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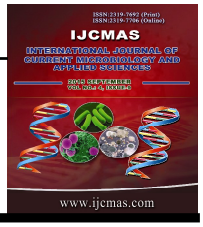

Original Research Article

Functional Characterization of Actinomycetes Isolated from the AM fungal (*Glomus mosseae*) Spores

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Glomus mosseae, Actinobacteria, PO_4 solublization, Hydrolytic enzymes, Organic acids

A B S T R A C T

Arbuscular mycorrhizae fungi play major role in plant growth promotion along with their associated bacteria. Ten actinomycetes sp from the AM fungal spores were characterized for their beneficial attributes. Most of the isolates grew well at pH 7 in 4% of NaCl which was found optimum. But *Streptomyces fradiae, S. cinnamonensis, S. violarus,* and *S. griseus* grew even at acidic and alkali pH with high salt concentration (16%). Studies on zinc solublization revealed that maximum solublization of all the three zinc substrates was caused by *S. cinnamonensis* (820.38±0.03 µg ml^{-1} ($ZnSo_4$): 668.23±0.01 µg ml^{-1} (ZnO_2): 504.15±0.04 µg ml^{-1} ($ZnCo_3$)) followed by *S.canus*. In enzyme assay *S. cinnamonensis* showed highest chitinase activity (87.10±0.08 U mg^{-1}) followed by *S. avermitilis*. *S. netropis* showed maximum amylase activity (4.8±0.24U mg^{-1}) followed by *S. cinnamonensis.* For cellulase, *S. canus* exhibited maximum activity (0.53±0.02 U mg^{-1}) followed by *S. griseus*. The highest lipolytic activity was found in *Leifsonia poea* (30.27±0.05 U mg^{-1}) followed by *S. scabiei* and the high protease activity was recorded in *S. albidoflaves* (121.9±0.04 U mg^{-1}) followed by *S. cinnamonensis*. Out of ten isolates *S.cinnamonensis* showed highest tri-calcium phosphate solublization (339.85±5.4 µg ml^{-1}) with the total acids production of 109127.8±22.7 µg ml^{-1} followed by *S. avermitilis* and *S. albidoflaves*. When aluminum phosphate was used as substrate *S.griseus* showed highest activity (423.78±2.5 µg ml^{-1}) with total acids production (2398.92±0.06 µg ml^{-1}) followed by *S. violarus* and *S. cinnamonensis*.

Introduction

Arbuscular mycorrhiza (AMF) fungi are well known for their effect on plant growth, nutrient mobilization and for their antagonistic effect on plant pathogens. The beneficial attributes of AMF are augmented through their associated bacteria. Among the associated bacteria, Actinobacteria are the most important due to their capacity to produce plant growth hormone, anti-microbial compounds and nutrient solublization by extracellular enzymes and organic acids (Thumar and singh, 2007: Dietera *et al.,* 2003: Salami, 2004: Padmadhas and Ragunathan, 2010 and

Santos *et al.,* 2012). The growth conditions like optimum pH and salinity may influence the growth and production of important secondary metabolites (Naik et al, 1982: Shesadhri et al, 2000) from microbes. Extracellular enzymes and organic acids from the soil microbes play major role in solublizing soil bound nutrients to facilitate plant growth (Oyeleke *et al.,* 2010 & Kafilzadeh *et al.,* 2012), improve of plant defense mechanism and support AM fungal colonization (El–Tarabily KA, 2003:Cherry *et al.,* 2004: Yassien and Astuur, 2011:Jang and Chen, 2003:Haki and Rakshit, 2003). Around 90% of Chitinase enzymes and their related compounds are produced by actinobacteria (Mukherjee G and Sen SK, 2004) against pathogenic fungi and insects.

They are studied for their production of α-amylase enzyme (Kafilzadeh *et al.,* 2012: Selvam. *et al.,* 2012) with thermo-stability (kar *et al.,* 2010: Burhan *et al.,* 2003). AM fungi colonizing actinobacteria aid in softening the cell wall of plant roots through their cellulase production there by facilitating the fungal hypal penetration and help in colonization (Arunachalam *et al.,* 2010). Major *streptomyces* sp are the great producers of lipase enzyme which use to synthesize organic chemicals (Hassan *et al.,* 2006: Luthara and Dubey, 2012) at large scale.

