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ANTIBIOTIC PRODUCTION BY RHIZOSPHERIC SOIL MICROFLORA - A REVIEW

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ABSTRACT: The rhizosphere represents the thin layer of soil surrounding plant roots and the soil occupied by the roots, supports large active groups of microorganisms. The vast organic compounds (amino acids, sugars etc.) secreted by plant roots in the rhizosphere provide a food source for microorganisms increasing microbial biomass and their activity in the rhizosphere. Antibiotics are antimicrobial compounds produced by living microorganism as secondary metabolites. These compounds are used therapeutically and sometimes prophylactically in the control of infectious diseases. The isolation of antibiotics from microorganisms is relatively easy as compared to chemical synthesis of antimicrobial agents. The isolation of antibiotics from microorganisms improved the discovery of novel antibiotics that could act as better chemotherapeutic agents. With the increased population pressure, costs and side effects and the development of resistance of pathogens to drugs for infectious diseases, there is an urgent need to explore microbes for development of new antimicrobial metabolites. As microorganisms grow in unique and extreme habitats, they may have the capability to produce unique and unusual metabolites. So rhizospheric soil gives an excellent option as a source for search of some new alternative medicines. This review highlights the recent developments in the production of antimicrobial compounds from rhizospheric soil microflora.

INTRODUCTION: Rhizosphere is a soil around a plant root which is inhabited by a diverse population of microbes, comprising bacteria, fungi, actinomycetes and algae affected by the chemicals which are released from the roots of plant [1]. The organic materials from roots provide the driving force for the development of active microbial biomass around the root than in the bulk soil [2]. The different compounds secreted by plant roots into the rhizosphere perform multiple functions.

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For example, Allelochemicals can inhibit the growth of other microorganisms in the rhizosphere, so plant microbes interactions are very complex[3]. The rhizosphere is divided into three zones which on the basis of their relative proximity to, and thus influence from, the root. Endorhizosphere includes parts of the endodermis and cortex where microbes and cations can occupy the "free space" between cells, rhizoplane is the medial zone which is directly adjacent to the root and includes the root epidermis and the mucilage and the outermost zone called the ectorhizosphere extends from the rhizoplane out into the bulk soil [4].

Populations of microbes can boom or reduce in the space in response to the changes in soil conditions i.e. moisture, temperature or substrates like carbon.

The aerobic bacteria are comparatively less in the rhizosphere as there is reduced oxygen level due to respiration by roots. The wide effect of rhizosphere is usually observed with bacteria than with the actinomycetes and fungi. The gram-negative, rod shaped and non-sporulating bacteria which respond to root exudates are dominant in the rhizosphere (*Pseudomonas*, *Agrobacterium*). Gram-positive, rods, cocci and aerobic spore forming (*Bacillus*, *Clostridium*) are comparatively rare in the rhizosphere.

The common genera of bacteria i.e. *Azotobacter*, *Micrococcus*, *Pseudomonas*, *Arthrobacter*, *Flavobacter*, *Mycobacterium*, *Agrobacterium*, *Alcaligenes*, *Cellulomonas* and others have been found to be either abundant or less populated in the rhizosphere. The bacterial population in the rhizosphere is enormous in the ranging from 10^8 to 10^9 per gram of rhizosphere soil. They cover about 4-10% of the total root area occurring on the root hair region and rarely in the root tips.

Rhizosphere effect is selective and significant on specific fungal genera (*Fusarium, Verticillium, Aspergillus* and *Penicillium*) which are stimulated. The mycelial forms are more dominant in the field. The lower fungi such as *Phytophthora, Pythium, Aphanomyces* are strongly attracted to the roots in response to particular chemical compounds excreted by the roots and cause diseases under favorable conditions. Among the actinomycete, the phosphate solublizers (eg. *Nocardia, Streptomyces)* have a dominant role to play [5].

Antibiotics produced by soil microflora:

Secondary metabolites are classically organic compounds produced from microorganisms during the alteration of primary metabolite synthesis. Secondary metabolites do not have a role in the growth and development of microbes and are usually formed in the stationary phase. Many among secondary metabolites have ecological functions; which include defense mechanisms, also function as antimicrobial agents or antibiotics [6]. The soil microbes are a major source of antibiotics (**Table 1**). Over 10000 different antibiotics have been isolated from cultures of gram-positive and gram-negative bacteria and of filamentous fungi. However, only about 100 of these have been commercially used to treat human, animal and plant diseases. The reason for this is that only compounds with selective toxicity can be used clinically [7].

