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Isolation and Screening of Effective Antibiotic Producing Actinomycetes from Rhizosphere Soil of Cipadessa baccifera and Clausena dentata

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Abstract: *Soil contain a vast number of microorganisms and it is the dynamic site of biological activity in nature in which many biochemical reactions occur for destruction of organic matter. Actinomycetes are one of the most important organisms which were widely used for producing secondary metabolites, and the actinomycetes were abundantly present in the rhizosphere soil. Consequently the present work aimed to identify the medicinally important Actinomycetes from the rhizosphere soil of Cipadessa baccifera and clausena dentata. The rhizosphere soil of Cipadessa baccifera and Clausena dentata have pH of 6.9 and 7.1, and different nutrients such as Carbon, Nitrogen, Nitrate, Ammonia, Phosphorous, Calcium, Magnesium and potassium. Actinomycetes from rhizosphere soil of Cipadessa baccifera and Clausena dentata showed $22.41X10^{-5}$ Colony/g of soil and $17.33X10^{-5}$ Colony/g of soil. All strains from rhizosphere soil of Cipadessa baccifera and Clausena dentata showed high percentage of inhibition in Bacillus subtilis. The different strains from the two types of rhizosphere soil efficient strains were selected by the antagonistic activity. The selected strains CBRSA1, CBRSA2, CBRSA4 and CDRSA1, CDRSA3, and CDRSA4 were grown under different temperature, pH, carbon source and different media in which all the strains showed highest growth at temperature of 40°C in 10^{th} day. The CBRSA1 strain showed better growth in D-mannitol as carbon source, CBRSA2 and CDRSA4 strain showed better growth in Cellulose. CDRSA1 strain showed better growth in sucrose. The CBRSA1 strain under showed better growth in yeast extract and CDRSA1 strain showed better growth in Oatmeal. The 16s rRNA sequencing of Actinomycetes strain CBRSA1 and CDRSA1 showed 970bp and 927bp and identified has Nocardiopsis lucentensis and Mycobacterium sp.*

Keywords: *Actinomycetes, Cipadessa baccifera, Clausena dentata, antagonistic activity and Rhizosphere soil.*

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I. Introduction

Soil is the rich source of microorganisms which produce variety of secondary metabolites which are later identified by researchers for pharmaceutical compounds. Microorganisms play an important role in maintain the soil fertility by oxidizing the organic matter and promoting the biogeochemical cycles of carbon (C), nitrogen (N), phosphorus (P), potassium (K), and sulphur (S) (Balloni & Favilli, 1987). The microbial activity in soil is controlled by several environmental factors such as availability of C, mineral nutrients, water availability, temperature growth factors, and pH, etc. However, composition of soil microorganisms and ecological interactions between them determine the nutrient supply and uptake by plants (Nannipieri *et al*., 2003, Barbosa *et al*., 2013).

Among the soil rhizosphere soil occupies a special position for diversity of microbes (Sorenson, 1997). Rhizosphere, a narrow zone, adjacent to the root of living plants and they are influenced by the plant exudates (Kennedy, 1999). These diverse microorganisms in turn influence growth and health of the plant through their involvement in biogeochemical cycle (Campbell and Greaves, 1990; Boehm *et al*., 1993). Further this diversity and composition of bacterial species in the rhizosphere is affected by several environmental factors like plant species (Miller *et al*., 1989), soil type (Hoitink and Boehm, 1999), soil management practices (Rovira *et al*., 1990), microbial interactions, etc.

Clausena dentata is one such medicinal plant, a small tree plant, belonging to the citrus family of Rutaceae and found in Kolli Hills Tamilnadu, India and also in Sri Lanka and China (Agarwal, 1981). The plant was first described by the Dutch botanist Nicolaas Laurens Burman in 1768 (Clausena, 1768). Genus Clausena

represent evergreen trees, which occur in tropical belt Asia (Burkill, 1966) and easily grown by farmers due to their pest and disease free nature and can withstand heavy lopping (Swarbrick, 1997). It is widely known as Anai chedi in Kolli Hills Tamilnadu. This plant is used by indigenous people for its medicinal and nutritive value in Kolli Hills, Yercaud and Boda Hills. Secondary metabolites from this plant was identified for a number of biological properties like anti cancer, antimicrobial, antioxidant, antidiabetic, pesticidal etc. (Arbab *et al*., 2012). The phytochemical studies of the plant revealed the presence of volatile oils furanoid terpenic compounds, α-clausenan, rosefuran (γ-clausenan) and diclausenans A and B (Rao and Subramanian, 1934) identified furanoterpenes and coumarins by Govindachari *et al*., (1968).

Cipadessa baccifera belongs to the family Melieceae, is one another plant found in Kolli Hills Tamilnadu and also in Western Ghats of India, Deccan, and North Circars. *C. baccifera* is used by the indigenous people of Kolli hill as insecticides and fertilizers in their rice field. It has been used treat ailments such as headaches, diabetes, diarrhea, piles and snake poison (Malarvannan *et al*., 2008). Malarvannanl *et al*., (2008) identified ovicidal activity of the crude extract of the plant against *Helicoverpa armigera* larvae. Liang *et al*., (1991) identified phytochemical compounds such as tetranortriterpenoids, glycosides in Cipadessa, flavanoids by Liang *et al*., (1994) and three diterpenoids by Rojatkar *et al*., (1994) and Rojatkar and Nagasampagi, (1994).

Among the microorganisms Actinomycetes are the dominant microbial species which have dominant role in the soil and also have huge quantity secondary of secondary metabolites which are economically important for their medicinal properties. These are present in the rhizosphere in large quantities and in diverse nature. This diversity also varies with plant to plant particularly the medicinal plants which stimulate production of therapeutic compounds in the Actinomycetes effectively. Hence the present research was designed to isolate and identify effective Actinomycetes species from rhizosphere soil of *C. baccifera* and *C. dentata* for antagonistic property.