Beneficial bacterial proteolytic enzymes play an important role in plant disease management (Illakiam *et al.,* 2013). Among the beneficial bacteria streptomyces sp are noteworthy for protease enzyme production (Deepthi et al, 2012: Al-Askar and Rashad, 2015) and broad spectrum antimicrobial activity against soil pathogens (Kieser *et al.,* 2000: Shahidi *et al.,* 2004: Oskay, 2009: Kaur *et al.,* 2013). In soil, phosphate is the second major nutrient for plants after the nitrogen, but being in insoluble form they are not absorbed by plants. The solublization of phosphate in soil in order to enable plants to absorb them is important. There are many soil microbial communities which have the potential to solubilize the bound phosphate by producing of organic acids, but the capacity to produce organic acids may differ from microbes to microbes. Actinobacteria are well documented for their ability to solubilize phosphate (Balakrishna, *et al.,* 2012: Kaviyarasi, *et al.,* 2011: Dastager and Damare, 2013) but the information on organic acid production by actinobacteria to solubilize phosphate is scanty. Therefore the present study was designed to study the ability of actinobacteria from the AM fungal spores to solubilize the phosphate, zinc and to produce hydrolytic enzymes and organic acids by using different substrates at optimum growth conditions.

Materials and Methods

Isolation of arbuscular mycorrhizae and its associated actinobacteria

The dominant AM fungal spores were isolated from the guava rhizosphere soil following Sukhada et al (2013) by using wet sieving and decantation method (Gerdemann and Nicolson, 1963). The molecular identity of the spores was confirmed by using ITS 1 & 4 primers. The identified spores were further surface sterilized (Tommerup and Kidby, 1980) and the crude extract of the spore was plated on nutrient agar media to isolate its associated bacteria.

Ten actinobacteria were isolated and further sub cultured. The molecular identity of the actinomycetes was done by using rPoB (RNA polymerase β-sub unit gene) primer (Kim *et al.,* 2003).The identified colonies were further purified on the Ken Knight agar media for the characterization studies.

Growth tolerance of actinobacteria at different pH and NaCl concentration

The Ken Knight medium (Dextrose- 0.1g, KH_2PO_4-0.11g, $NaNO_3$-0.01g, NaCl- 4-16g, $MgSo_4$-0.01g, pH-5-10, Agar-2.0g) was prepared in two sets and the pH of one set was maintained in the range of 5-10 to check acidic and alkali nature of the isolates. Another set with the different NaCl concentration (4-16 %) was used to check the saline tolerance of the isolates. A loop full of each purified actinobacteria was streaked on both the media and incubated at 28 $\pm 2^0$C for 7 days.

Phosphate and Zinc solublization by actinobacteria

The capacity of actinobacteria to solubilize phosphate and zinc was tested by using the following media. For phosphate solublization the media contained Glucose-1.0g, Ca_3PO_4-0.5g, (NH_4) SO_4-0.05g, Kcl-0.02g, $MgSO_4$-0.01g, yeast extract-0.05g,and Agar-2.0g at pH-7 (Ayyaku and Chandramohan, 1971) and for zinc solublization the media contained glucose – 1.0g, NH_4SO_4- 0.01g, Kcl-0.02g, KH_2PO_4-0.01g, $MgSO_4$-0.02g, $ZnSO_4/ZnO_2/ZnCO_3$- and 1.0g, Agar-2.0 at pH 7.0 (Saravanan *et al.*, 2003).

One ml of each isolates at 10^8 cfu ml^{-1} was inoculated in broth (100ml) and incubated at 30^0C for 7 days at 60rpm and the media without inoculation was used as control. After incubation, cultures were centrifuged at 10000rpm for 10mts and the supernatant was taken for the further assay. The solubilized tri-calcium phosphate and Zinc from the culture supernatant were quantified through Atomic Absorption Spectrophotometer (AAS) (Murphy & riley 1962: Saravanan *et al.*, 2003).

Quantification of organic acids production by actinobacteria

For the quantification of organic acids, 100 ml of broth containing 10 g glucose, 1g of $Ca_3(PO_4)$ $_2/AlPO_4$-0.5g, $MgCl_2.6H_2O$-0.5g, $MgSO_4$ $\cdot 7H_2O$-0.025g, KCl-0.02g and $(NH_4)_2SO_4$-0.01g was prepared and 1ml (10^8 cfu/ml) each of individual isolate was inoculated into the media and incubated at 30^oC for 7days in shacking incubator at 60rpm along with control broth. After seven days incubation the culture was centrifuged at 10000 rpm for 10 minutes at room temperature and the supernatant was collected. The drop in pH from 7 indirectly indicated the acidic nature of supernatant. Further the supernatant was filtered through 0.25µM filters (Pall Bioscience, USA) and different organic acids produced were analyzed using HPLC (Model: Prominence, Shimadzu, Japan). The reference standards for the organic acids were purchased from the SIGMA (Sigma-Aldrich, USA) with the 98% of purity for the detection and quantification of organic acid form the samples.