TABLE 1: SOME CLINICALLY IMPORTANT ANTIBIOTICS PRODUCED FROM SOIL MICROBES

Antibiotic	Producing microbe	Spectrum of Activity
Penicillin	*Penicillium chrysogenum*	Gram-positive bacteria
Streptomycin	*Streptomyces griseus*	Gram-negative bacteria
Cephalosporin	*Cephalosporium acremonium*	Broad spectrum
Bacitracin	*Bacillus subtilis*	Gram-positive bacteria
Erythromycin	*Streptomyces erythreus*	Gram-positive bacteria
Neomycin	*Streptomyces fradiae*	Broad spectrum
Tetracycline	*Streptomyces rimosus*	Broad spectrum
Vancomycin	*Streptomyces orientalis*	Gram-positive bacteria
Kanamycin	*Streptomyces kanamyceticus*	Gram-positive bacteria, negative bacteria and mycobacteria
Amphotericin B	*Streptomyces nodosus*	Fungi
Trichomycin	*Streptomyces hachijoensis*	Fungi
Polymyxin	*Bacillus polymyxa*	Gram-negative bacteria
Gramicidin	*Bacillus brevis*	Gram-positive bacteria
Zwittermicin	*Bacillus cerus*	Gram-positive, negative prokaryotic microorganism
Fusidic acid	*Acremonium fusidioides*	*Staphylococci* and gram negative bacteria
Cochliodinol	*Chaetomium cochlioides*	Fungi and bacteria

Many medically useful antibiotics are produced by members of the genus *Bacillus* e.g. polymyxin and bacitracin produced by *B. polymyxa* and *B. licheniformis* respectively. The classical β-lactam antibiotics, penicillin and cephalosporin, are synthesized by the filamentous fungi *Penicillium* and *Cephalosporium*, but can also be produced by some actinomycetes and other bacteria. However, the actinomycetes, mainly *Streptomyces* species, are responsible for the synthesis of more than 60% of the known antibiotics while a further 15% are made by members of the related actinomycetes:

Micromonospora, *Actinomadura*, *Actinoplanes*, *Nocardia*, *Streptosporangium*, *Streptoverticillium* and *Thermoactinomyces* [8]. Thus, almost all commercially used antibiotics are produced by three groups of microorganisms – *Streptomyces*, *Bacillus* and the filamentous fungi [9].

Antibiotics produced by bacteria:

In an investigation on the potent bacteria producing antibiotics against pathogenic microorganisms from the rhizosphere soil samples of Kanjamalai hills, Salem district, Tamilnadu, India, a total of 6 isolates of bacteria was obtained and screened for inhibitory activity against selected opportunistic pathogens. Potent bacterial isolate (A6) was identified as *Pseudomonas aeruginosa* by cultural, morphological and biochemical characterization. The antimicrobial active compound was partially purified from the broth culture and its concentration was estimated to be 373.086 g/ml. The obtained protein was separated in SDS-PAGE and its molecular weight was determined as 66.4 kD, 44.3 kD, 29.0 kD, 20.1 kD and 14.3 kD using standard molecular markers. The results showed that the isolated strain has high potential against *Bacillus megaterium* and *Aspergillus niger* [10].

In a study carried out by Mhatre [11], 22 rhizospheric soil samples of *Curcuma longa* from western Vidharbh region of Maharashtra state was collected and total 26 bacteria were isolated by serial dilution and analyzed for production of antibiotics. Out of 26 only 3 were potent isolates on the basis of antibiogram test. Further characterization was done by following the Bergey's Manual of Systematic Bacteriology. Accordingly rhizospheric characterized isolates were *Bacillus megaterium*, *Pseudomonas fluorescens* and *Globicatella sulfidifaciens*. These potent isolates could be further exploited for the production of metabolites in production media.

Lactic acid bacteria (LAB) were isolated from rhizosphere soil samples in one study by Kaur *et al* [12]. And metabolite produced from LAB was checked for antibacterial activity at different temperature, pH and at different incubation periods against different bacteria viz., *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. 12 different isolates of *Lactobacillus* sp. were identified on the basis of various morphological, cultural and biochemical characters. Bacteriocin produced from isolate LB 12 showed maximum antibacterial activity against *E.coli* followed by *S. aureus* and *P. aeruginosa.* The optimized temperature for antibacterial activity of bacteriocin was 60°C as showed highest zone of inhibition at against *E.coli, S.aureus* and *P. aeruginosa* (26, 24 and 23 mm respectively) and least activity was observed at 121°C. Antibacterial activity of bacteriocin was retained at pH 2-10 but maximum activity was observed at pH 6 against *E.coli, S.aureus* and *P. aeruginosa* (24, 22 and 18mm) and incubation period of 24 hrs at 37°C was observed to be the most suitable for bacteriocin activity against test bacteria.

