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Growth characteristics and production of secondary metabolites from selected streptomyces species isolated from the Rhizosphere of Chili Plant

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ABSTRACT: The aim of this study is to investigate the antimicrobial activity of actinomycete isolated from the rhizosphere of chilli plants and to characterize the growth of Streptomyces isolates which was produced by secondary metabolites. Antimicrobial products were extracted using ethyl acetate, and the crude secondary metabolite extracts analyzed using gas Chromatography- Mass Spectrophotometer (GC-MS). 0.54 g/l, 0.62 g/l, 0.41 g/l, 0.3 g/l and 0.14 g/l yields of crude secondary metabolites extracted from the isolates. The crude secondary metabolites have different levels of inhibition against Fusarium oxysporium f. Sp. Lycopersici fungi. During the investigation, it is found that the extracts had effective against the test fungi, and the mycelial growth of fungi is inversely proportional to the concentration of the extract. Characterization of the crude secondary metabolites indicated the availability of the chemical compounds such as amides, phynol, amines, acids, pyrrolizidines, butenolides, alcohols and hydrocarbons.

Keywords: Streptomyces, antimicrobial activity, gas chromatography, mass Spectrophotometer, secondary metabolites.

Introduction

Actinomycetes belong to the order Actinomycetales, a division of the Gram positive bacteria **[**1**]** (Ames et al., 1984). Actinomycetes species are well-known as saprophytic bacteria that decompose organic matter, especially biopolymers such as lignocelluloses, starch, and chitin in soil **[**2**]** (Crawford et al., 1993). Actinomycetes represent a high proportion of the soil microbial biomass and can produce a wide range of antibiotics and extracellular enzymes. Several strains of actinomycetes have been found to protect plants against plant diseases. They play different roles: as (a) source of Agra active compounds, (b) plant growth promoting organisms and (c) biocontrol agents of plant diseases. Actinomycetes are a gram-positive, aerobic, high GC-(guanine –cytosine) content and 0.5–1.0 μm in size. They are filamentous, sporulation colonies and recognized as a transition group between primitive bacteria and fungi **[**3**]** (Kuster, 1968). Actinomycetes were also known as slow growing bacteria and micro goldmine with useful secondary metabolites. They are sources of approximately 70% antibiotics, extracellular enzymes and other commercially important bioactive compounds **[**4**]** (Willey et al. 2008). Several actinomycetes have characteristic biological features such as mycelia growth that culminates in sporulation , they also process the ability to biosynthesize a wide variety of antibiotics as secondary metabolites **[**5**]** (Franklin et al.,1989).

Actinomycetes represent a high proportion of the soil microbial biomass and can produce a wide variety of antibiotics and extracellular enzymes. Several strains of actinomycetes have been found to protect plants against plant diseases **[**6**]** (El-Tarabily et al., 2000). Many species of Actinomycetes, particularly those belonging to the genus Streptomyces, are well known as antifungal biocontrol agents that inhibit several plant pathogenic fungi.

The antagonistic actives of Streptomyces to fungal pathogens is usually related to the production of antifungal compounds and extracellular hydrolytic enzymes like Chitinase and protease are considered to be important hydrolytic enzymes in the

lysis of fungal cell walls, as for example, cell walls of Fusarium oxysporum, Sclerotinia minor, and S. rolfsii **[**7**]** (Benjaphorn et al .,2008).

Potential uses of naturally occurring bacteria, Actinomycetes and fungi replacement or supplements for chemical pesticides have been addressed in many studies **[**8**]** (Kamil et al.,2007). Actinomycetes are Gram-positive bacteria with a high guanine plus cytosine content in their DNA (> 55 mol %). Despite the well-documented history of Streptomyces in biocontrol and preliminary evidence of their capacity to enhance plant growth **[**9**]** (Aldesuquy et al., 1908). Streptomyces species have been poorly investigated specifically for their potential as PGPR. **[**10**]** (Alexander,1977).