Production and quantification of Hydrolytic enzymes from actinobacteria

All the isolates were tested for their ability to produce hydrolytic enzymes (Chitinase, Cellulase, Amylase, Protease, and Lipase) by using specific media with respective substrates. For Chitinase production the media (100ml) contained $(NH_4)_2SO_4$-0.1g, $KH_2PO_4$0.02g, K_2PO_4-0.16g, $MgSO_4.7H_2o$,-0.001g, $CaCl_2.2H_2o$-0.002g, colloidal chitin-1.2%, pH-7.0: agar-2.0g (Skujins *et al.*, 1965: Hoster *et al.* 2005). For testing cellulase production, media (100ml) contained KH_2PO_4-0.05g, $MgSO_4$-0.025g, CMC-0.2g, Congo red- 0.02g, Gelatin-0.2g, pH-7: Agar-2.0g (Ariffin *et al.*, 2006) was used. For testing amylase production, media

(100ml) contained yeast extract-0.05g, $(NH_4)_2SO_4$-0.1g, $MgSO_4.7H_2o$-0.03g, KH_2PO_4-0.136g, soluble starch-2%, pH-7: Agar-2.0g (Smibert & Krieg 1994). Protease enzyme production was quantified by using media (100ml) containing, Casein -0.5g, yeast extract-0.25g, Dextrose-0.1g, KNO_3 0.2g, NaCl 0.2g, pH-7.0, Agar-2.0g (Berg *et al.,* 2005). For the lipase production the cultures were inoculated in media (100ml) containing yeast extracts-0.4g, malt extract-1.0g, dextrose-0.4g, olive oil-1ml, pH-7, Agar-2.0g for (Kouker and Jaeger, 1987). For the enzyme specific activity, 1ml of each isolates were inoculated in 100ml of the respective broths and incubated at 28± 2^o C at 60rpm for 7 days. After incubation period all the broths broth was centrifuged individually at 10000 rpm for 10mins at room temperature. All the supernatants were filtered through 0.45µM filters and tested for their specific activity (Chitinase- Boller and Mauch, 1998: Cellulase- Breuil and Saddler, 1985: Amylase- Rick and Stegbauer, 1974: Protease- Tsuchida et al, 1986: Lipase- Wantanabe et al, 1977).

Statistical analysis

The data were analyzed using Web Agri Stat Package version WASP1.0 and Graph pad prism 6.

Results and Discussion

Isolation of arbuscular mycorrhizae and its associated actinobacteria.

The major microbes associated with *Glomus spores* were identified as act1-*Streptomyces fradiae,* act 2- *S.avermitilis,* act3- *S.cinnamonensis,* act4- *Leifsonia poea,* act5- *S.canus,* act6-*S.netropis,* act7-*S.scabiei,* act12- *S.gresius,* act11- *S.violarus* and act13- *S.albidoflaves* and purified on the selective media for further studies.

Growth tolerance of actinobacteria at different pH and NaCl concentration

The growth tolerance was observed from the third day of inoculation on both types (NaCl and pH) of media. Growth at pH 5-10 indicates the acidic and alkali nature of the isolates. Compared with other isolates *L.poea, S.canus, S.violarus* and *S.griseus* were grown well at pH 5-9 (Table 1). Out of ten isolates high saline tolerance was observed in *S.fradiae, S.cinnamonensis, S.violarus* and *S.griseus* at 16% of NaCl (Table 2).

Phosphate and Zinc solublization by actinobacteria

Phosphate and Zinc solublization by the actinomycetes in the liquid media was evident from the significant pH drop from the initial pH 7.0. After incubation the total amount of solubilized phosphate was around 64.88±0.1- 369.83±5.4 µg ml^{-1} followed by $ZnSO_4$ (540.13±0.10-942.25±0.04 µg ml^{-1}), ZnO_2 (51.17±0.02-750.21±0.00 µg ml^{-1}) and $ZnCO_3$ (65.23±0.02-504.15±0.04 µg ml^{-1}).