Microorganisms were isolated from soil sample by Khatkar and Kaur [13] collected near the root of *Calotropis* sp. by serial dilution, plating method and grown on nutrient agar medium. All isolated strains were tested for antimicrobial activity using agar well diffusion method. Out of 8 isolates 7 strains showed antimicrobial activity. The characterization of isolates was carried out by morphological method, biochemical method and molecular characterization techniques. The isolate having highest antimicrobial activity was identified as *Bacillus cereus* by16S rRNA sequence analysis.

Malleswari [14] studied *in vitro* antagonistic activity of diverse bacteria isolated from the rhizosphere soil samples of some medicinal and aromatic plants viz., *Coleus forskohlii*, *Andrographis paniculata*, *Withania somnifera*, *Ocimum sanctum*, *Aloe vera*, *Mimosa pudica*, *Artemisia vulgaris*, *Acorus calamus* and *Mentha spicata* collected from different locations in Andhra Pradesh against *Macrophomina phaseolina,* a fungus that causes charcoal root rot in many plant species and considered as one of the most important pathogens in forest nurseries showed that among the 219 isolates 43 strains had antagonistic activity against pathogen but one isolate showed maximum inhibition (52.22%) against mycelial growth of the pathogen by dual culture plate technique.

On the basis of colony morphology and biochemical characteristics and 16S rRNA gene sequencing isolate was identified as *Bacillus subtilis* (Cf 60).

In a study, it was tried to find a new antimicrobial agent producing bacteria from rhizospheric soil which might be active against multi drug resistant clinical pathogens viz. *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae,* isolated from different clinical samples. All the isolates were subjected to antibiotic susceptibility testing by disc diffusion method. Out of 86 isolates 62.79 % isolates were found to be multi drug resistant (MDR). The rhizospheric isolates S2 (*Bacillus* sp.) and S5 (*Pseudomonas fluorescens*) were found to exert good antimicrobial activity against 8 most resistant clinical isolates. Because of huge emergence of multidrug resistant (MDR) bacteria as reported in many studies, there is an urgent need to discover new therapeutics that would be effective against MDR strains[15].

Antimicrobial activity of a bacterial isolate collected from *Medicago sativa* rhizosphere field of Helwan region, Cairo, Egypt was evaluated against pathogens by Abada *et al* [16]. According to Bergey's manual of systematic bacteriology, the isolate was identified as *Bacillus circulans*. The isolate *Bacillus circulans* showed antimicrobial activity against gram-positive, gram-negative bacteria and plant pathogenic fungi. The optimized temperature, pH, carbon and nitrogen source for maximum antimicrobial production was observed after 24 h at 30ºC and pH 8, starch and DL-methionin respectively. The GC-mass analysis showed that the compound responsible for antimicrobial activity is 4-(Diphenylmethyl)-6 ethoxycarbonyl-1-phenyl1H-pyranolo [4, 3-c] pyridine with molecular formula $C_{28}H_{23}N_3O_2$.

The Minimum Inhibitory Concentration (MIC) of antimicrobial compound against Gram positive, Gram negative bacteria and unicellular fungi was in range of 0.5-2µg/ml. Due to the antimicrobial activity of *Bacillus circulans*, it could be used in industry for production of antibacterial compound and in biological control against different plant pathogens [16].

Screening of bacteria isolated from the rhizosphere soil of tomato seedlings was done by Walia *et al* [17] for having broad spectrum antifungal activity against *Fusarium oxysporum*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum***.** *In vitro* antifungal antibiotic study revealed that among eleven isolates N11 showed maximum inhibition against *F. oxysporum* (82.85%), *R. solani* (76.45%) and *S. sclerotiorum* (74.71%) after seven days of incubation. The per cent growth inhibition increased with increase in bacterial cell density from O.D 0.25 to 1.50. It is also concluded that the antibiotic production is induced only in the presence of fungal host and lack of antifungal activity in culture filtrate. Bacterial isolate N11 was identified to species level by biochemical characterization and 16S rRNA sequencing as *Bacillus subtilis* CKT1[17].

Different Soil samples of *Calotropis procera* and *Catharanthus roseus* were subjected for antibacterial activity of microbes, extraction of secondary metabolites i.e both intracellular and extracellular, and characterization by Arora *et al* [18]. Three type of colony were found i.e white, off-white and yellow. Further using Bergeys' manual *Macrococcus luteus*, *Neisseria sicca* were obtained. Secondary metabolites i.e intracellular and extracellular were extracted using solvents chloroform and methanol. Antibiotic sensitivity test was performed against pathogen (*Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus. aureus*) and the best Zone of inhibition was of culture of *Catharanthus roseus* i.e 28.5 mm [18].

