# COMPARATIVE STUDIES ON ANTIBACTERIAL ACTIVITY OF *ACTINOMYCETES* FROM DIFFERENT SAMPLE

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**Abstract**

Earthworm and microorganisms are interdependent on each other. The interactions between them help to regulate the biogeochemical cycle of terrestrial life. A large diversity of fungi, bacteria, yeast, actinomycetes and protozoa are found to be present in the gut and cast of earthworms. Their number and species depend on their feed substrates obtained in soil. The higher numbers of microorganisms observed in the gut sections and casts of the earthworms examined in this work reinforce the general concept that the gut and casts of earthworms show higher microbial diversity and activity than the surrounding soil. Antibacterial activity producing actinomycetes can be identified from soil and earthworm gut from two different locations.

**Keywords**: *Earthworm, Isolation actinomycetes, Antimicrobial activity, Antibiotics, Microorganisms.*

## Introduction

Soil ecology studies are focused on the prospects of linking microbes and fauna (Brown *et al*., 2004; Coleman *et al*., 2004). Without doubt, earthworms are the most important soil invertebrates in the soil ecosystem in terms of biomass and activity (Rombke *et al*., 2005), being often considered as ecosystem engineers (Lavelle, 1988). Moreover, soil contains a large diversity of microorganisms (Torsvik *et al*., 2002). The microorganisms available in the gut of earthworm species are mostly related to the soil micro flora. If microorganisms were to act as symbionts in digestion by this earthworm, they would probably be associated with its gut (Brown *et al.*, 2004).

Actinomycetes are gram positive bacteria frequently filamentous and sporulating with DNA rich in G+C from 55-75% (Lai *et al*., 2002).The name actinomycetes derived from Greek aktis (a ray) and mykes (fungus) was given to these organisms from initial observation of their morphology. *Streptomyces* is the dominant among actinomycetes. They are responsible for the production of about half of the discovered bioactive secondary metabolites (Berdy, 2005), antibiotics (Strohl, 2004), antitumor agents (Cragg *et al*., 2005), immunosuppressive agents and enzymes (Mann, 2001).The diversity of terrestrial actinomycetes is of extraordinary significance in several areas of science and medicine, particularly in antibiotic production Magarvey *et al*., 2004)

Actinomycetes are unparalleled sources of bio-active metabolites including antibiotics, plant growth factors, and other substances (Shahidi *et al.*, 2004). *Streptomyces* and other actinomycetes are major contributors to biological buffering of soils and have roles in organic matter decomposition conductive to crop production (Dhingra *et al.*, 1995). Actinomycetes are known to produce bioactive substances, especially antibiotics that are effective against phytopathogenic bacteria (Crawford *et al*., 1993). Among microorganisms, actinomycetes are the most economically and biotechnologically useful prokaryotes (Lam, 2006; Valli *et al.*, 2012). They produce antibiotics and other industrially important secondary metabolites (Kekuda *et al.*, 2010; Naine *et al*., 2011).

**Materials and Method**

**a. Collection of Sample**

The earthworm and soil were collected from paddy field at Thiruppampuram Village, Thiruvarur (Dist) and dumping site at Karikulam near by Kumbakonam, Thanjavur (Dist), Tamilnadu, India. Healthy adult earthworms were collected and allowed to starve for 24 hrs.

A healthy sexually mature, clitellated worm was taken, washed with tap water and were then cleaned externally with 70% ethanol. Sterile dissecting pins were used to hold the earthworm down on a board and an incision was made longitudinally along the earthworm. The gut was then freed from surrounding blood vessels and nephridia and separated into gut sections.