II. Materials And Methods

Soil Sample Collection

Soil samples were collected about 15 cm below the surface of the soil. All the soil samples were collected randomly from Kolli hills, Namakkal. Soil sample were then packed in a ziplock bag and stored in a container with icepack during transportation.

Soil Analysis Procedure

The pH of the soil was analyzed using Davis and Freitas (1970), method. Organic carbon was determined with Walkey Black method which was described by Singh *et al*., 1999. The nitrogen was estimated by means of Kjeldal method (Black, 1965). Then the nitrate was analyzed with PDA method. Using Singh *et al.,* 1999 technique ammonium and phosphate concentrations in the soil were determined. Finally the calcium and magnesium were deliberate by using Ghosh et al, 1983.

Actinomycetes Isolation and Colony count

Actinomycetes were isolated from the soil sample using starch casein agar medium by the method described by Oskay *et al*., (2004). Then Actinomycetes were picked and streaked onto fresh Starch casein agar plates and incubated at 30^{o}C for 1 week. Colony forming unit (cfu) per one gram of soil was determined for all the samples collected.

Biochemical Characterization of Actinomycetes

Biochemical tests like gram staining, gelatin Liquification, starch hydrolysis, casein hydrolysis, catalase test, nitrate reduction test, indole test and hydrogen sulfide production tests were performed for the strains isolated from *C. baccifera* and *C. dentata*.

Antibacterial Activity

Isolated actinomycetes strains were grown in actinomycetes broth (BD BBLTM, USA) supplemented with cycloheximide (0.05 g/l), nalidixic acid (0.02 g/l) and nystatin (0.05 g/l). These cultures were grown in a rotary shaker at 200 rpm for seven days. The resulting culture broths (approximately 50 ml), obtained following growth of each isolate in the culture media, separated from the cell by centrifugation at 10,000 g for 15 min. The cell free supernatant was collected and freeze dried. Freeze dried materials were re suspended into appropriate concentration for antimicrobial activity by the antimicrobial activities of those extracts were tested against different test organisms by using agar disc diffusion method as described by Kirby-Bauer with modification (Boyle *et al.,* 1973) against test microorganisms. The test organisms were *Vibrio cholera, Salmonella typhi, Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Staphylococcus pneumoniae, Pseudomonas aeruginosa, Proteus mirabilis Klebseilla pneumoniae, Candida albicans, Aspergillus fumigates, Fusarium*

graminearum respectively. The diameter of the zone of inhibition was then recorded on day three. A clear zone suggests that the sample has properties that can inhibit the growth of the test organism. No clear zone indicates complete resistance.

Optimization of pH and temperature

Modified Nutrient broth was prepared at different pH range respectively 5, 6, 7, 8, 9 and temperature at 20°C, 25°C, 30°C, 35°C, 40°C to detect the tolerance level of the selected strains. The flasks were incubated in a shaker for 24 hrs at 28 ± 20 °C and the OD value for each pH and temperature flask was noted (Rohban *et al*., 2009). The OD values are taken at 2nd day, 4th day, 6th day, 8th day and 10th day respectively for both pH and temperature.

Optimization of Carbon Source

The ability of the strain to utilize various carbon compounds as a source of energy was studied as described in the ISP. Various carbon sources, D-Glucose, D-fructose, D-mannitol, D-cellulose and D-sucrose were tested for the growth of the isolate on Carbon utilization medium supplemented with 1% carbon sources (Nonomura *et al*., 1974). The OD values are taken at 2nd day, 4th day, 6th day, 8th day and 10th day respectively.

Media Optimization

Five different liquid media: Sucrose nitrate broth, Tyrosine broth, Yeast extract broth, Malt extract broth, Oatmeal broth was used in this study. The effect of various liquid media was studied by submerged culture flasks method using 250 ml Erlenmeyer flask. The selected strains were inoculated into various flasks containing different media of 100 ml each. The flasks were kept at 28ºC for 8 days at 200 rpm on a rotary shaker. Total growth of different strains in the broth was quantified by double beam spectrophotometer at 500 nm absorbance (Kamijo *et al*., 1999). The OD values are taken at 2nd day, 4th day, 6th day, 8th day and 10th day respectively.

16sRNA Sequencing

The DNA of the sample was isolated using cTAB method. The 16S rRNA of isolate was amplified using the universal primers 8F (5′- AGAGTTTGATCCTGGCTCAG) and 1541R (50-AAGGAGGTGATCCAGCCGCA-3′). The amplicon was run on the agarose gel and they were eluted and sequenced. The sequence similarity was analysed by using BLAST. The sequence was submitted in the NCBI Gen Bank.

III. Results

Physiochemical Characteristics of the rhizosphere soil of *Cipadessa baccifera* and *Clausena dentata*

The Physiochemical characteristics of the rhizosphere soil of *Cipadessa baccifera* and *Clausena dentata* have pH of 6.9 and 7.1, percentage of carbon of 2.7 and 3.5, percentage of Nitrogen content of 0.533 and 0.583, nitrate content of 1.777 and 2.037 (mg kg^{-1}), Ammonia content of 3.577 and 3.983 (mg kg^{-1}), Phosphorous content of 15.947 and 17.450(mg kg^{-1}), Calcium content of 0.831 and 0.882 (cmol+ kg^{-1}), Magnesium content of 0.414 and 0.391 (cmol+ kg^{-1}), and potassium content of 0.271 and 0.357 (cmol+ kg^{-1}) (Table 1).

Isolation of Actinomycetes

Actinomycetes from rhizosphere soil of *Cipadessa baccifera* and *Clausena dentata* showed $22.41X10^{-5}$ Colony/g of soil and $17.33X10^{-5}$ Colony/g of soil.

