg ml$^{-1}$) and gentamycin (100 mg ml$^{-1}$); arsenic trioxide (0.05 mg L$^{-1}$), crystal violet (0.0001%w/v), sodium azide (0.02% w/v), phenol (0.1% w/v) dettol (0.1% w/v) mercuric chloride (0.1% w/v) and sodium chloride ( 4%, 7%, 10% and 13% w/v ). They found that *Streptomyces* were resistant to all tested inhibitory agent, where as *Streptoverticillum, Thermoactinomycetes* and *Nocardia* were resistant to maximum number of tested inhibitory agents.

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 *Nocardiodes* sp. They observed that during simultaneous degradation in the low concentration of phenol, after the exhaustion of phenol, some P-nitrophenol was transformed by the catechol pathway and 4-nitrocatechol was transiently accumulated. Kinetically, the addition of phenol greatly enhanced the apparent P-nitrophenol degradation rate, which may be due to the increased cell mass by the assimilation of phenol. Sustained degradation of trichloroethylene in a suspended growth gas treatment reactor by actinomycetes enrichment was reported by Lee *et al.,* (2000). They also observed competitive inhibition between growth substrate, phenol and trichloroethylene due to actinomycetes cis- DCE degradation capacity.

**Table 1:** Antibiotics susceptibility pattern of actinomycetes isolated from soil under cultivation of *Curcuma longa* L.

| Isolate No. | Chloramphenicol | Penicillin | Tetracycline | Gentamycin | Streptomycin |
|---|---|---|---|---|---|
| 1 | + | + | + | - | - |
| 2 | + | - | - | - | - |
| 3 | + | + | - | - | - |
| 4 | + | - | + | - | - |
| 5 | + | + | + | - | - |
| 6 | - | + | - | - | - |

Where + = resistant, - = sensitive

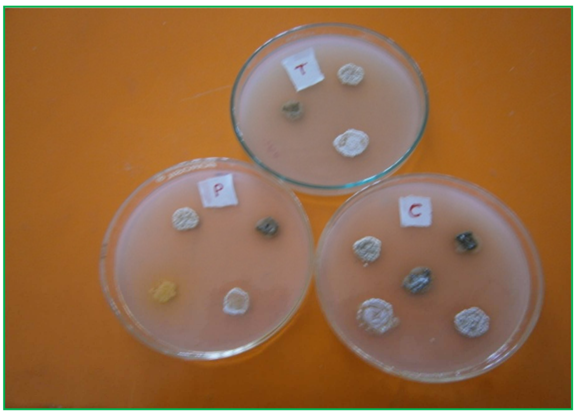

**Figure 2: Actinomycetes resistant to antibiotics**
C= chloramphenicol P= penicillin T= tetracycline

Hamid M. E. (2011) reported variable antibiotic susceptibility pattern among *Streptomyces* species causing actinomycetoma in man and animals in Sudan. They were having various phenotypic groups. All of the studied strains were inhibited by novobiocin, gentamycin, doxycycline, and were found resistant to amphotericin B Penicillin and sulphamethoxazole, He also reported that fusidic acid inhibited 94.4% of the strains; bacitracin, streptomycin, cephaloridine, clindmycin, ampicillin, rifampicin and tetracycline, inhibited between 61.1 and 77.8% of the strains, Chaudhari *et al.,* (1997) studied antibiotic sensitivity patterns of actinomycetes isolated from patients of actinomycetoma. They reported the effectiveness of eight antibiotics against 30 human isolates of actinomycetoma agents belonging to 7 different species. They were tested by agar dilution and disc diffusion methods to evaluate the susceptibility patterns and to study drug resistance among the organisms. They were found that many of the isolates had developed partial or complete resistance to conventionally used antibiotics like cotrimoxazole, streptomycin and ampicillin, but almost all were sensitive to amikacin and ciprofloxacin.

Chaphalkar and Dey (1993) reported penicillin G (10IU) resistant in 3 isolates of 11 actinomycetes isolates studied. All studied isolates were *Streptomyces.* They also reported that sodium azide tolerance in 3 isolates and phenol tolerance in 6 isolates of the 11 *Streptomyces.* Lavrova (1971) was observed that *Actinomadura* resistance to Streptomycin, to Penicilin in actinomycetes was observed by Williams and Davies (1965) and to gentamycin in *Micropolyspora* was observed by Ivanitskaya *et al.,* (1971). Comparative to these studies we found that out of total actinomycetes isolates 83.33% were showed growth on chloramphenicol, 66.66% on penicillin and 50% on tetracycline, while all the actinomycetes isolates were showed no growth on gentamycin and streptomycin. This data were recorded in table 1. Actinomycetes resistant to penicillin, chloramphenicol and tetracycline were shown in figure-2. We found that all actinomycetes were sensitive to gentamycin and streptomycin.

## 4.0 Conclusion:

From the result it is concluded that the actinomycetes resistant to penicillin, chloramphenicol and tetracycline may produce antibiotics in this category and they will studied further for the production of this type of antibiotics. Antibiotics produced from actinomycetes under cultivation of *Curcuma longa* L. will be used to prevent human and plant bacterial and fungal diseases. They may have ability to degrade chemical, pesticide and antibiotics and may be used to control soil pollution to some extent.

## 5.0 Acknowledgement:

Researchers are thankful to the Shriman Bhausaheb Zadbuke Mahavidyalya Barshi for providing laboratory and library facilities to carry out this study.

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