[11**]** Merriman et al., (1974) reported the use of a Streptomyces griseus (Krinsky), Waksman and Henrici isolate as a seed treatment of barley, oat, wheat and carrot, in order to increase their growth. The isolate originally selected for the biological control of Rhizoctonia solani. Though the S. griseus isolate did increase the average grain yield, dry foliage weight, tiller number, and advanced head emergence for wheat and oat over the controls, the differences were not statistically significant. Nearly 20 years later, though studies by **[**12**]** El-Abyad et al., (1993) and **[**11**]** Merriman et al., (1974), reported plant growth enhancement as a function of inoculation with Streptomyces, they did not investigate the possible mechanisms of Streptomycete- mediated growth promotion.

Methodology

Soil sample collections

The specimens (actinomycetes) used in this study were isolated from a different region of chilli roots (healthy plants). Soils about 10-20 cm depth from the surface and near to the root area was selected for sampling. **[**13**]** (Bonjar et al, 2005).

Isolation of Actinomycetes:

CaCO3 enrichment methods were used to isolate Actinomycetes, the soil samples were mixed with CaCO3 at the ratio of 10:1, and were incubated under moisture rich conditions for seven days at room temperature **[**14**]** (Hayakawa et al., 2004). One gram of the soil sample was serially diluted up to 10–7 dilution. Aliquots of 0.1 ml of each dilution was spread plated on Actinomycetes agar plates in triplicates and incubated at room temperature for seven days. After an incubation period, the plates were examined for the presence of actinomycetes colony. The suspected colonies was picked up and purified on International Streptomyces Project (ISP-2) agar by **[**15**]** Waksman, (1961) media and incubated at room temperature for about seven days. The suspected pure actinomycetes culture were inoculated on ISP-2 slants; after the incubation period, the slants were taken for further identification and antifungal screening. The stock culture was preserved in 15% glycerol (v/v) at −20°C **[**16**]** (Maniatis, 1989).

Characterization of Actinomycetes:

The potent Actinomycetes isolates selected from primary screening was characterized by morphological, biochemical and physiological methods. The morphological method consists of macroscopic and microscopic characterization**.** Macroscopically the Actinomycetes isolates were differentiated by their colony characters, e.g. size, shape, color, consistency, etc. For the microscopy, the isolates were grown by a cover slip culture method [17] (Kawato & Sinobu 1959).

Identification by PCR amplification and sequencing of 16s rRNA gene

The identification of actinomycetes was done on the basis of morphology of spare chain, pigment production, color of aerial mycelium, color of substrate mycelium, consistency, gram's staining, growth of Actinomyces media, **[**18**]** (Lim et al., 2000). PCR operations were also carried out for diagnostics (molecular study) Actinomycetes 16S rRNA gene was amplified from the extracted genomic DNA using the following universal bacterial 16SrRNA gene PCR was performed in a 50 µL reaction mixture containing 2 µL (10ng) of DNA as the template, each primer at a concentration of 0.5mm, 1.5mm Mgcl2 and each deoxynucleotide triphosphate at a concentration of 50mM, as well as 1U of Taq polymerase. The thermal cycler were programmed for one cycle of the initial denaturation for 5 minutes at 950C. It was followed by 30 cycles programmed for denaturation at 940C for 1 minute, annealing at 550C for 1 minute and extension at 720C for 1 minute. An additional cycle at 720C for 5 minutes were used for the final extension. For the purification dissolved the unpurified DNA sample (at least 10- 15µl) in 50µl of PCR cleanup solution. It was mixed well and incubated at 55 ºC for 15-20 minutes. Centrifuged the mixture at 12000 rpm for 15 minutes, during which time the contaminants are released into the supernatant and discarded at the end of the centrifugation. The DNA was further precipitated by the addition of 600µl of 80% ethanol

and centrifugation at the same conditions as before. The residual cleanup solution/contaminants was removed along with ethanol by discarding the supernatant. Finally, the DNA pellet was dried and dissolved in 10-15µl of MilliQ water.
The amplification of 16S rRNA gene was confirmed by running the amplification product in 1.5% agarose gel in 1xTris – Borate – EDTA buffer. The sequencing of the 16S rRNA gene was done using Big Dye Chemistry, and performed as per the manufacturer's protocols (Applied Biosystems 3730xl DNA Analyzer) in the lab. The obtained sequence was compared with the BLAST.