Biochemical characterization of the isolated strain

Randomly selected 6 distinct colonies from each rhizosphere soil all showed gram positive. CBRSA1, showed positive for Gelatin, Starch hydrolysis, Casein hydrolysis, Catalase and negative for Nitrate, indole and H_2S production. CBRSA2, CBRSA6, CDRSA5, CDRSA6 showed positive for Gelatin, Starch hydrolysis, Casein hydrolysis, Catalase, Nitrate and negative for indole and H_2S production. CBRSA3, CBRSA4 and CDRSA2 showed positive for Gelatin, Catalase, indole and negative for Starch hydrolysis, Casein hydrolysis, Nitrate and H_2S production. CDRSA1 showed positive for Catalase, Nitrate, Starch hydrolysis, and negative for Gelatin, Casein hydrolysis, H_2S production and indole. CBRSA5 showed positive for Gelatin, Catalase, indole and negative for Starch hydrolysis, Casein hydrolysis, H_2S production and Nitrate. CDRSA4 showed positive for Gelatin, Catalase, and Nitrate and negative for Starch hydrolysis, Casein hydrolysis, H_2S production and indole (Table 2).

Antagonistic activity of the isolated strain

Antagonistic activity of strain CBRSA1 isolated from rhizosphere soil of *Cipadessa baccifera* showed high percentage inhibition in *Bacillus subtilis* (11.56 mm), followed by *Vibrio cholera, Fusarium graminearum* and low percentage in *Pseudomonas aeruginosa*. Antagonistic activity of strain CBRSA2 isolated from rhizosphere soil of *Cipadessa baccifera* showed high percentage inhibition in *Bacillus subtilis* (22.38 mm), followed by *Klebsiella pneumonia*, and low percentage in *Salmonella typhi*. Antagonistic activity of strain CBRSA3 and CBRSA4 isolated from rhizosphere soil of *Cipadessa baccifera* showed high percentage inhibition in *Bacillus subtilis* (19.50 mm and 18.36 mm), followed by *Klebisella pneumonia* and low percentage in *Salmonella typhi* and *Aspergillus.* Antagonistic activity of strain CBRSA5, CBRSA6 isolated from rhizosphere soil of *Cipadessa baccifera* showed high percentage inhibition in *Bacillus subtilis* (18.10 mm), followed by *Candida albicans* and low percentage in *Pseudomonas aeruginosa* .

Antagonistic activity of strain CDRSA1 isolated from rhizosphere soil of *Clausena dentata* showed high percentage inhibition in *Bacillus subtilis* (16.40 mm), followed by *Salmonella typhi* and low percentage in *Staphylococus aureus.* Antagonistic activity of strain CDRSA2 isolated from rhizosphere soil of *Clausena dentata* showed high percentage inhibition in *Bacillus subtilis* (18.28 mm), followed by *Klebsiella pneumoniae* and low percentage in *Salmonella typhi.* Antagonistic activity of strain CDRSA3 isolated from rhizosphere soil of *Clausena dentata* showed high percentage inhibition in *Bacillus subtilis* (14.33 mm), followed by *E. coli* and low percentage in *Proteus mirabilis.* Antagonistic activity of strain CDRSA4 isolated from rhizosphere soil of *Clausena dentata* showed high percentage inhibition in *Bacillus subtilis* (17.29 mm), followed by *Aspergillus, Fusarium graminearum* and low percentage in *Vibrio cholera.*Antagonistic activity of strain CDRSA5 isolated from rhizosphere soil of *Clausena dentata* showed high percentage inhibition in *Bacillus subtilis* (14 mm), followed by *E. coli* and low percentage in *Salmonella typhi.* Antagonistic activity of strain CDRSA6 isolated from rhizosphere soil of *Clausena dentata* showed high percentage inhibition in *Bacillus subtilis* (13.53 mm), followed by *E. coli* and low percentage in *Salmonella typhi.* All strains from rhizosphere soil of *Cipadessa baccifera* and *Clausena dentata* showed high percentage of inhibition in *Bacillus subtilis* (Figure 1).

Effect of temperature on the isolated strain

The strains which showed better inhibition percentage were studied. The growth of Actinomycetes strains CBRSA1, CBRSA2, CBRSA4 and CDRSA1, CDRSA3, and CDRSA4 under different temperature highest growth was observed in 40°C in 10^{th} day.

Effect of pH on the isolated strain

The growth of Actinomycetes species CBRSA1, CBRSA2 and CBRSA4 strain under different pH better growth was observed at pH 7 in 10^{th} day. The growth of CDRSA1, and CDRSA3 strains under different pH showed better growth in 10^{th} day at pH 8, and CDRSA4 strain showed better growth in 10^{th} day at pH 8 and 9.

Effect of carbon source on the isolated strain

The growth of Actinomycetes species CBRSA1 strain under different carbon source showed better growth in D-mannitol at 10^{th} day followed by Cellulose, D-fructose, sucrose and D-glucose. CBRSA2 strain under different carbon source showed better growth in Cellulose at 10^{th} day followed by D-mannitol, sucrose, D-fructose, and D-glucose. The growth of CDRSA1 strain under different carbon source showed better growth in sucrose at 10^{th} day followed by Cellulose, D-mannitol, D-fructose, and D-glucose and CDRSA4 strain under different carbon source showed better growth in Cellulose at 10^{th} day followed by sucrose, D-mannitol, D-fructose, and D-glucose (Figure 2)

Effect of different culture media on the isolated strain

The growth of Actinomycetes species CBRSA1 strain under different culture media showed better growth in yeast extract, at 10^{th} day followed by Malt extract, tyrosine and Sucrose-nitrate and in Oatmeal the growth was better in 8^{th} day. The growth of CDRSA1 strain under different culture media showed better growth in Oatmeal at 10^{th} day followed by Malt extract, yeast extract, tyrosine, and Sucrose-nitrate(Figure 3).

