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Original Research Article

Isolation, characterization and biodiversity of actinomycetes from rhizosphere soil of some medicinal plants

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Abstract

The actinomycetes are strange group of organisms in bacterial taxonomy. Actinomycetes are widespread in all type of soils. The present study focus on biodiversity of actinomycetes from rhizosphere soils of some medicinal plants which are available at local area of Barshi, Dist. Solapur. M.S, India. The rhizosphere soil of medicinal plants were screened for the study of actinomycetes. Rhizosphere soil of medicinal plants viz ; *Aloe barbadense*, *Emblica officinalis*, *Zingiber officinale*, *Tinospora cardifolia*, *Nerium oleander*, *Eucalyptus camaldulensis*, *Mentha arvensis, Santalum album, Hibiscus – rosa- sinensis*, *Ocimum sanctum* and *Curcuma longa* were used for screening of actinomycetes. Serial dilution technique was used for the isolation of actinomycetes using Glycerol asparagine agar as a nutrient medium. Total seventy- one isolates were obtained. These isolates were studied morphologically, culturally and biochemically. The obtained isolates were identified as actinomycetes by MICRO – IS software and also 16srRNA. Among these majority of isolates belonged to *Streptomyces* (70%), *Streptoverticillium* (9%), *Nocardia* (7%), *Micromonospora* (4%), and *Micropolyspora* (10%).

Key Words: Actinomycetes, Medicinal plants, *Streptomyces,* Rhizosphere soil.

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INTRODUCTION

Soils are major support system of human life and welfare. Different kind of soils are spread across different landscapes having complex mixture of mineral matter, organic matter and living organisms. The major groups of microorganisms are bacteria, fungi, actinomycetes, protozoans and soil nematodes. These soil biota show vast variation quantitatively and qualitatively in different sites of collection and at different depths. Actinomycetes from rhizosphere soils are quantitatively and qualitatively important and numerous in plant rhizosphere than away from roots. The rhizosphere is defined as a narrow zone, adjacent to and influenced by living plant root (Kennedy,1999), is a site of high microbial activity in and around roots in soil (Sorenson, 1997). India is one of the richest plant medical cultures in the world and considers "all" plants as potential sources of medicinal substances. Rhizosphere soil of medicinal plants can serve as an important source of pharmaceutically valuable microorganisms. Actinomycetes are prokaryotic organisms and called as "ray bacteria". They are widely distributed in soil next to bacteria in abundance. Actinomycetes are free living, Gram- positive organisms in nature and are known as saprophytic soil inhabitants (Takizawa *et al*., 1993). Actinomycetes are unicellular like bacteria which shows fungus like characteristics with branching filaments in culture or tissue. On culture media actinomycetes colonies grow slowly, show powdery consistency and stick firmly to agar surface. They are also responsible for earthy or musty odor. Actinomycetes plays an extremely useful role in degradation of waste material and as an integrant part of the recycling of materials in nature (Kulkarni and Deshmukh, 2002). The richly diverse flora and fauna of India provided a wealth of medicinal substances. Human have used their local plants for medicinal effects since prehistoric times. In literature, there are few reports on microorganisms from rhizosphere of medicinal plants. For the present study

locally available medicinal plants are selected and rhizosphere soil of these plants have been screened for the study of actinomycetes for example - *Aloe barbadense, Emblica officinalis, Zingiber officinale, Tinospora cardifolia, Nerium oleander, Eucalyptus camaldulensis, Mentha arvensis, Santalum album, Hibiscus – rosa-sinensis*, *Ocimum sanctum* and *Curcuma longa.*

MATERIAL AND METHODS

1. Rhizosphere soils from selected medicinal plants available at local area of Barshi, Dist. Solapur, M.S India.
2. Glycerol asparagine agar.