Growth of Streptomyces Isolates

The isolates was fermented in a tryptone soy broth media in a shaker incubator (Gallen Kamp, Germany) (200 rpm, 28ºC) for five days. The original stocks of the isolates from which the working stocks were prepared was kept in the freezer (Sanyo MDF-594 AT, Japan) at – 80 C.

Antifungal activity of actinomycetes

In vitro antagonism test, dual culture method was used to examine the inhibition of F.orysporum we was measured the inhibition of pathogen on the PDA medium than actinomycetes isolates. Then; were showing the highest inhibitory percentages; moreover, which isolates produced more extracellular metabolites and volatile compound. These isolates which it has a higher inhibition against hyphal pathogen was used.

Preparation Of Crude Cell Extract (CCE)

Antibacterial compounds were recovered from the filtrate by solvent extraction method following the process described by **[**19**]** Liu et al., (1986), Ethyl acetate was added to the filtrate in the ratio of 1:1 (v/v) and shaken vigorously for 1 hour for complete extraction. The ethyl acetate phase that contains antibiotic were separated from the aqueous phase. It was evaporated to dryness in the water bath at 80°-90°C, and the residue obtained was weighed. Thus obtained compound was used to determine antimicrobial activity, minimum inhibitory concentration and to perform bio autography. **[**20**]** (Dev Sharma et al,2013).

Antimicrobial Bioassay (Agar Well Diffusion Assay)

To check antimicrobial activity of cell extract (PDA) media was produced in plats, small wells (dim,6mm) were made in agar plates and bottom of wells separately and allowed to diffuse in agar media . Indicator fungal strains were spread plated on separate PDA agar plates.Ethyle acetate (100)ml was used as a control to check inhibitory effect.

Purification of Antibiotics

The purification of the antimicrobial substance was carried out using silica gel (2.5x50) chromatography. Chloroform and Methanol 95:5 (v/v) mixtures were used as an eluent. The column was left overnight until the silica gel (pro labor) was completely settled. 1 ml crude extract to be fractionated were added to the silica gel, and 15 eluents were collected (each of 10 ml). The 15 eluents were tested for their antimicrobial activity **[**21**]** (Afifi et al., 2012).

GC-MS Analysis of Secondary Metabolites from the Actinobacteria Isolates

Chemical screening of the active compounds present in the crude extracts was done by use of a GC-MS to detect the active compounds, as well as their quantity and quality ratios. The sample was reconstituted using 1ml DCM (Dichloromethane (≥99.8%; Aldrich Chemical Co. Ltd., USA.) and passed through a glass wool to remove solid materials. 40 µl of the collection in triplicate were transferred into Autosampler glass vials having Teflon caps and analyzed using GC-MS.Agilent Technologies 7890A system were used. Oven conditions set during the analysis were: 1 minute for equilibration time; 35 °C for 5minutes, 10 °C/minute, to 280 °C for 10.5 minutes and 50 °C/min to 285 °C for 9.9 minutes, as the oven program while the running time was 50 minutes.

The Injection was done in splitless mode and the conditions used were as follows: 250 °C for the heater, 8.8271 Psi as the pressure , a total flow of 10.2 ml/ min, septum purges flow of 3 ml/ min, gas saver at 20 ml/ min after 2 minutes and Purge flow to split vent at 6mL/ min at 0.8minutes. The column used was HP-5MS, (5% methyl silox), (30 m × 250 µm × 0.25 µm). The compounds identified was generated by a computer program that involved calculation by the data system of a similarity index, match factor or purity between the unknown spectrum and library (reference) spectra. For this analysis,

NIST/EPA/NIH MASS SPECTRAL LIBRARY (NIST 05) and NIST MASS SPECTRAL SEARCH PROGRAM Version 2.0d were used.

Data Analysis

ANOVA tests were used to analyze the data. The SAS 9.1 version was the software used to perform the analysis and separation of means was done by use of Tukeys' test. Means were used to draw graphs and tables.