16s rRNA sequencing

The 16s rRNA sequencing of actinomycetes CBRSA1 and CDRSA1 showed 970bp and 927bp. Sequence alignment for similarity of other ten best aligned species CBRSA1 showed maximum score and total score both ranged from 1648 to 1711 without any error value with maximum query cover of 100% with *Nocardiopsis lucentensis* same was also reflected in the phylogenetic tree. Sequence alignment for similarity of other ten best aligned bacterial species CDRSA1 showed maximum score and total score both ranged from 1592

to 1631 without any error value with maximum query cover of 100%with *Mycobacterium sp.* same was also reflected in the phylogenetic tree.

IV. Discussion

The rhizosphere soil microflora includes bacteria, fungi, nematodes, protozoa, algae and microarthrops (Raaijmakers *et al*., 2001) vary from thousands to millions. The *Proteobacteria* and the *Actinobacteria* form the most common of the dominant populations in the rhizosphere soil.(Singh *et al*., 2009). The bacterial population in the rhizosphere soils of the medicinal plants were also vary according to the seasons and type of plant species. The impact of microorganism according to the season and plant species were reported by Mahasneh *et al.,* (1984), Smalla *et al*., (2001), Koeberl *et al*., (2013), Rigobelo and Nahas, (2004). However, bacterial colony count in soil ranges from $4X10^6$ to $2x10^9$ per gram of soil. In Agricultural and garden soils bacteria range from 196 $X10^3$ to 304 $X10^4$, fungi range from 14 $X10^3$ to 3 $X10^4$ and Actinomycetes range from 48 $X10^3$ to 20 $X10^4$ (Williams and Cross, 1971). Conversely, present study was able to show 22.41 $X10^4$ colonies in *C. baccifera* and 17.33 $X10^4$ colonies in *C. dentata* rhizosphere soil which shows its richness.

Randomly selected 6 distinct colonies from each rhizosphere soil all showed gram positive. The identified genus of the strains are *Nocardiopsis, Actinomycetes, Arthrobacter, Streptomyces* and *Mycobacterium*. A study by Tamilarasi *et al*., 2008 enumerated rhizosphere bacteria from the rhizosphere soil of medicinal plants showed maximum of 280 x 104 CFU/g in Acacia nilotica and minimum of 18 x 104 CFU/g in *Cassia auriculata*. Duine *et al*. (2005) who found the maximum rhizosphere population of 8.92 x 108 CFU /g and minimum rhizosphere count was 2.35 x 108 CFU/g of *Carex arenaria*. Wamberg *et al*. (2003) reported that the bacterial count in the rhizosphere was 7.45 x 107 CFU/g in *Pisum sativum*. Khanikar *et al*., 2015 studied the fungal population in the rhizosphere of five medicinal plants varied from 17x103 to 33x103 CFU/g dry soil. Basil *et al.,* (2004) reported occurrence plant specific species of Actinomycetes in the rhizosphere of big sagebrush (*Artemisia tridentata*). Gonzalez-Franco *et al.,* (2009) studied on temporal variation of Actinomycetes communities in rhizosphere soil of the desert plant *Artemisia tridentate* (sagebrush).

Khanikar *et al*., 2015 studied the rhizospheric soil of five medicinal plants such as *A. racemosus, C. colebrookianum, V.* negundo, *C. teeta* and *T. sinensis* studied pH of the soil from 3.2 to 6.5, organic carbon 6.3%, nitrogen, phosphorus ranges from 0.07 to 0.29 % among the different rhizosphere.

The medicinal plant rhizosphere soil rich in microorganism may vary due to influence of root exudates released by the plant roots (Broeckling *et al*., 2008). Collado *et al*., (1999) and Gao *et al*., (2005) also reported seasonal influence of plant species on distribution of the rhizospheric microorganisms. There are reports on higher bacterial population followed by fungus and Actinomycetes recorded from the rhizosphere of different medicinal plants (Tamilarasi *et al*., 2008; Karthikeyan *et al*., 2008).

Laishram Shantikumar identified that Glucose Soyabean meal broth was found to be the optimum medium for antimicrobial agent production by *S. sannanensis*. Thus the *S. sannanensis* positively affected by the nature and type of carbon and nitrogen sources in the medium and the antimicrobial agent production was also high when the glucose and soyabean meal added as carbon and nitrogen source respectively. Cruz *et al*., 1999 also found that *S. griseocarneus*, are able to produce antibiotic by using suitable carbon source. Calvo *et al.,* 2002 also consider that carbon source, simple sugar such as glucose, fructose, sucrose as sole carbon source enhanced the growth of the organism as well as bioactive metabolite production. Singh *et al*., 2009 also confirmed that soya bean meal to be a suitable carbon source for antibiotic production by *S. tanashiensis* A2D. From our result CBRSA1 strain showed better growth in D-mannitol as carbon source, CBRSA2 and CDRSA4 strain showed better growth in Cellulose. CDRSA1 strain showed better growth in sucrose.

The natures of the carbon and nitrogen sources strongly influence antibiotic production in different organisms (Vilches *et al.,* 1990). Singh *et al* (2014) identified that *S. sannanensis* SU118 demonstrate narrow range of incubation temperature for better growth and antimicrobial agent production and reported that highest growth and antimicrobial agent production at 28°C. The temperature range adequate for good production of secondary metabolites is narrow (Iwai and Omura, 1982). From our result selected strains were grown under different temperature, pH, carbon source and different media in which all the strains showed highest growth at temperature of 40°C in 10^{th} day. CBRSA1, CBRSA2 and CBRSA4 strain showed better growth at pH 7 in 10^{th} day. CDRSA1, CDRSA3 and CDRSA4 strains showed better growth at pH 8 in 10^{th} day. The maximum growth and highest antimicrobial activity was also demonstrated with *S. sannanensis* at pH 7 (Singh *et al.,* 2014), similarly Guimaraes *et al.,* (2004) reported the influence of pH on production of bioactive metabolite by microorganisms.