METHODS

1. Collection of soil samples – soil samples were collected at depth of 10-20 cm from selected medicinal plants.
2. Isolation of actinomycetes – For the present study eleven soil samples of medicinal plants viz; *Aloe barbadense* (Aloevera), *Emblica officinalis* (Avala), *Zingiber officinale* (Ginger), *Tinospora cardifolia* (Gulvel), *Nerium oleander* (Nerium), *Eucalyptus camaldulensis* (Nilgiri), *Mentha arvensis* (Pudina), *Santalum album* (Sandal), *Hibiscus – rosa- sinensis* (Shoeflower), *Ocimum sanctum* (Tulasi) and *Curcuma longa* (Turmeric) from local area of Barshi, Dist. Solapur M.S.India were collected. Collected samples were serially diluted and aliquots of dilution were transferred to glycerol asparagine agar (L-asparagine – 0.1g, K_2HPO_4-0.1, Glycerol -1g, Trace salt solution -0.1 ml, Agar-2.5 g, Distilled water- 100ml, pH -7.4). The plates were incubated at room temperature for 4-7 days.
3. Identification of actinomycetes – Tough colonies were selected and identified as actinomycetes with the help of cultural, morphological and biochemical studies up to genus level by using Bergey's Manual of Systematic Bacteriology volume 4.The morphological characters were observed by coverslip culture technique and slide culture technique for actinomycetes.

Coverslip culture technique – This technique also called as "inclined coverslip technique". For the study of morphological characteristics with reference to aerial mycelium, substrate mycelium and sporulation this technique were used. The isolate were cultivated on Glycerol asparagine agar, sterile coverslip was inserted at angle of about 45^0C into a solidified medium in a Petri dish so that the half the coverslip is in the medium. The inoculum was spread along the line, where the upper surface of the coverslip meets the agar, with a fine wire needle and plates were incubated at ambient temperature for 7 days. After incubation period the isolates were grown on both, the medium and coverslip. The coverslip was carefully withdrawn from the microscope to differentiate between substrate and aerial mycelium. The observation were also recorded about the presence of colour of spore mass (grey, blue, red, violet, yellow or white), spore chain morphology (rectiflexibiles, spirals or retinaculiaperti) and presence of sclerotia. Total isolates were identified as actinomycetes with the help of MICRO-IS Software and also 16srRNA.

RESULTS AND DISCUSSION

Total seventy-one isolates were obtained from eleven rhizosphere soil samples collected from *Aloe barbadense, Emblica officinalis, Zingiber officinale*, *Tinospora cardifolia*, *Nerium oleander*, *Eucalyptus camaldulensis*, *Mentha arvensis, Santalum album, Hibiscus – rosa-sinensis*, *Ocimum sanctum* and *Curcuma longa* etc. All isolates code were labelled according to their vernacular name in short form. Eleven medicinal plants were selected according to their medicinal compounds present for therapeutic purpose.

Table 1: Distribution of actinomycetes isolates in various rhizosphere soil samples of medicinal plants.

Sr. No	Medicinal plants	Number of actinomycetes isolates belonging to genus				
		Str.	Strv.	Noc.	Mm.	Mpol.
1	*Aloe barbadense* (Aloe Vera-AL)	4	1	1	-	1
2	*Emblica officinalis* (Avala-AV)	2	1	1	1	1
3	*Zingiber officinale* (Ginger-GI)	5	1	-	-	-
4	*Tinospora cardifolia* (Gulvel-GL)	7	-	-	-	-
5	*Nerium oleander* (Nerium-NE)	2	1	1	1	1
6	*Eucalyptus camaldulensis*(Nilgiri-NI)	4	1	-	1	1
7	*Mentha arvensis* (Pudina-PD)	5	1	-	-	-
8	*Santalum album* (Sandal-SD)	4	1	1	-	-
9	*Hibiscus–rosa sinensis* (Shoeflower-SH)	5	-	-	1	1
10	*Ocimum sanctum* (Tulasi-TL)	3	1	-	1	1
11	*Curcuma longa* (Turmeric –TR)	6	1	-	-	-
	Total and (%)	**47 (%)**	**09 (%)**	**04(%)**	**05(%)**	**06 (%)**

Str = *Streptomyces*, Strv. = *Streptoverticillium*, Noc. = *Nocardia*, Mm = *Micromonospora*, Mpol= *Micropolyspora.*