Results and Discussions

In the present study, the endophytic Bacteria were isolated by using three different Mycological media, namely Trepton soy agar (TSA), Actinomycetes media Agar and (ISP2) Media. Maximum endophytes was isolated in Actinomyces media. The bacterial culture was extracted with ethyl acetate, and the crude extract was used for checking antimicrobial activity. The crude bacterial extract showed inhibitory activity against fungal pathogens F.oxysporium. In this study, bacterial molecular identification of the most active isolate was done by 16S ribosome RNA sequence analysis. On the basis of blast analysis of the 16S rRNA sequence, the strain identified it belonged to the Streptomyces sp. Group. The sequence of strain submitted in NCBI and obtained an accession number KJ872546 with name Streptomyces indiaensis. The sequence of the isolate were compared by alignment against 16S rDNA sequences, available in the GenBank Database using the BLAST program. Alignment and similarity comparison were initially conducted by the Clustal W method12 and the phylogenetic tree was constructed using Clustal W with the neighbour joining method. A boost strap analysis of 1000 replicates was carried out using the MEGA 5.05 software. The organism showed 100% gene sequence similarities with Streptomyces indiaensis. In GC-MS analysis 77 compounds were identified based on peak area percentage, Retention time, then the molecular formula, molecular weight (Table1). The major constituents are Chloroxylenol, 3-Pyrrolidin-2-yl-propionic acid , Pyrrolo{1,2-a}pyrazine-1,4-dioe,hexahydro-, Pyrrolo{1,2-a}pyrazine-1,4-dione,hexahydro-3-(2-methylpropyl)- ,5,10-Diethoxy-2,3,7,8-tetrahydro-1H,6H-dipyrrolo{1,2-a;1,2-d}pyrazine , Tetradecane,2,6,10-trimethyl and Benzamide, N-propyle the major constituents alone or along with minor constituents may provide the antibacterial activity.