From the present study *Nocardiopsis lucentensis* isolated from rhizosphere soil of *Cipadessa baccifera* showed effective growth with pH 7, 40°C, D-mannitol as carbon source and yeast extract has effective media. *Mycobacterium sp* isolated from rhizosphere soil of *Clausena dentata* showed effective growth with pH 8, 40°C, D-mannitol as carbon source and Oatmeal has effective media.

Thangapandian *et al.* (2007) isolated *Streptomyces* from medicinal plant rhizosphere soils with antipathogenic activity. Crawford *et al.,* (1993) isolated 12 Actinomycetes strains isolated from *Taraxicum officinale* rhizosphere were active against *Pythium ultimum.* Sutthinan Khamna *et al.* (2009) isolated *Actinomycetes* from rhizosphere of medicinal plants and it has the antagonistic activity. Kanini *et al.* (2013) isolated and identified potential antifungal *Streptomycetes* sp. from rhizosphere and non rhizosphere soil and carried out in vivo experiments on beans. Srividya *et al.* (2012) evaluated *Streptomyces sp*. as effective biocontrol against chilli soil borne fungal phytopathogens.

The Actinomycetes are capable producing primary and secondary metabolites with economically important. The metabolic process of Actinomycetes provides interesting area of research in secondary metabolites production. It is also capable of producing industrially important enzymes such as amylase, lipase, cellulose, etc. Certain enzymes produced by Actinomycetes helps as therapeutic agents in human cancer, mostly in acute lymphoblastic leukemia. Some of the antibiotics produced by them are novobiocin, amphotericin, vancomycin, neomycin, gentamycin, chloramphenicol, tetracycline, erythromycin, nystatin, etc. It also produces plant growth promoting agents, acts as biocontrol tools, biopesticides and antifungal compounds.

V. Conclusion

The present study reveals that two different effective actinomycetes were isolated from the rhizosphere soil *Cipadessa baccifera* and *Clausena dentata* namely *Nocardiopsis lucentensis* and *Mycobacterium sp. Nocardiopsis lucentensis* isolated from rhizosphere soil of *Cipadessa baccifera* showed effective growth with pH 7, 40°C, D-mannitol as carbon source and yeast extract has effective media. *Mycobacterium sp* isolated from rhizosphere soil of *Clausena dentata* showed effective growth with pH 8, 40°C, D-mannitol as carbon source and Oatmeal has effective media.

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References

[1]. Agarwal, S. R. 1981. Trees, flowers and fruits in Indian folk-songs, folk-proverbs and folk-tales. In: Jain SK editors. Glimpses of Indian Ethnobotany. Oxford and IBH Publishing Co., New Delhi, pp. 3-12.
[2]. Arbab Ismail Adam, Ahmad Bustamam Abdul, Mohamed Aspollah, 2012. A review of traditional uses, phytochemical and pharmacological aspects of selected members of Clausena genus Rutaceae Journal of Medicinal Plants Research, 6: 5107-5118.
[3]. Balloni, W., & Favilli, F. 1987. Effects of agricultural practices on physical, chemical and biological properties of soils: part 1 - effect of some agricultural practices on the biological soil fertility. In: H. Barth & P. L'Hermite (Eds). Scientific Basis for Soil Protection in the European Community. (pp. 161–175).
[4]. Barbosa, E. R. M., Tomlinson, K. W., Carvalheiro, L. G., Kirkman, K, de Bie S, Prins H. H. T., et al. 2013 Short-Term Effect of Nutrient Availability and Rainfall Distribution on Biomass Production and Leaf Nutrient Content of Savanna Tree Species. PLoS ONE 93: e92619
[5]. Basil, A.J., Strap, J.L., Knotek-Smith, H.M. andCrawford, D.L. 2004. Studies on the microbial populations of the rhizosphere of big sagebrush Artemisia tridentata. J Ind. Microbiol. Biotechnol, 316:278-88.
[6]. Black, C. A. 1965. Methods of Soil Analysis, Part-2, American Society of Agronomy, INC. Soil Society of America, Madison, Wisconsin, USA.
[7]. Boehm, M., Madden V. and Hoitink, H. A J. 1993. Effect of organic matter decomposition level on bacterial species diversity and composition in relation to Pythium damping off severity. Appl. Environ. Microbiol., 59, 4171- 4179.
[8]. Boyle, V. J., Fancher, M. E. and Ross, R.W., 1973. Jr. Rapid, modified Kirby-Bauer susceptibility test with single, high-concentration antimicrobial disks. *Antimicrob. Agents Chemother., 3*, 418–424.
[9]. Broeckling, C. D., Broz, A.K., Bergelson, J., Manter, D. K. and Vivanco, J. M., 2008. Root Exudates Regulate Soil Fungal Community Composition and Diversity. Applied and Environmental Microbiology, 743: 738–744.
[10]. Burkill, I.H. 1966. A dictionary of the economic products of the Mala Peninsula (Vol.1 and 2). Min. Agric. & Coop. Govt. of Malaysia and Singapore.
[11]. Calvo, A. M, Wilson, R. A., Bok, J. W., Keller, N. P 2002. Relationship between secondary metabolism and fungal development. Microbiol Mol Biol Rev, 66:447–459.
[12]. Campbell, R. and Greaves: M. P. 1990. Anatomy and community structure of the rhizosphere. In: The rhizosphere (Ed.: J.M. Lynch). Wiley, New York. pp. 11-34.
[13]. Clausena, N. L., Burman, Fl. And Indica, F. l. 1768. China 11: 83–85
[14]. Collado, J., Platas, G., Gonzalez, I., Pelaez, F. 1999. Geographical and seasonal influences on the distribution of fungal endophytes in Quercus ilex. New Phytologist 144: 525–532
[15]. Crawford, D.L., *et al.* 1993. Isolation and characterization of *Actinomycetes* antagonists of a fungal root pathogen. *Appl. Environ. Microbiol.*, 5911.
[16]. Cruz, R., Arias, M. E., Soliveri, J. 1999. Nutritional requirements for the production of pyrazoloisoquinolinone antibiotics by Streptomyces griseocarneus NCIMB 40447. Appl Microbiol Biotechnol, 53:115–119.
[17]. Davis, J. and Freitas, F. 1970. In Physical & Chemical Methods of Soil & Water Analysis, FAO of United Nations, Rome, Soil Bulletin. 10, III-1 : 65-67.
[18]. Duine, A.S.R., G.A. Kowalchuk, P.J.A.K. Gunnewick, W. Smant, J.A. Vanveen and W. Boer, 2005. Rhizosphere bacterial community composition in natural stands of Carex arenaria sand sedge is determined by bulk soil community composition. Soil Biol. Biochem., 37, 349-357