Distribution of actinomycetes in various rhizosphere soil samples of some medicinal plants is given to Table 1 and they belongs to genus *Streptomyces*, *Streptoverticillium, Nocardia, Micromonospora* and *Micropolyspora. Streptomyces* is best known genus and found commonly in any type of soil. *Streptomyces* were obtained more from all medicinal plants rhizosphere soil samples. As compare to *Streptomyces*, the percentage of *Streptoverticillium* was quiet less. They were found in all medicinal rhizosphere soil except *Tinospora cardifolia* and *Hibiscus-rosa-sinensis.* The results were showed that, the percentage of *Nocardia* was less than these above mentioned genera. *Nocardia* was obtained from *Aloe barbadence, Emblica officinalis, Nerium oleander* and *Santalum album* in lower extent. *Micromonospora* were obtained from only *Emblica officinalis, Nerium oleander, Eucalyptus camaldulensis, Hibiscus-rosa-sinensis* and *Ocimum sanctum. Micropolyspora* were obtained in less percentage as compared to all genera. These were obtained from only *Aloe barbadense*, *Emblica officinalis, Nerium oleander*, *Hibiscus rosa-sinensis* and *Ocimum sanctum.*

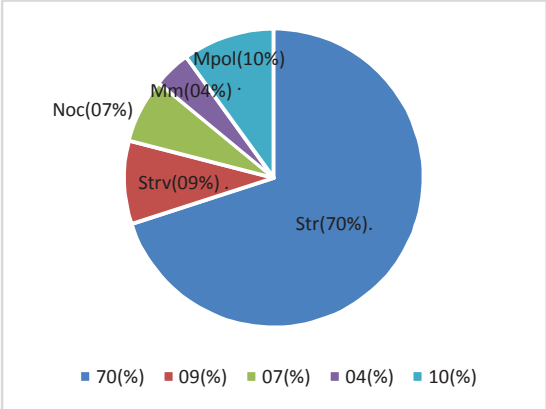

Figure 1: Percentage of actinomycetes isolates from medicinal rhizosphere soil

1st Qtr. =*Streptomyces,* 2nd Qtr. = *Streptoverticillium*, 3rd Qtr. =*Nocardia*, 4th Qtr. = *Micromonospora,* and 5th Qtr. = *Micropolyspora.* Out of seventy-one actinomycetes majority of isolates were identified as *Streptomyces* (70%). On the other hand isolates belonging to *Streptoverticillium* (09%), *Nocardia* (07%), *Micromonospora* (04%) and *Micropolyspora* (10%). Figure 1 explains the generic distribution of actinomycetes in different medicinal plants rhizosphere soil. *Streptomyces* are found in numerous in rhizosphere soil of medicinal plants than away from root and have been poorly studied as biocontrol agents (Thangapandian *et.al*, 2007).

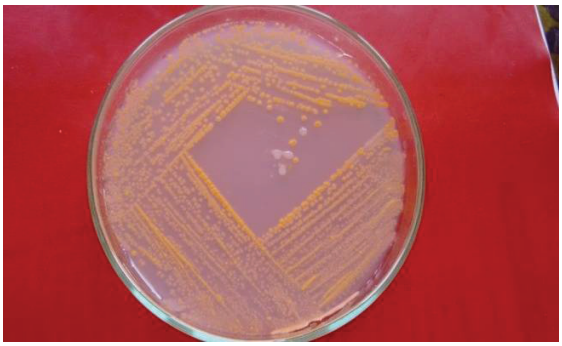

Figure 2: Plate 1 Isolation of actinomycetes on Glycerol asparagine agar

Figure 3: Plate 2 – Coverslip culture technique for actinomycetes

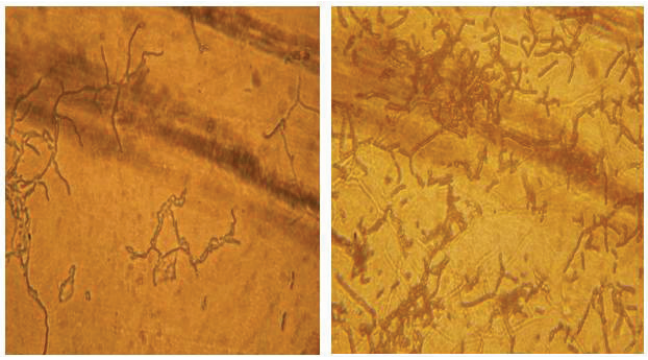

Figure 4: Plate 3 Plate 4

Plate 1 and 2 shows isolation of actinomycetes on glycerol aspargine agar and slide culture technique respectively.Glycerol aspargine agar is best medium for growth of actinomycetes. The microscopic examination of actinomycetes seen in Plate 3 and 4.