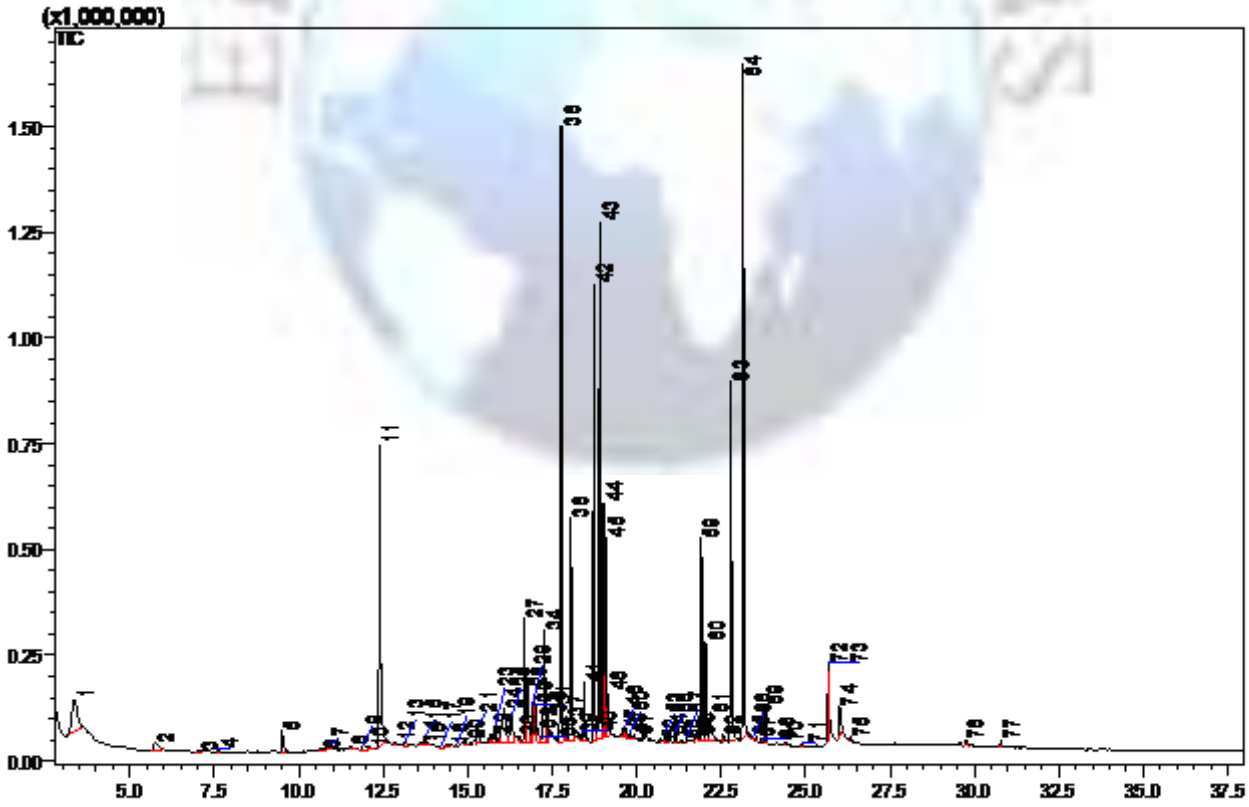

Figure (1) : GC-MS chromatogram of the ethyl acetate bacterial crude extract

Table (1): Compounds identified in the ethyl acetate bacterial crude extract by GC-MS

Peak	Retention Time (RT)	Area%	Height%	Compounds separated (Name of the Analytes)
1	3.345	2.77	0.65	Butanoic acid, 3-methyl
2	5.741	0.55	0.16	Dimethyl trisulfide
3	7.049	0.09	0.04	Pantolactone
4	7.608	0.12	0.08	Undecane
5	9.505	0.58	0.44	3-Cyclohexene-1-methanol,alpha,alpha,4-trimethyl
6	10.688	0.13	0.04	dl-Proline
7	10.889	0.10	0.06	5-Thiazoleethanol, 4-methyl
8	11.515	0.23	0.08	dl-Ornithine
9	11.902	0.36	0.11	2,4-Dimethoxy-5-methyl pyrimidine
10	12.072	0.11	0.06	Ethanone, 1-(2,4-dihydroxyphenyl)
11	12.386	6.66	5.98	Chloroxylenol
12	12.846	0.12	0.07	Pyrrolidine-1-acetonitrile,2,5-dioxo
13	13.155	0.15	0.10	2,4-Dihydroxy-5,6-dimethylpyrimidine
14	13.613	0.09	0.07	Formamide,N-{1-{(1-cyanopropyl)hydroxyamino}-2methylpropyl}
15	13.719	0.19	0.18	Phenol,2,6-bis(1,1-dimethylethyl)-4-methyl-,methylcarbamate
16	13.839	0.08	0.04	4,5-Pyrimidinediamine,6-methyl-
17	14.228	0.14	0.07	Pyrazine,2-thoxy-5-(2-methylpropyl)
18	14.455	0.16	0.07	3,6-Dimethylpiperazine-2,5-dione
19	14.670	0.05	0.05	3-Ethyl-4-hydroxybicyclo{3.3.1}non-3-en-2-one
20	14.924	0.23	0.13	Diazepan-2,4-dione
21	15.273	0.62	0.39	Pyrimido{1,2-a}azepine,2,3,4,6.7,8,9,10-octahydro
22	15.686	0.29	0.18	2,5-Piperazinedione,3-methyl-6-(1-methylethyl)
23	15.809	0.42	0.34	1,3-Dimethyl-3,4,5,6-tetrahydro-2(1H)- pyrimidinone
24	16.081	2.05	0.68	1-Pyrrolid-2-one,N-carboxyhydrazide
25	16.297	1.23	0.54	3-Ethyl-5-methylindolizidine
26	16.513	0.08	0.09	2,2-Dimethyl-N-phenethylproionamide
27	16.684	2.65	2.45	3-Pyrrolidin-2-yl-propionic acid
28	16.793	1.25	1.10	dl-Alanyl-1-leucine
29	16.893	0.64	0.70	dl-Alanyl-1-leucine
30	16.970	0.55	0.64	3-Pyrrolidin-2-yl-propionic acid
31	16.991	0.36	0.56	Benzene,1,2,3-trimethoxy-5-methyl
32	17.085	0.45	0.29	4-Pyrimidinol, 5-methoxy-
33	17.181	0.02	0.04	Maltol propionate
34	17.270	2.94	2.20	Pyrrolo{1,2-a}pyrazine-1,4-dioe,hexahydro-
35	17.362	0.57	0.59	DL-Threonine,N-glycyl-
36	17.767	12.12	12.16	Pyrrolo{1,2-a}pyrazine-1,4-dione,hexahydro-3-(2-methylpropyl)-
37	17.931	0.42	0.51	Piperazine-3,5-dione,1-tetradecanoyl-
38	18.057	4.21	4.42	Pyrrolo{1,2-a}pyrazine-1,4-dione,hexahydro-3-(2-methylpropyl)-
39	18.261	0.11	0.13	2-Piperidinone,1-(3,4,5,6-tetrahydro-2-pyridinyl)-
40	18.368	0.13	0.19	Hexadecanoic acid,methyl ester
41	18.451	1.08	1.13	1-(4-Aminofurazan-3-yl)-1-(1-pyrrolidinyl)methanone o-acetyloxime
42	18.736	8.22	9.03	Pyrrolo{1,2-a}pyrazine-1,4-dione,hexahydro-3-(2-methylpropyl)-
43	18.906	9.18	10.22	Pyrrolo{1,2-a}pyrazine-1,4-dione,hexahydro-3-(2-methylpropyl)-
44	19.018	4.29	4.64	Pyrrolo{1,2-a}pyrazine-1,4-dione,hexahydro-3-(2-methylpropyl)-
45	19.075	2.89	3.95	Pyrrolo{1,2-a}pyrazine-1,4-dione,hexahydro-3-(2-methylpropyl)-
46	19.127	1.01	0.91	5,10-Diethoxy-2,3,7,8-tetrahydro-1H,6H-dipyrrolo{1,2-a;1,2-d}pyrazine
47	19.591	0.21	0.17	Ethyl undecyl ether
48	19.659	0.22	0.21	Octadecanamide