**b. Isolation of actinomycetes from the earthworm gut and soil**

The gut sections were washed in sterile distilled water, weighed and homogenized for 5 minutes with a pistal mortal in sterile 0.85% of (0.85g Nacl mixed with 100 ml distilled water to prepare 0.85% sterile saline ) Nacl solution. The gut and soil homogenate was serially diluted ($10^{-1}$, $10^{-7}$) and settle for 1 hour and plated on to (Petri plates were cleaned methanol and sterilized in autoclave with 20 minutes in 120 psi) The isolation of actinomycetes from starch casein agar was supplemented with amphotericin B, and streptomycin of each 0.25 µg/250 ml medium to inhibit the normal bacterial and fungal flora. The plates were incubated at $23^0$C / $28^0$C and observed for 7 days for the growth and sporulation of actinomycetes.

Whitish pin – point powdery like structure colonies which are the characteristic of actinomycetes with clear zone of inhibition around it were observed in the plates. The pinpoint colonies with inhibitory or clear zone of inhibition were selected and the suspected colonies were selectively isolated and transferred to starch casein agar medium with the help of loop inoculation in streak plate method (Hamaki *et al.*, 2005). The plates were kept for incubation at 25 to $30^0$ C for 2- 3 days in bacteriological incubator an inverted position. The actinomycetes isolates were purified by pure culture techniques. The colonies were refrigerated in starch casein agar slants by frequent sub culture for further studies.

To characterize the taxonomic position of the selected earthworm gut actinomycetes isolate, a range of tests were carried for morphological, physiological and biochemical out according to the guidelines by (Bergey 1989), International streptomyces project (Shirling and Gottlieb, 1966 and Williams *et al*., 1983).

All the actinomycetes isolates obtained were tested for their antibacterial activity against several bacteria Gram- negative bacteria and Gram -positive bacteria. Screening for antimicrobial activity of pure isolates was determined by perpendicular streak method using Starch Casein agar Medium.

**Result**

A total of 22 isolates were isolated from Earthworm gut and soil samples. The numbers of samples and isolates in each sample were presented in Table 1, 2.