In a study, bacterial strains were evaluated for antifungal activity in the sorghum rhizosphere. The bacterial isolate UM96 showed strong growth inhibition in bioassays against the pathogens *Diaporthe phaseolorum*, *Colletotrichum acutatum*, *Rhizoctonia solani*, and *Fusarium oxysporum*. Further molecular characterization by partial 16S rDNA sequencing showed isolate UM96 in a cluster with *Bacillus amyloliquefaciens.* As highest identity match found in databases of Bacillus species was 91% identity so this suggested that *Bacillus sp.* UM96 might be a novel species [19].

Rekha *et al* [20]. isolated proteobacterium from rhizospheric soil and identified using morphological, cultural and biochemical characteristics as *Pseudomonas fluoresecens*. Antibacterial activity of *Pseudomonas fluorescens* was screened against ten target bacterial pathogens

of health significance like *Staphylococcus aureus, Streptococcus mutans, Bacillus subtilis, Aeromonas hydrophila, Vibrio cholerae, Escherichia coli, Klebsiella pneumoniae, Shigella sonnei, Salmonella typhi* and *Serratia marcescens* by *in vitro* techniques. The result indicated that strains of *Pseudomonas fluorescens* presented a significant value against S. *typhi* (20.7 ± 0.58mm), *S. mutans* (19.7±0.58mm), *B. subtilis* (15.3 ±0.58mm), *S. sonnei* (15.3 ± 0.58mm) and no activity against *S. aureus, A. hydrophila, V. cholerae, E. coli, K. pneumoniae* and *S. marcescens.*

In a study of antifungal activity of thirty *Pseudomonas fluorescens* isolated from rice rhizosphere against *Magnaporthe grisea*, *Dreschelaria oryzae*, *Rhizoctonia solani* and *Sarocladium oryzae* that are known to attack rice plants showed one *P. fluorescens* isolate (Pf 003) effectively inhibited the mycelial growth in all these fungi in dual culture tests (62–85%). The antifungal compounds extracted with ethyl acetate from *P. fluorescens* at 5% completely inhibited the pathogens. The antifungal compounds were identified on thin layer chromatography (TLC) at R_f 0.22, 0.35, 0.42 and 0.51. These compounds were individually purified by column chromatography and re-tested for antifungal activity. One compound with R_f 0.35 on TLC completely inhibited the mycelial growth of all test fungi at 0.5%.

This compound showed melting point at 168-173ºC. The proton nmr and C^{13} nmr confirmed its identity as 2, 4- diacetylphloroglucinol (DAPG). IR spectrum of this sample showed OH functional group at 3341 cm^{-1} and the molecular weight was estimated as M/z 210 by mass spectrometry, which agreed with the 2,4- DAPG composition of $C_{10}H_{10}0_5$. This antifungal compound can be effectively used against rice fungal pathogens [21].

Antibiotics produced by Actinomycetes:

In a study carried out by Kesavan and Hemlatha [22], six soil samples were serially diluted and plated on starch casein agar supplemented with nalidixic acid and cyclohexamide for inhibition of bacteria and fungi, respectively. Agar well diffusion method was done to determine the antimicrobial activity of the crude extract. On primary screening, 13 out of 22 actinomycete isolates showed potential antimicrobial activity against one or more test bacteria and/or fungus. The isolate BN8 showed antagonistic activity against all the tested bacteria and fungi, isolates BN5 and BN16 were active against only bacteria not fungi, and isolate BN2 was active against all tested fungi. The crude extract produced by isolate BN8 showed zone on inhibition against all the tested bacterium in 100 µg/ml against *Pseudomonas aeruginosa* (22 mm), *Klebsiella pneumonia* (25 mm), *Bacillus cereus* (20 mm), *Staphylococcus aureus* (22 mm), *Escherichia coli* (15 mm), *Aspergillus flavus* (14 mm), *Aspergillus niger* (20 mm), *Aspergillus fumigatus* (10 mm), respectively. The crude extracts of isolates BN2, BN5, and BN16 did not exhibit any zone of inhibition against the test microbes on agar well diffusion assay.

The minimum inhibitory concentrations (MIC) also quantified for the crude extract by microtiter plate assay. The MIC of the crude extract against *P. aeruginosa* (50 µg/ml), *S. aureus* (25 µg/ml), *K. pneumonia* (25 µg/ml) and *B. cereus* (25 µg/ml) and *E. coli* was 12.5 µg/ml. The MIC of 12.5 µg/ml was observed for the crude extract against *A. flavus*, 25 µg/ml against *A. niger*, and 50 µg/ml against *A. fumigatus*, respectively.