[19]. Gao, X.X., Hui, Z., Xu, D.Y., Chen, Y.Q., Qu, L.H. 2005. High diversity of endophytic fungi from pharmaceutical plant Heterosmilax japonica Kunth revealed by cultivation independent approach. FEMS Microbiology Letters, 249, 255–266.

[20]. Ghosh, A. B., Bajaj, J.C., Finanul Hasan and Dhyan Singh. 1983. Soil and water testing methods. Laboratory Manual. P. 21-22 (ICAR Publication).

[21]. Gonzalez-Franco, A.C., Robles-Hernandez, L., Nunez-Barrios, A., Strap J.L. and Crawford, D.L., 2009. Molecular and cultural analysis of seasonal actinomycetes in soils from Artemisia tridentate habitat, international Journal of Experimental Botany, 78: 83-90.

[22]. Govindachari, T. R., Pai, B. R., Subramaniam, P. S. and Muthukumaraswamy, N. 1968. Coumarins of Clausena dentata (Willd.) R. and S. Tetrahedron, 24: 753-757.

[23]. Guimaraes, L. M., Furlan, R. L., Garrido, L. M., Ventura, A., Padilla, G., Facciotti, M. C. 2004. Effect of pH on the production of the antitumor antibiotic retamycin by Streptomyces olindensis. Biotechnol Appl Biochem, 401:107–111.

[24]. Hoitink, H. A. J. and M. J. Boehm. 1999. Biocontrol within the context of soil microbial communities: A substrate dependent phenomenon. Annu. Rev. Phytopathol., 37, 427-446.

[25]. Iwai, Y., Omura, S. 1982. Cultural conditions for screening of new antibiotics. J Antibiot 1982, 35:123–141.

[26]. Kamijo, M., Suzuki, T., Kawai, K., Fujii, T. and Murase, H. 1999. Ytterbium-decreasing *Streptomyces sp* and its naphthoquinone pigment production in the presence of rare earth elements. J. Biosci Bioeng. 87, 340–343.

[27]. Kanini, G.S., Katsifas, E.A., Savvides, A.L., Hatzinikolaou, D.G., Karagouni, A.D. 2013. Greek indigenous streptomycetes as biocontrol agents against the soil-borne fungal plant pathogen *Rhizoctonia solani. J. Appl. Microbiol.,*

[28]. Karthikeyan, B., Jaleel, C. A., Lakshmanan, G. M. A. and Deiveekasundaram, M., 2008. Studies on rhizosphere microbial diversity of some commercially important medicinal plants. Colloids and Surfaces B: Bioinerfaces, 62:143-145.

[29]. Kennedy, A.C. 1999. Bacterial diversity in agro ecosystems. Agricultural Ecosystems and Environment 74: 65-76.

[30]. Khanikar, C, Sorokhaibam Sureshkumar Singh, 2015. Seasonal and Spatial Variation of Microbial Population In the Rhizosphere Soils of Five Traditionally Important Medicinal Plant Species in NE India. International Journal Of Scientific Research. 46, 366-369.

[31]. Koeberl, M, Schmidt, R, Ramadan, E. M., Bauer, R., Berg, G. 2013. The microbiome of medicinal plants: diversity and importance for plant growth, quality, and health. Frontiesr in Microbiology, 4:400

[32]. Liang, L., Zhong, C. C. and Xiao, Z. Y. 1991. Chemical components from leaves of greyhair Cipadessa (Cipadessa cinerascens). Zhongcaoyao, 22, 6 – 8.

[33]. Liang, L., Zhong, C. C. and Xiao, Z. Y., 1994. Chemical constituents of leaves of greyhair Cipadessa (Cipadessa cinerascens) (III). Zhongcaoyao 25, 236 – 237.

[34]. Mahasneh, A., Budour, S., and Doddema, H., 1984. Nitrification and seasonal changes in bacterial populations in the rhizosphere of Suaeda and Arthrocnemum species growing in saline soils. Plant and soil, 82:149- 154.

[35]. Malarvannan, S., Senthil Kumar, S., Prabhavathy, V. R. and Sudha Nair. 2008. Individual and Synergistic Effects of Leaf Powder of a Few Medicinal Plants against American Bollworm, Helicoverpa armigera (Hubner) (Noctuidae: Lepidoptera). Asian Journal of Experimental Sciences. 22(1), 79-88.