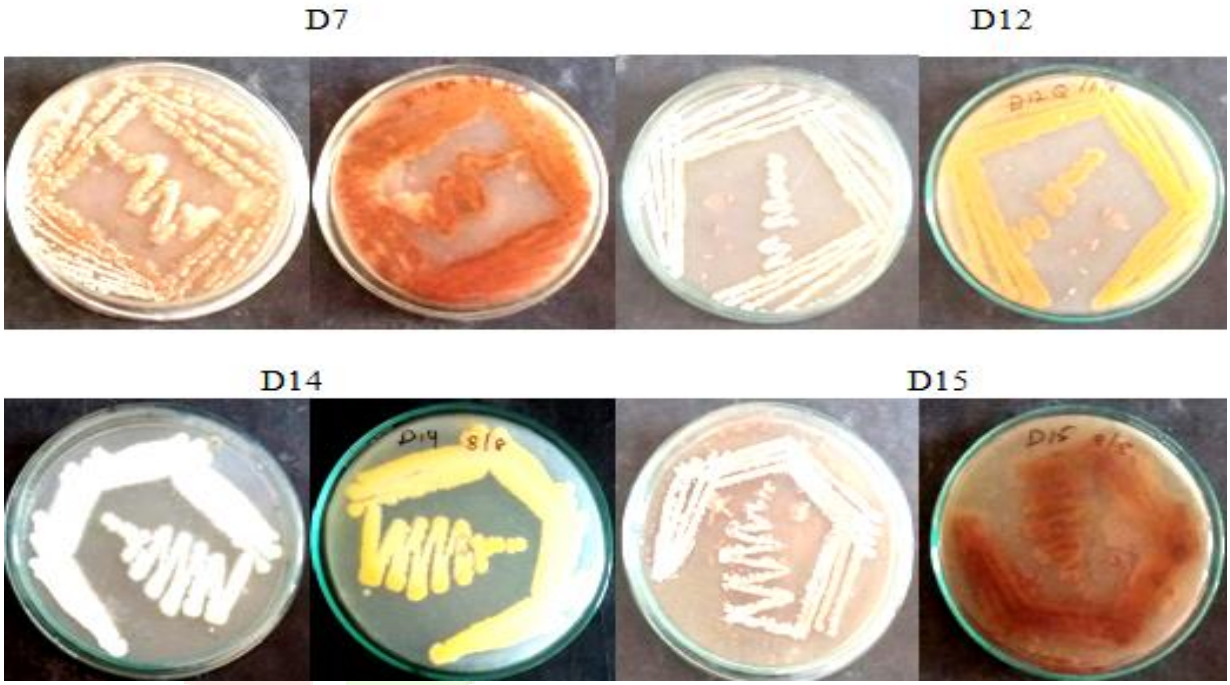


**Figure 1** Actinomycetes isolation plate

T**able 1** Total number of isolates active against sensitive strains

| Sl. No. | Sample source | Number of isolates screened | Number of isolates active |
|---|---|---|---|
| 1. | Paddy soil – Thiruppampuram Village | 7 | 1 |
| 2. | Paddy soil – Earthworm Gut | 8 | 2 |
| 3. | Dumping site soil – Kurikulam, Kumbakonam | 3 | - |
| 4. | Dumping site – Earthworm Gut | 4 | 1 |
| Total | | 22 | 4 |

**Table 2** Colony characteristics of actinomycetes isolates

| Sl. No. | No. of isolates | Sample source | Growth | Sporulation | Aerial mycelium colour | Substrate mycelium colour | Diffusible pigment | Melanoid Pigment |
|---|---|---|---|---|---|---|---|---|
| 1. | D1 | P S | Good | Poor | White Yellow | Yellow | - | - |
| 2. | D2 | | Good | Moderate | Yellowish | Yellow | - | - |
| 3. | D3 | | Good | Good | White Yellow | White Yellow | - | - |
| 4. | D4 | | Good | Good | Blackish Grey | Pale Yellow | - | - |
| 5. | D5 | | Good | Good | Grey | Brown | - | - |
| 6. | D6 | | Moderate | Poor | Grey | Pale Yellow | - | - |
| 7. | D7 | | Good | Good | Brownish | Brown | - | Dark Brown |
| 8. | D8 | P S E G | Good | Good | Rough White | White | - | - |
| 9. | D9 | | Good | Good | Blue | Blue | - | - |
| 10. | D10 | | Poor | poor | Green | Green | - | - |
| 11. | D11 | | Good | Moderate | Jelly Yellow | Light Yellow | - | - |
| 12. | D12 | | Good | Good | Yellow White | yellow | - | - |
| 13. | D13 | | Good | Good | Whitish Grey | Brown | - | Brown |
| 14. | D14 | | Good | Good | White | Pale Yellow | - | - |
| 15. | D19 | | Good | Moderate | White | White | - | - |
| 16. | D15 | D S E G | Good | Good | Grey | Brown | - | Brown |
| 17. | D16 | | Good | Poor | Grayish Brown | Brown | - | - |
| 18. | D17 | | Good | Good | White | Pale Yellow | - | - |
| 19. | D18 | | Good | Poor | Grey | Dark Brown | - | Reddish |
| 20 | D20 | D S | Good | Good | Grey | Grey Black | Black | - |
| 21. | D21 | | Good | Poor | Yellow | Yellow | - | - |
| 22. | D22 | | Good | Good | Grey | Black | Black | - |

*** PS** – Paddy Soil, **PSEG** - Paddy Soil Earthworm Gut, **DSEG** – Dumping Site Earthworm Gut, **DS** – Dumping Site Soil

**Antibacterial activity**

In screening for actinomycetes with antibacterial activity, twenty two isolates were screened. The potent actinomycetes were characterized by morphological and biochemical methods. The observed structure was compared with Bergeys manual of systematic Bacteriology (Williams *et al.,* 1989 and Waksman *et al.,* 1943). Four isolates showed activities against the test bacteria. Actinomycetes (isolate No.D12) had maximum activity against the tested bacteria in comparison with others based on larger zone of inhibition and was selected for further evaluations.