Antimicrobial potential of 13 soil actinomycetes isolated from Karachi was evaluated by Kiran *et al* [23]. In Primary antagonistic screening performed by cross streak and over lay method, out of 13 isolates, 8 isolates inhibited the Gram positive and Gram negative test bacterial strains. The antimicrobial activity of fermented broth of eight strains was evaluated by agar well diffusion method. Based on the antagonistic spectrum, the selected strain GZ024 produced Retinaculum sporangium with oval shaped spores (with marty surface), arranged in clusters, citrate, catalase and gelatinase positive, produced acidic butt and alkaline slope on Triple Sugar Iron test and only ferment the glucose hence named *Streptomyces* sp. GZ024. The optimized growth conditions for isolate were temp. 25˚C, pH 9 and 3% NaCl concentration. The minimum inhibitory concentration of antimicrobial substance of *Streptomyces* sp. GZ024 was found to be 1:8. Ethyl acetate solvent was proved to best solvent for the antibiotic metabolite extraction. The extract was

stable at 100˚C and pH 9. The organism starts producing antibiotics at log phase and showed maximum activity when organism entered in stationary phase. It can be concluded *Streptomyces* sp. GZ024 could be an important producer of antimicrobial compounds.

The study was performed for the production of antibiotics from pigment producing actinomycetes isolated from the rhizosphere of three different plant soils (Foxtail millet, Groundnut and Mulberry) from different localities of Anantapur region, Andhra Pradesh, India by Varalakshmi *et al* [24]. Out of 30 actinomycete isolates, 10 promising isolates (A2, A5, A8, A12, A14, A17, A21, A24, A27 and A29) showed activity against pathogenic bacteria and fungi by primary screening.

Out of ten active isolates, potential isolate A2 was subjected to secondary screening, identification and fermentation methods. According to morphological, biochemical and molecular methods, strain belongs to the genus *Streptomyces* and was designated as *Streptomyces rameus* KCTC 9767. RNA secondary structure predicted for 16S rRNA gene of *S. rameus* showed the free energy of -98.5kcal/mol and the restriction site analysis predicted by Genebee and NeBCutter online software's showed the GC and AT content to be 59% and 41%.The active metabolite extracted using ethyl acetate was screened for antimicrobial activity. A maximum zone of inhibition were observed (29 mm) for Gram positive bacteria and (30 mm) for fungi. The MIC was 3.01 mg/ml against *Staphylococcus aureus* and 1.55mg/ml against *Klebsiella pneumonia* and *Saccharomyces cerevisiae*. So it can be said that rhizosphere actinomycetes have an immense potential as a source of antibacterial compounds.

Antibacterial and antioxidant activity was reported for ethyl acetate extract of *Streptomyces* species SRDPH03 isolated from rhizosphere soil of Hosudi, Karnataka, India in one study. The isolate SRDP-H03 was assigned to the genus *Streptomyces* based on the cultural and microscopic characteristics. The ethyl acetate extract of the isolate SRDP-H03 showed marked inhibition of Gram positive bacteria than Gram negative bacteria. The extract was found to possess dose dependent DPPH free radical scavenging and Ferric reducing activity. UV spectral data of ethyl acetate extract showed strong absorption maxima (λ_{max}) at 267 and 340nm. So isolate could be a potential candidate for the development of novel therapeutic agents active against pathogens and free radicals [25].

In a study fifteen soil samples were collected by Bizuye *et al* [26]. from waste disposal (at Taxi Mazoria, Johannes, Gibirina and Condominium) and rhizosphere (at Tewodros Campus) Gondar town, Ethiopia areas to isolate and screen antibiotic producing actinomycetes. Samples were serially diluted and spread on starch casein and oat meal agar supplemented with amoxicillin and cyclohexamide for inhibition of bacteria and fungi, respectively. Cross streak method showed three isolates (Ab18, Ab28 and Ab43) had high antagonistic activity. Solid state fermentation and crude extraction were used for the production of antibiotics from isolates.

Inhibition zones obtained from agar well diffusion test of crude extracts showed significance differences when compared with standard antibiotics tested against test organisms ($P<0.05$). Inhibition zone of crude extracts from isolate Ab18 against *Klebsiella pneumonia* ATCC 7000603 and *Escherichia coli* ATCC 25922 were (14±1) mm and (35±1) mm, respectively which were strong active when compared to amoxicillin (0 mm) and tetracycline [(13±1) mm for *Klebsiella pneumonia* ATCC 7000603 and (33±1) mm for *Escherichia coli* ATCC 25922]. Crude extracts from isolate Ab18 showed (20±1) mm and (15±1) mm inhibition zones against methicillin resistant *Staphylococcus aureus* strains 2 (MRSA2) and MRSA 4, respectively. Crude extract from isolate Ab43 has shown inhibition zones of (16±1) mm and (17±1) mm against MRSA 2 and MRSA 4, respectively. Combination of Ab18 and Ab43 has shown high antimicrobial activity (18±1) mm against MRSA 2 and MRSA 4.