Table 2: Identification of actinomycetes isolates by using MICRO-IS PIBWIN Software

Sr. No	Medicinal plants	Isolate code	Score as per MICRO-IS PIBWIN Software	Actinomycetes identified as
1.	*Aloe barbadense*	AL1	0.97823	*Streptomyces chattanoogensis*
2.		AL2	0.94134	*Streptomyces cellulosae*
3.		AL3	**	*Streptomyces enissocaesilis*
4.		AL4	*	*Nocardia sp.*
5.		AL5	0.99473	*Streptoverticillium olivoverticillatum*
6.		AL6	*	*Micropolyspora sp.*
7.		AL7	0.91165	*Streptomyces fulvissimus*
8.	*Emblica officinalis*	AV1	0.99913	*Streptomyces chattanoogensis*
9.		AV2	0.99954	*Streptoverticillium olivoverticillatum*
10.		AV3	*	*Nocardia sp.*
11.		AV4	*	*Micropolyspora sp.*
12.		AV5	0.99867	*Streptomyces xanthochromogenes*
13.		AV6	*	*Micromonospora sp.*
14.	*Zingiber officinale*	GI1	0.93699	*Streptomyces chattanoogensis*
15.		GI2	0.99694	*Streptomyces aureofaciens*
16.		GI3	0.92589	*Streptomyces antibioticus*
17.		GI4	0.99941	*Streptoverticillium olivoverticillatum*
18.		GI5	0.92748	*Streptomyces fulvissimus*
19.		GI6	0.93885	*Streptomyces flaveolus*
20.	*Tinospora cardifolia*	GL1	0.99920	*Streptomyces thermovulgaris*
21.		GL2	0.99945	*Streptomyces roseus*
22.		GL3	0.99867	*Streptomyces xanthochromogenes*
23.		GL4	0.91536	*Streptomyces cellulosae*
24.		GL5	0.91467	*Streptomyces fulvissimus*
25.		GL6	0.99069	*Streptomyces chattanoogensis*
26.		GL7	0.93351	*Streptomyces olivaceoviridis*
27.	*Nerium oleander*	NE1	0.93885	*Streptomyces flaveolus*
28.		NE2	0.90421	*Streptomyces chattanoogensis*
29.		NE3	0.98677	*Streptoverticillium olivoverticillatum*
30.		NE4	*	*Nocardia sp.*
31.		NE5	*	*Micropolyspora sp.*
32.		NE6	*	*Micromonospora sp.*
33.	*Eucalyptus camaldulensis*	NI1	0.98605	*Streptomyces fulvissimus*
34.		NI2	**	*Streptomyces flavoviridis*
35.		NI3	0.98592	*Streptomyces thermovulgaris*
36.		NI4	0.99927	*Streptoverticillium olivoverticillatum*
37.		NI5	0.93189	*Streptomyces chattanoogensis*
38.		NI6	*	*Micromonospora sp.*
39.		NI7	*	*Micropolyspora sp.*
40.	*Mentha arvensis*	PD1	0.99927	*Streptoverticillium olivoverticillatum*
41.		PD2	0.89909	*Streptomyces alboflavus*
42.		PD3	0.98013	*Streptomyces thermovulgaris*
43.		PD4	0.97823	*Streptomyces chattanoogensis*
44.		PD5	*	*Micromonospora sp.*
45.		PD6	0.99843	*Streptomyces aureofaciens*
46.	*Santalum album*	SD1	0.91452	*Streptomyces microflavus*
47.		SD2	0.93914	*Streptomyces thermovulgaris*
48.		SD3	0.96095	*Streptomyces chattanoogensis*
49.		SD4	0.94469	*Streptomyces aureofaciens*
50.		SD5	0.99950	*Streptoverticillium olivoverticillatum*
51.		SD6	*	*Nocardia sp.*
52.	*Hibiscus-rosa-sinensis*	SH1	0.94030	*Streptomyces fulvissimus*
53.		SH2	0.92589	*Streptomyces antibioticus*