[36]. Miller, H.J., G. Henken and J.A. VanVeen. 1989. Variation and composition of bacterial populations in the rhizosphere of maize, wheat and grass cultivars. Can. J. Microbiol., 35, 656-660.

[37]. Nannipieri, P., Ascher, J., Ceccherini, M.T., Landi, L., Pietramellara, G., & Renella, G. (2003). Microbial diversity and soil functions. European Journal of Soil Science, 54: 655– 670.

[38]. Nonomura, H. 1974. Key for classification and identification of 458 species of the *Streptomycetes* included in ISP. J. Ferment. Technol. 52(2): 78-92.

[39]. Oskay, M., Tamer, A. U. and Azeri, C. 2004. Antibacterial Activity of Some Actinomycetes Isolated from Farming Soils of Turkey. African Journal of Biotechnology. 3, 441-446.

[40]. Raaijmakers J. M., Weller, D. M., 2001. Exploiting genotypic diversity of 2,4-diacetylphloroglucinol-producing *Pseudomonas* spp.: characterization of superior root-colonizing *P. fluorescens* strain Q8r1-96. *Appl. Environ. Microbiol*., **67**, 2545-2554.

[41]. Rao, B. S. S. and Subramanian, K. S. 1934. The Occurrence of Furan derivatives in volatile oils. Proc. Indian Acad Sci., 1: 189-200.

[42]. Rigobelo, E. C. and Nahas, E., 2004. Seasonal fluctuations of bacterial population and microbial activity in soils cultivated with eucalyptus and pinus. Scientia Agricola, 611.

[43]. Rohban, R., Mohammad, A. A. and Ventos, A. 2009. Screening and isolation of halophilic bacteria producing extracellular hydrolyses from Howz Soltan Lake, Iran. Journal of Industrial Microbiology and Biotechnology. 36, 333–340.

[44]. Rojatkar, S. R. and Nagasampagi, B. A. 1994. Diterpenes from Cipadessa fruticosa. Phytochemistry 37, pp 505 – 507

[45]. Rojatkar, S. R., Chiplunkar, Y. G. and Nagasampagi, B. A. 1994. A diterpene from Cipadessa fruticosa and Grangea maderaspatana. Phytochemistry, 37, pp 1213 – 1214.

[46]. Rovira., L.F. Elliot and Cook, R. J. 1990. The impact of cropping systems on rhizosphere organisms affecting plant health. In: The rhizosphere (Ed.: J. M. Lynch). Wiley, New York. pp. 389-436.

[47]. Singh Dhyan, Chhonkar, P. K. and Pande, R. N. 1999. Soil Organic Carbon in "Soil, plant, water Analysis" A methods manual. Indian Agricultural Research Institute, Indian Council of Agricultural Research, New Delhi. 1.4.6: 19-19.

[48]. Singh, L. S, Mazumder, S., Bora, T. C. 2009. Optimisation of process parameters for growth and bioactive metabolite produced by a salt-tolerant and alkaliphilic, Streptomyces tanashiensis strain A2D. J Myc Med, 19:225–233

[49]. Smalla, K., Wieland, G., Buchner, A., Zock, A., Parzy, J., Kaiser, S., Roskot, N., Heuer, H. and Berg, G., 2001. Bulk and Rhizosphere Soil Bacterial Communities Studied by Denaturing Gradient Gel Electrophoresis: Plant-Dependent Enrichment and Seasonal Shifts Revealed. Applied and Environmental Microbiology, 6710: 4742- 4751.

[50]. Sorensen, J. 1997. The rhizosphere as a habitat for soil microorganisms.In: Modern soil microbiology Eds.: J.D Van Elas., J.T. Trevors and E.M.H. Wellington. Marcel Dekker, New York. pp. 21-45

[51]. Srividya, S., Adarshana Thapa, Deepika V. Bhat, Kajingailu Golmei, Nilanjan Dey, 2012. Streptomyces sp. 9p as effective biocontrol against chilli soilborne fungal phytopathogens. *Eur.J. Exp. Biol.,* 21: 163 173.

[52]. Sutthinan Khamna, Akira Yokota, John F. Peberdy, Saisamorn Lumyong, 2009. Antifungal activity of *Streptomyces sp*p. isolated from rhizosphere of Thai medicinal plants. *Int. J. Integrative Biol.,* 63: 143.

[53]. Swarbrick, J. T. 1997. Environmental weeds and exotic plants on Christmas Island. Indian Ocean: A report to Parks Australia.

[54]. Tamilarasi, S., Nanthakumar, K., Karthikeyan, K. and Lakshmanaperumalsamy, P. 2008. Diversity of root associated microorganisms of selected medicinal plants and influence of rhizomicroorganisms on the antimicrobial property of Coriandrumsativum. Journal of Environmental Biology, 291: 127-134.

[55]. Thangapandian, V., Ponmuragan, P., Ponmuragan, K. 2007. *Actinomycetes* diversity in the rhizosphere soil of different medicinal plants in Kolli Hills, Tamilnadu, India, for secondary metabolite production. *Asian J. Plant Sci.,* 6: 66 70.

[56]. Vilches, C., Mendez, C., Hardisson, C., Salas, J. A. 1990. Biosynthesis of oleandomycin by Streptomyces antibioticus: influence of nutritional conditions and development of resistance. J Gen Microbiol, 136:1447–1454.
[57]. Wamberg, C., S. Christensen, I. Jakobsen, A.K. Muller and S.J. Sorensen, 2003. The mycorrhizal fungus Glomus intraradices affects microbial activity in the rhizosphere of pea plants Pisum sativum. Soil Biol. Biochem., 35, 1349-1357.
[58]. Williams, S.T and Cross, T. 1971. Actinomycetes. *In:* Methods in Microbiology J.R. Norris and D.W. Ribbons, Eds. Academic Press, New York,London. 4, 295-334.