Bacteria was isolated from agricultural soil from village Khuda Lahora near Chandigarh (India), found to inhibit the growth of clinical isolates including *Staphylococcus* (resistant to amikacin, ciprofloxacin, clindamycin, clinafloxacin, erythromycin, gentamicin and methicillin) and

Candida (resistant to fluconazole and itraconazole). The culture was identified as *Burkholderia gladioli* and based on TLC profile and bioautography studies, the chloroform extract of *B. gladioli* OR1 consisted of at least three anti-staphylococcal and two anti Candida metabolites which were highly stable at high temperature (121ºC) and in the broad pH range (3.0-11.0) [27].

Antimicrobial activity of ninety-seven actinomycete strains isolated from fifty soil samples collected from the Taif City, Kingdom of Saudi Arabia was checked. Only one actinomycete culture T-4 was active *in vitro* against some microbial pathogenic viz: *Staphylococcus aureus*, NCTC 7447; *Micrococcus lutea*, ATCC 9341; *Bacillus subtilis*, NCTC 10400; *Bacillus. pumilus*, NCTC 8214; *Klebsiella pneumonia*, NCIMB 9111; *Escherichia coli*, NCTC 10416; *Pseudomonas aeruginosa*, ATCC 10145; *Saccharomyces cerevisiae* ATCC 9763; *Candida albicans*, IMRU 3669; *Aspergillus flavus*, IMI 111023; *Aspergillus niger* IMI 31276; *Aspergillus fumigatus* ATCC 16424; *Fusarium oxysporum*; *Rhizoctonia solani*; *Alternaria alternata*; *Botrytis fabae* and *Penicillium chrysogenium*.

According to the morphological, cultural, physiological and biochemical characteristics, and 16S rDNA sequence analysis, strain T-4 was identified as *Streptomyces torulosus*. The optimized conditions for maximum yield of secondary metabolites are as temperature 35ºC, incubation period five days, glucose as best carbon source and KNO_3 as best nitrogen source. The metabolites were extracted using n butanol (1:1, v/v) at pH 7.0. The chemical structural analysis with UV, IR, and MS spectral analyses confirmed that the compound produced by *Streptomyces torulosus*, T-4 is tunicamycin antibiotic [28].

Three soil samples were collected such as ant mound soil (VAS), sugarcane rhizospheric soil (SRS) and termatorium soil (VT) from Vengodu (village) in Kanchipuram district, Tamil Nadu, India to isolate novel actinomycetes to evaluate their antibacterial activity. Total 35 actinomycetes were isolated on basis of colony characteristics using serial dilution and plating method on actinomycetes isolation agar. All the isolates were screened for antibacterial activity by cross streak method. Out of six isolates (SRS 2, 3, 6, VAS 9, 10 and 16) taken for further studies based on their antibacterial activity, only one isolate VAS 10 showed maximum activity. Medium (Actinomycetes Isolation Broth) and day (3^{rd}) was optimzed for the potent strain using Nathan's agar well diffusion method. The most active isolate VAS 10 was identified as *Actinobacterium Loyola* PBT VAS 10 (accession No. JF501398) using 16s rRNA sequence method.

The maximum antibacterial activity was observed in dichloromethane and ethyl acetate; maximum zones of inhibition were observed against *Enterococcus durans*. The rRNA secondary structure and the restriction sites of *Actinobacterium Loyola* VAS 10 were predicted using Genebee and NEBCutter online tools respectively. It showed the free energy of the predicted structure to be 204.9 kkal/mol, threshold energy to be 4.0 by using Genebee and restriction site analysis showed GC 58% and AT 42% content [29].

In a study 25 soil samples were collected from rhizosphere regions of different plants from a farm in sungai ramal luar, Malaysia. These samples were divided into two sets for the isolation of actinomycetes: one receiving the treatment with calcium carbonate and other set without calcium carbonate. A total of 300 actinomycete isolates with different morphology were obtained. Out of 50 fast-growing isolates, four potential antibiotic producing isolates were obtained by employing primary and secondary screening. The antibacterial activity of crude compounds extracted from the actinomycetes was tested against *Bacillus subtilis*, S*taphylococcus aureus*, S*almonella* sp. and S*erratia* sp. Two actinomycete cultures, LM1A and MG1A that antagonized most bacteria with largest inhibition zones *(B. subtilis*: 20.5 mm, *Salmonella* sp.: 13.0 mm, *Serratia* sp.: 13.0 mm, *S. aureus*: 19.0 mm) during screening were selected for further study. Both of the isolates were found to be growing at pH and temperature ranges of 5.0-9.0 and 30-37°c respectively and tolerated NaCl concentrations as high as 7%. The isolates were presumed as *Streptomyces* sp.[30].