The actinomycetes isolates showed various types of colour pigments like white, yellow, orange, black and brown which may have potential use in industries. Out of 22 actinomycetes isolates, 4 of them showed significant inhibition of different Gram positive and Gram negative bacteria. Actinomycetes isolate no. D7,

D12, D14, D15 inhibited *Staphylococcus aureus, Escherichia coli, Protease vulgaris, Vibrio cholarae, Klebsiella pneumonia* and *Shigella bodyii*. Actinomycetes populations in soil and earthworms may help in developing a strategy for discovery additional antimicrobials were presented in Table 3, 4.

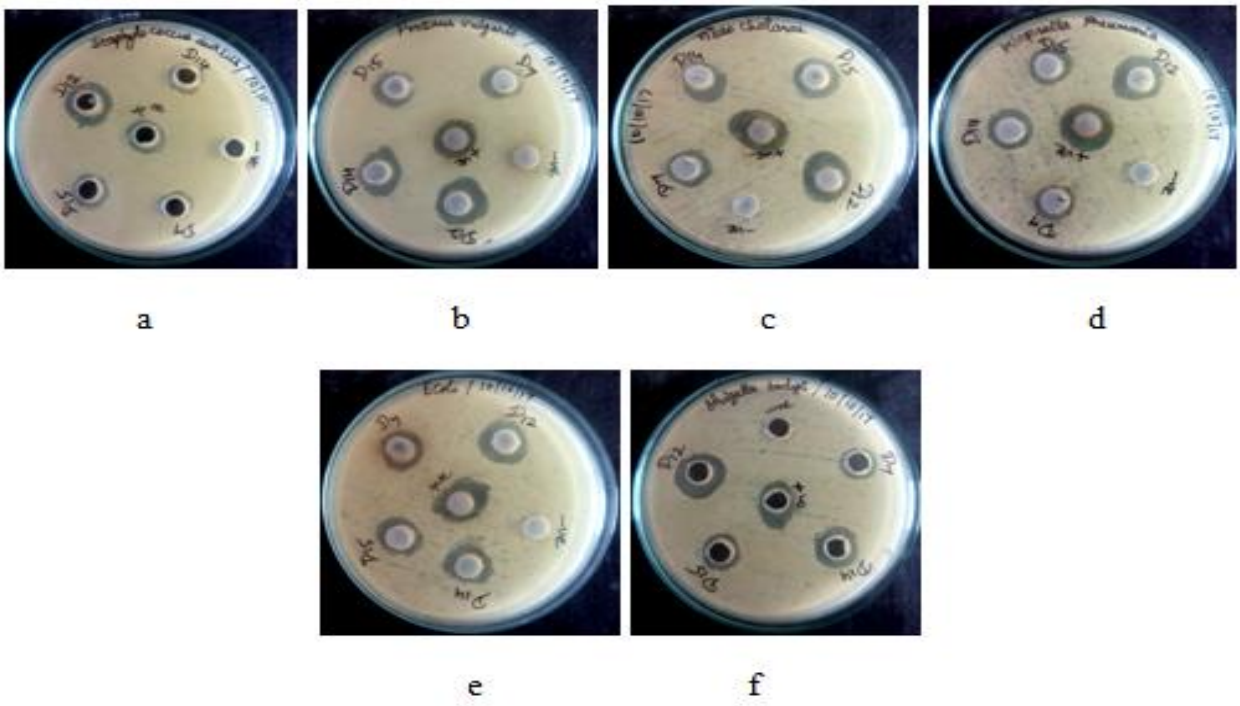


**Figure 2** Antibacterial activity (a. *Staphylococcus aureus* b. *Protease vulgaris* c .*Vibrio cholarae* d. *Klepsella pnemoniae* e. *Escherichia coli* f. *Shigella bodyii*)