49	19.726	0.06	0.09	4-(Pyrrolidin-2-ylcarbonyl)morpholine
50	19.806	0.07	0.09	1,2-Propanediol,3-(4-chlorophenoxy)-
51	19.925	0.07	0.05	N-Methyl-6-{4-methylphenyl}-1,2,4,5-tetrazinamine
52	20.710	0.09	0.07	2,5-Piperazinedione,3-methyl-6-(phynelmethyl)
53	20.840	0.18	0.19	Octadecanamide
54	20.963	0.12	0.13	2,5-Piperazinedione,3-methyl-6-(phynelmethyl)
55	21.140	0.20	0.21	N1-Isopropyl-2-methyl-1,2-propanediamine
56	21.332	0.06	0.07	2,5-Piperazinedione,3-(phynelmethyl)
57	21.480	0.08	0.09	2,5-Piperazinedione,3-benzyl-6-isopropyl-
58	21.748	0.08	0.10	Pyrrolo{1,2-a}pyrazine-1,4-dione,hexahydro-3-(2-methylpropyl)-
59	21.914	3.44	4.02	Pyrrolo{1,2-a}pyrazine-1,4-dione,hexahydro-3-(2-methylpropyl)-
60	22.043	1.86	1.98	5,10-Diethoxy-2,3,7,8-tetrahydro-1H,6H-dipyrrolo{1,2-a;1,2-d}pyrazine
61	22.174	0.94	0.56	Cyclo-(1-leucyl-1-phenylalanyl)
62	22.556	0.11	0.15	Ergotaman-3,6,18-trione,9,10-dehydro-12-hydroxy-2-methyl-5-(phynelmethyl)-,(5.alpha,10.alpha)
63	22.794	6.70	7.12	Tetradecane,2,6,10-trimethyl
64	23.158	11.14	13.30	Benzamide, N-propyle
65	23.383	0.03	0.06	1-Propanol,2-{2-(benzoyloxy)propoxy}-,benzoate
66	23.452	0.03	0.05	Diethylene glycol dibenzoate
67	23.556	0.06	0.08	1,2-Benzenedicarboxylic acid,mono(2-ethylhexyl) ester
68	23.706	0.05	0.07	Ile-pro-ile,trimethylsilyl ester
69	23.818	0.52	0.76	Nonadecane,9-methyl-
70	24.414	0.05	0.06	Decanedioic acid, bis (2-ethylhexyl)ester
71	24.902	0.07	0.08	5-Nitroso-2,4,6-triaminopyrimidine
72	25.675	0.77	1.36	Eicosane
73	25.685	0.90	1.36	10-12-Pentacosadiynoic acid
74	26.010	0.81	0.60	1,4-Dimethyl-8-isopropylidenetricyclo{5.3.0.0(4,10)}decane
75	26.315	0.06	0.08	
76	29.747	0.15	0.13	
77	30.786	0.15	0.14	