Antimicrobial activities was identtified of two actinomycete isolates, designated as B8 and C2, isolated from a patch of soil in the peripheral area of University Putra Malaysia by streaking on starch casein agar after standard serial dilution procedures. Their antimicrobial activities were first evaluated against eight clinical laboratory strains namely *Bacillus* sp., *Enterococcus* sp., *Escherichia coli*, *Klebsiella* sp., *Pseudomonas* sp., *Salmonella* sp., *Staphylococcus aureus*, and *Staphylococcus epidermidis* by perpendicular streak method on Mueller Hinton and Tryptic Soy agar.

In both media, a broad-spectrum antibacterial activity was observed for both isolates, with B8 against all the test bacteria and C2 against five of them (*Bacillus* sp., *E. coli*, *Pseudomonas* sp., *S. aureus* and *S. epidermidis*). Subsequently, the two isolates were identified by PCR/sequencing techniques and phylogenetic analysis to be *Streptomyces* species (>93% homology based on 16S rRNA and rpoB genes). Characterization on cultural characteristic and viable count at different temperatures (37ºC and 28ºC), on different microbiological media (Actinomycete Isolation Agar, ISP-2, Muller Hinton Agar, Nutrient Agar, Potato Dextrose Agar and Tryptic Soy Agar), were performed.

More morphological features were observed on ISP-2 for both isolates. A higher growth yield was also observed at 28ºC in all media but in comparing that between the two isolates, isolate B8 outnumbered C2 at all experimental conditions. The observed variation in cultural traits and growth yield indicated unique properties between the two antibiotic-producing isolates [31].

In vitro antagonistic activity of *Streptomyces* isolates from the rhizosphere of sixteen medicinal plants against six plant pathogenic fungi; *Alternaria brassicicola*, *A. porri*, *Colletotrichum gloeosporioides*, *Fusarium oxysporum*, *Penicillium digitatum* and *Sclerotium rolfsii* was assesesd. Out of 27 isolates, *Streptomyces spectabilis* CMU-PA101 recovered from soil associated with pandanus palm (*Pandanus amaryllifolius*) was very effective in producing bioactive metabolites against the six test fungi. The culture filtrate of strain CMU-PA101 was extracted using various solvents (chloroform, n-butanol, diethyl ether, ethyl acetate, and n-hexane). The n-butanol extract demonstrated the highest activity against the test organisms, with a minimum inhibitory concentration (MIC) of 0.781 to 6.250 mg/ml [32].

The evaluation of inhibitory activity of *Streptomycete* strains from different fields (rhizosphere of plants, agricultural soil, preserved areas and forest soils) in Mugla, Turkey was performed on seven microorganisms including multiple antibiotic resistant *Staphylococcus aureus* and *Stenotrophomonas maltophilia*. Out of 15 isolates, five isolates were highly active against *S. aureus* strains including meticillin resistant *Staphylococcus aureus* (MRSA). Twelve Streptomycete isolates showed anticandidal activity against *Candida albicans*. Ten isolates were highly active with an inhibition zone more than 30 mm in diameter. Most of the isolates inhibited growth of the Gram negative bacteria tested. Eight isolates showed antibacterial activity on *S. maltophilia* MU64. The inhibition zones of two were higher than 30 mm for *S. maltophilia* [33].

Indigenous actinomycetes from rhizosphere soils were assessed for *in vitro* antagonism against *Colletotrichum gloeosporioides* and *Sclerotium rolfsii*. A potent antagonist against both plant pathogenic fungi was designated SRA14, identified as *Streptomyces hygroscopicus*. Culture filtrates of strain SRA14 collected from the exponential and stationary phases inhibited the growth of both the fungi tested, indicating that growth suppression was due to extracellular antifungal metabolites present in culture filtrates. The percentage of growth inhibition by the stationary culture filtrate was significantly higher than that of exponential culture filtrate [34].

Antibiotics produced by Fungi:

In a study, fungi was isolated from rhizospheric soil sample from Kuttralam hills station. Fungal isolate F-4 identified as *Aspergillus terreus* based on molecular 18S rRNA assay and phylogenetic tree construction. F-4 strain showed highest antibacterial activity against *Staphylococcus* (29mm). Further, Bioactive fraction F-4was identified by FT-IR and HPLC analysis. Based on the GC-MS analysis, ten compounds were

identified, out of ten compounds, one compound (Tetracontane) used in anti-microbial effect [35].

Ten different rhizosphere soils of healthy groundnut plants was selected for isolation of fungi having antimicrobial property. Out of 25 isolates assayed for antagonism, ten different fungi significantly inhibited colony growth of potential charcoal rot pathogen *Macrophomina phaseolina* in dual culture plates. Antagonistic fungi showed 25.6-41% mycelial growth inhibition in M. phaseolina. Colony growth and sclerotia production of M. phaseolina ceased effectively by *Emericella nidulans* and *Emericella rugulosus*, respectively. Mutual inhibition between *Alternaria alteranata*, *Aspergillus flavus*, *Aspergillus terreus*, *Penicillium chrysogenum* and *M. phaseolina* was observed [36].