54.		SH3	*	*Micropolyspora sp.*
55.		SH4	*	*Micromonospora sp.*
56.		SH5	0.93469	*Streptomyces aureofaciens*
57.		SH6	0.92502	*Streptomyces olivaceoviridis*
58.		SH7	0.97378	*Streptomyces chattanoogensis*
59		TL1	0.99927	*Streptoverticillium olivoverticillatum*
60.		TL2	*	*Micromonospora sp.*
61.		TL3	0.92469	*Streptomyces aureofaciens*
62.	*Ocimum sanctum*	TL4	0.96092	*Streptomyces chattanoogensis*
63.		TL5	0.94959	*Streptomyces cellulosae*
64.		TL6	*	*Micropolyspora sp.*
65.		TR1	0.89469	*Streptomyces aureofaciens*
66.		TR2	0.97524	*Streptomyces chattanoogensis*
67.		TR3	0.91737	*Streptomyces antibioticus*
68.	*Curcuma longa*	TR4	0.99473	*Streptoverticillium olivoverticillatum*
69.		TR5	0.91409	*Streptomyces fulvissimus*
70.		TR6	0.96843	*Streptomyces poonensis*
71.		TR7	0.92469	*Streptomyces aureofaciens*

*= Identification of isolate by using Bergey's Manual of Determinative Bacteriology (Vol-4)
** = Identification of isolate by Phylogenetic analysis (16srRNA Segquencing Report)

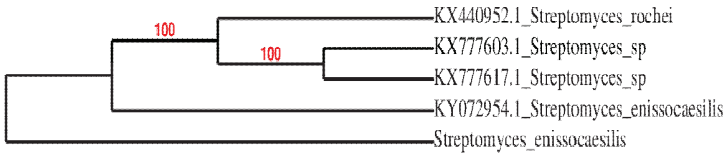

Figure 2: Phylogenetic tree of AL3 isolate by 16srRNA analysis.

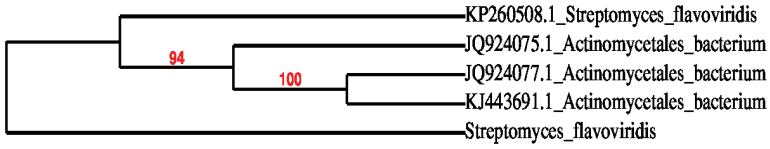

Figure 3: Phylogenetic tree of NI2 isolate by 16srRNA analysis

Isolated actinomycetes were identified by using MICRO-IS Software and Bergey's Manual Of Determinative Bacteriology (vol-4) on the basis of their morphological,cultural and biochemical charactristics.Members of *Streptomyces* genus were identified by using PIB WIN Software. Table 2 represents identification of actinomycetes from medicinal rhizosphere soil. Out of Seventy-one isolates studied fifty-five isolates were identified upto species level while remaining sixteen were identified up to genus level. Actinomycetes are more numerous in plant rhizosphere than away from root. The actinomycetes in rhizosphere soil of medicinal plants have a both higher and lower occurrences depending on the species (McCarthy and Williams, 1990*)*. *Streptomyces* and *Nocardia* are abundant in the rhizosphere of plant stabilizing and dunes (Webley *et al*., 1952). The flora of Indian medicinal plants is rich in biodiversity and product from these plants as medicines though neglected in the recent past are now gaining importance again. Actinomycetes are a valuable resource for drugs. In literature there are very few reports on actinomycetes from rhizosphere of medicinal plant such as *Aloe barbadense* (Aloe vera), *Curcuma longa* (Turmeric*), Eucalyptus camaldulensis* (Nilgiri), *Santalum album* (Sandal), *Mentha arvensis* (Pudina) etc. This study gives clear idea about rhizosphere soil as an important source for the research of a large number of actinomycetes.

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