**Table 3** Antibacterial activities of actinomycetes in secondary screening

| Sl.No | Bacterial Pathogens | Antagonistic activity of isolated actinomycetes strains | | | |
|---|---|---|---|---|---|
| | | D7 | D12 | D14 | D15 |
| 1. | *Staphylococcus aureus* | + | +++ | + | + |
| 2. | *Escherichia coli* | ++ | +++ | +++ | + |
| 3. | *Klepsella pnemoniae* | ++ | +++ | ++ | ++ |
| 4. | *Protease vulgaris* | ++ | ++ | + | ++ |
| 5. | *Vibrio cholarae* | + | ++ | ++ | + |
| 6. | *Shigella bodyii* | ++ | +++ | ++ | ++ |

+++ Very Good, ++ Good, + Poor

**Table 4** Antibacterial activities of actinomycetes in zone formation (mm)

| Sl.No | Bacterial Pathogens | Zone of inhibition (mm) | | | |
|---|---|---|---|---|---|
| | | D7 | D12 | D14 | D15 |
| 1. | *Staphylococcus aureus* | 09 | 25 | 08 | 12 |
| 2. | *Escherichia coli* | 14 | 22 | 13 | 08 |
| 3. | *Klepsella pnemoniae* | 17 | 20 | 21 | 16 |
| 4. | *Protease vulgaris* | 19 | 27 | 11 | 17 |
| 5. | *Vibrio cholarae* | 07 | 17 | 14 | 09 |
| 6. | *Shigella bodyii* | 12 | 20 | 12 | 20 |