Rhizospheric and non-rhizospheric region of two medicinal plants-*Oscimum tenuiflorum* and *Aloe barbadensis* was explored for the production of antibiotics. The results of Antibiotic Sensitivity Test revealed the activity of isolates which further led to characterization of isolates through Gram's staining and Bergey's manual. The isolates obtained were *Proteus vulgaris*, *Streptococcus epidermis*, *Lactobacillus fermentum*, *Bacillus cereus*, *Neisseria mucosa*, *Sterptococcus equisimilis*, *Streptococcus faecalis* and *Bacillus subtilis*. These isolates were cultured in optimized production media in suitable nutrient sources, temperature conditions and pH range. Glucose and sucrose as carbon sources and ammonium chloride and ammonium sulphate as nitrogen sources at 37ºC and pH-7 were suitable for growth of majority isolates.

This further led to secondary screening by intracellular and extracellular metabolite extraction from the cultures obtained in production media. These antimicrobial properties of the metabolites were tested by Antibiotic sensitivity test which gave positive results. The results i.e. Rf values obtained from the silica gel Thin Layer Chromatography proved the possible presence of beta-lactum antibiotics in the secondary metabolites extracted from the cultures of *Lactobacillus fermentum*, *Bacillus cereus* and *N. mucosa* [1].

The screening of bacteria, fungi and *Streptomyces* was performed by Sethi *et al* [37]. for potential antibiotic activity. Among the microbes isolated from soil and identified as *Bacillus subtilis*, *Penicillium chrysogenum* and *Streptomyces* sp. were selected on the basis of their anti-bacterial activity. The inhibitory activities of the isolated microorganisms were checked against *Staphylococcus aureus* (ATCC 29213), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922) and *Klebsiella pneumoniae* (ATCC 4352). *Penicillium chrysogenum* metabolites showed maximum antimicrobial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumoniae* with a zone of inhibition of 17mm, 11mm, 19.8mm and 8.2mm respectively. *Bacillus subtilis* metabolites showed activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* measured as zone of inhibition of 13.4mm and 13.8mm respectively whereas *Streptomyces* sp. showed minimum activity against all the four tested organisms.

Future Prospects:

The use of antimicrobial drugs for prophylactic or therapeutic purposes regularly in human, veterinary and agricultural purposes is favoring the survival and spread of resistant organisms. Due to this multidrug resistant pathogenic strains [24], it is indisputable that new antibiotics are urgently needed. The screening of microbial natural products continues to represent an important route to the discovery of valuable chemicals, for the development of new therapeutic agents and for evaluates of the potential of new microbial taxa [22].

As already studied, soil is rich in microorganisms capable of antibiotic synthesis, but quantity and quality of nutrients as Nutrients are not dispersed uniformly throughout soil, but rather, are localized in the rhizosphere of plants and the ability to compete successfully for them are major determinants of microbial population size and their metabolic activity which regulates antibiotic synthesis. So it was found that the nutritional status of the different soil types could affect on the distribution of antimicrobial agent producing microorganisms in the soil [38]. As One gram of soil may harbour up to 10 billion microorganisms of

possible thousands of different species, only a tiny fraction of soil microbes are readily cultured, so soil ecosystems are to large extent, unexplored. So soil might be the greatest untapped resource for novel chemistry. Although several compounds with antibiotic activity have been isolated from microorganisms over the years, but only a few of them are clinically useful. The reason is that they must be highly effective against a microorganism but have minimal toxicity to human[39].

In future different soil ecosystems must be explored for the discovery of new strains, development of effective antimicrobial drugs with novel mechanisms of action and bioactive metabolites to overcome possibilities of spreading of drug resistant and new pathogenic strains.

CONCLUSION: The rhizosphere is a densely populated area in which the microbes, including bacteria, fungi and insects feeding compete for space, water, and mineral nutrients. So there is a population of microbes which kill other microbes for survival and exhibit antimicrobial property. Most of the antibiotics in current use for the treatment of various infectious diseases are microbial products. There is an emerging menace of drug resistance among microorganisms due to inappropriate use of antibiotics by general health practitioners worldwide. This situation has become an alarming condition to drug manufacturers and public health practitioners. Natural products from microorganisms have been the most successful source that has found many applications in the fields of medicine, pharmacy and agriculture.

There has been continue search for more effective antibiotics that can stand this crisis. Therefore, there is an urgent need to investigate indigenous soil resources with potential of antimicrobial production that could be used to produce new product with better efficacy.

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