VI. Tables And Figures

Table 1. Physico-chemical characteristics of rhizosphere soil of medicinal plants in Kolli Hills

Parameters	*Cipadessa baccifera*	*Clausena dentata*
pH	7.107 ± 0.093	6.923 ± 0.031
% C	2.710 ± 0.160	3.577 ± 0.172
% N	0.533 ± 0.025	0.583 ± 0.025
NO_3- (mg kg^{-1})	1.777 ± 0.045	2.037 ± 0.087
NH_4+ (mg kg^{-1})	3.577 ± 0.166	3.933 ± 0.051
PO_4^{3-} (mg kg^{-1})	15.947 ± 0.388	17.450 ± 0.130
Ca^{2+} (cmol+ kg^{-1})	0.831 ± 0.046	0.882 ± 0.020
Mg^{2+} (cmol+ kg^{-1})	0.414 ± 0.024	0.391 ± 0.006
K^+ (cmol+ kg^{-1})	0.271 ± 0.014	0.357 ±0.019

Table 2. Morphological and biochemical characterization of Actinomycetes strains

S. No.	Actinomycetes strain	Gram reaction	Gelatin liquification	Starch hydrolysis	Casein hydrolysis	Catalase test	Nitrate reduction	Indole production	H2S production	Strains
1	CBRSA1	+	+	+	+	+	-	-	-	*Nocardiopsis*
2	CBRSA2	+	+	+	+	+	+	-	-	*Actinomycetes*
3	CBRSA3	+	+	-	-	+	-	+	-	*Arthrobacter*
4	CBRSA4	+	+	-	-	+	-	+	-	*Arthrobacter*
5	CBRSA5	+	+	+	+	+	+	-	+	*Streptomyces*
6	CBRSA6	+	+	+	+	+	+	-	-	*Actinomycetes*
7	CDRSA1	+	-	+	-	+	+	-	-	*Mycobacterium*
8	CDRSA2	+	+	-	-	+	-	+	-	*Arthrobacter*
9	CDRSA3	+	+	+	+	+	+	-	-	*Actinobacterium*
10	CDRSA4	+	+	-	-	+	+	-	-	*Streptomyces*
11	CDRSA5	+	+	+	+	+	+	-	-	*Actinomycetes*
12	CDRSA6	+	+	+	+	+	+	-	-	*Actinomycetes*

Figure 1. Comparative analysis of antagonistic activity of Actinomycetes isolated from *C. baccifera* and *C. dentata* rhizoshpere soil

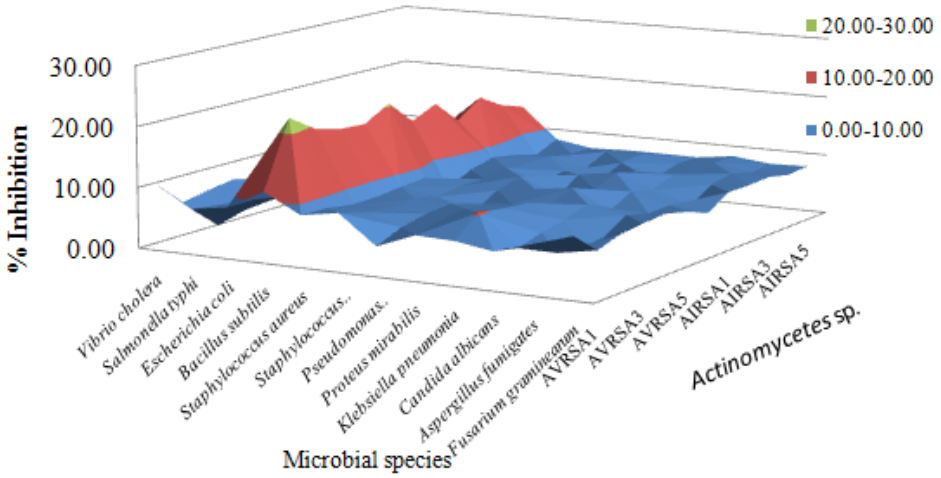

Figure 2. Average growth rate of different Actinomycetes with different carbon source for ten days at two days interval

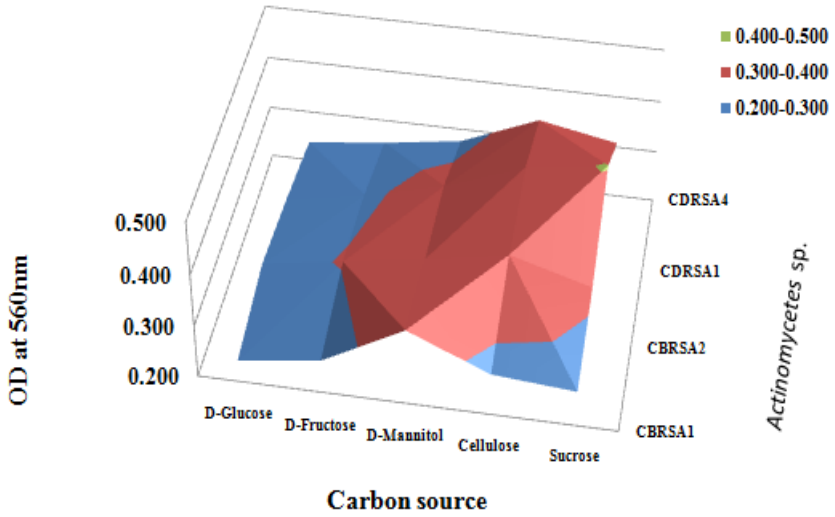

Figure 3. Average growth rate of different Actinomycetes with different culture media for ten days at two days interval