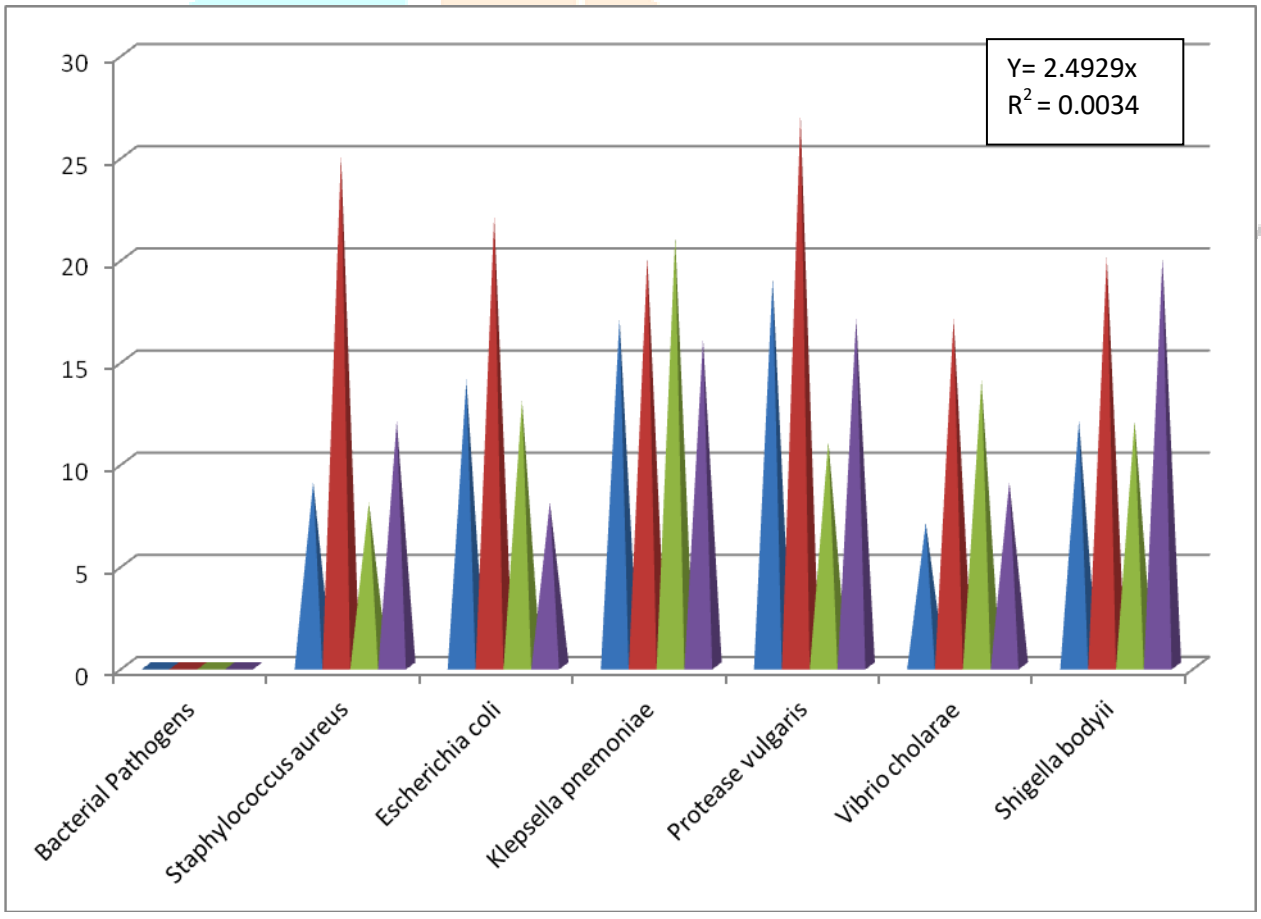


**Figure 3** Antibacterial activities of actinomycetes in zone formation (mm) against pathogenic bacteria

**Discussion**

The bacterial counts in guts was higher than the surrounding soil ecosystem (Edwards and Bohlen, 1996; Munnoli, 1998 and 2007; Munnoli *et al.,* 2000; Suthar, 2008; Kulinska,1961) and as the organic matter ingested passes through the gut, it undergoes biochemical changes effected by gut-inhibiting bacteria Suthar. (2008).

Karsten and Drake (1997) who found number of microorganisms (bacteria, actinomycetes, fungi) in alimentary tract earthworms were six times higher in comparison with the surrounding soil. The antibacterial resistance is presently an urgent biological control of focus of research and new antibiotics are necessary to combat pathogens. The emergence and dissemination antibacterial resistance is well documented as a serious problem worldwide (Gold *et al*., 1996). The emergence of identification of newly bacterial resistance threatens to return us to the era before the development of antibiotics (Smith *et al*., 1999). This situation production, recommends the need for the investigation of new, safe and effective antimicrobials for replacement of invalidated antimicrobials (Gerding *et al.,* 1991). Actinomycetes have been recognized as source of several secondary metabolites, antibiotics and lytic enzymes among which *Streptomyces* spp. have been shown to have characteristics which make them useful as antagonistic agents against pathogens.

**Conclusion**

The gut of the earthworm constitutes a unique microenvironment in soils. The selective digestion of microbes in the gut influences the type of nutrients that are available for subsequent assimilation by both the earthworm and members of the gut microflora. The variation in the microbial populations in the earthworm gut may be because of their nutritional needs and digesting ability of the earthworms. The present study reported the antimicrobial activities exhibited by different isolates of actinomycetes from two different samples. Out of the 22 isolates, isolate D12 showed maximal antagonistic activity against the microorganisms used. These findings and indicated that our produced substance might be the alternative antimicrobial substance as a tool for controlling human diseases.

In conclusion, actinomycetes were isolated from two ecological habitats and Earthworm gut using a combination of physical and chemical pretreatment methods. Actinomycetes from earthworm gut and paddy soil possessed appreciable antimicrobial activities as compared to others. Isolates showed noticeable antimicrobial activities not only against Gram positive bacteria but also against Gram negative bacteria. In this respect, present data suggest that the number and diversity of actinomycetes in paddy soil and paddy soil earthworm gut is high, thus representing a vast unexplored resource for biotechnology.

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