Actinomycetes are a prolific source of secondary metabolites. Around 23,000 bioactive secondary metabolites produced by microorganisms have reported, and Actinomycetes produce over 10,000 of these compounds, representing 45% of all bioactive microbial metabolites discovered **[**22**]** (Berdy, 2005). Among Actinomycetes, around 7,600 compounds are produced by Streptomyces species **[**22**]** (Berdy, 2005). Streptomyces species are distributed widely in aquatic and terrestrial habitats **[**23**]** Pathom-aree et al. (2006) and are of commercial interest due to their unique capacity to produce novel bioactive compounds. The main source for the bioactive secondary metabolites is soil streptomycetes , but a wide variety of structurally unique and biologically active secondary metabolites have recently been isolated from marine Actinomycetes, including those from the genus Streptomyces **[**24**],[**25**],[**26**],[**27**]**(Cho et al., 2001; Sanchez-Lopez et al., 2003; Lee et al., 2005; Jensen et al., 2005).

Production of secondary metabolites commonly precedes the development of aerial hyphae, when the growth rate of bacterial filaments has decreased and sporulation starts **[**28**]** (Bibb, 2005). Much of the published data indicate that the most important environmental signal triggering secondary metabolism is nutrient starvation, particularly that of phosphate **[**29**]** (Sola-Landa et al., 2003). The signaling networks behind the regulation of secondary metabolism in streptomycetes have recently been reviewed **[**28**]** (Bibb, 2005). Many of these secondary metabolites are potent antibiotics, which has made streptomycetes the primary antibiotic-producing organisms exploited by the pharmaceutical industry **[**22**]** (Berdy, 2005).
In this research, were studied Actinobacteria isolates for the production of secondary metabolites. Yields of the secondary metabolites produced were determined and isolate.The isolate was also tested for their in vitro activity on type culture collection of the pathogen F.oxysporium(Table 2), The Isolate showed stronger inhibition against F.oxysporium with an inhibition diameter of 71mm.

Table 2: In-vitro inhibition of Fusarium oxysporum by rhizobacterial isolates from chilli's rhizosphere on the basis of dual culture technique

Serial No.	Isolate	Radial growth (in mm)	Percentage inhibition of radial growth
1	At1	35_b	50.0%
2	At5	20_a	71.0%
3	At6	40_d	42.0%
4	At8	40_d	42.0%
5	At11	37_c	47.1%
6	At17	40_d	42.0%
7	At18	35_c	50.0%

Mean percentage inhibition :a The values given are mean (n= 3) with standard deviation. Means in the same column followed by same letter are not significantly different at P< 0.05 (Tukey's Honestly Significant Difference test). The results were an indication that although all the isolates showed activity on the test microorganism, the degree of activity was varying due to the different or varying concentrations of the active ingredient. GC-MS analysis of the secondary metabolites was carried out, and profiles of the fractions indicated the presence of different number of chemical compounds with different retention times and abundance. Among the compounds, 77 identified comprised of amides, amines, acids, pyrrolizidines, ketones, quinones, alcohols and hydrocarbons. Some of these compounds have been detected from Actinobacteria and documented. This isolated Streptomyces indiaensis with KJ872546 were isolated from different soil of chilli rhizosphere. Streptomyces sp has also produced These compounds together with resistomycin and tetracenomycin D. B8005 isolated from sediments of the Laguna de Términos at the Gulf of México **[**30**]** (Kock et al., 2005). The bioactive compounds in the bacterial crude extract had to be isolated, purified, and further in vivo studies have to carry out for future study.

CONCLUSION

The present investigation was an attempt to search for antifungal compounds from an alternate natural source endophytic bacteria. The study revealed the presence of good antifungal activity for the crude extracts of streptomyces. Against Fusarium oxysporium. So the Streptomyces indiaensis bacteria with accession number KJ872546 could be a good source of bioactive compounds and the isolated compounds may be a further check in in vivo model as an antifungal agent.

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