The maximum phosphate solublization was observed in *S.* cinnamonensis (369.85±5.4 µg ml^{-1}) with the pH of 3.5 followed by *S.avermitilis* (162.74±1.2 µg ml^{-1}) at pH 4.5. *L.poea showed* maximum $ZnSO_4$ (942.25±0.04 µg ml^{-1}) solublization with pH drop 3.0 followed by *S.violarus* (875.23±0.03 µg ml^{-1}) and *S.cinnamonensis* (820.38±0.03 µg ml^{-1}) with the pH 3.5. ZnO_2 was solubilized to the maximum extent by *S.albidoflaves* (750.21±0.00 µg ml^{-1}) and *S.cinnamonensis* (668.23±0.01 µg ml^{-1}) at pH 3.5. In $ZnCO_3$ media *S.cinnamonensis* showed maximum activity (504.15±0.04 µg ml^{-1}) followed by *S.canus* (446.25±0.04 µg ml^{-1}) with the pH of 4-4.5 (Table 3).

Quantification of Organic acids production from the actinobacteria

HPLC analysis of culture supernatant showed the presence of organic acids released by the actinobacteria. Totally 10 different organic acids gluconic acid, 2-Keto glutaric acid, tartaric acid, formic acid, Malic acid, Malonic acid, lactic acid, citric acid, succinic acid, propionic acid were detected by using two different substrates. The numbers of acids from the actinobacteria was significantly differed from each other. When using tri-calcium phosphate media, *S.fradiae, L.poea, S.canus, S.scabiei,* and *S.gresius* secreted more than 8 acids in the broth. Compared with other strains the total organic acid production was found maximum in *Streptomyces cinnamonensis* (109127.8±22.7 µg ml^{-1}) followed by *Streptomyces scabiei* (102094.6±26.1 µg ml^{-1}) and the lowest was found in *Streptomyces netropsis* (1356.77±14.1 µg ml^{-1}).

When aluminum phosphate was used as substrate *S.gresius* produced very high level of Gluconic acid (2397.69±0.03 µg ml^{-1}) with the total acids production (2398.92±0.06 µg ml^{-1}), followed by *S.violarus* (387.82±0.15 µg ml^{-1}) and *S.cinnamonensis* (311.00±0.1 µg ml^{-1}) (Table 4 a&b).

Production and quantification of Hydrolytic enzymes from actinobacteria

The production of hydrolytic enzymes by the actinobacteria varied significantly from each other (Fig. 1). After incubation period, the maximum specific activity of chitinase (87.10±0.08 U mg^{-1}) was observed with *S.cinnamonensis* followed by *S.avermitilis* (81.59±0.00 U mg^{-1}). There was no prominent production of cellulase enzyme found in the cultures. Among the cultures *S.canus* and *S.gresius* showed maximum cellulase activity (0.53±0.02 U mg^{-1}) followed by *S.avermitilis* (0.43±0.04 U mg^{-1}). Highest specific activity of amylase (4.8±0.24 U mg^{-1}) was observed with *S.netropis* and *S.cinnamonensis* (3.02±0.01U mg^{-1}). *S.albidoflaves* showed maximum specific activity of protease (121.9±0.04 mg^{-1}) followed by *S.cinnamonensis* (117.09±0.07 U mg^{-1}). Lipase specific activity was found maximum in *L.poea* (30.27±0.05 U mg^{-1}) followed *S.canus* (28.42±0.01 U mg^{-1}) and *S.scabiei* (24.67±0.05U mg^{-1}).

Actinobacteria are well known for impacting beneficial effect on plant growth and protecting against pathogens. The present study was conducted with ten actinomycetes species isolated from the *Glomus mosseae* to demonstrate the growth stability of actinobacteria in different pH and NaCl level, their ability to solubilize phosphate and zinc, and produce organic acids and hydrolytic enzymes.

Phosphate and zinc solublization

pH and salt concentrations are the main factors for the growth of bacteria and their secondary metabolites production (Ventosa *et al.*, 1998). Dastager and Damare, (2013) have reported the ability of Actinobacteria specially streptomyces sp to solubilize phosphate, they recorded the solubilized phosphate in the range of 89.3±3.1 to 164.1±4.1 µg ml^{-1} after the six days of incubation with gradual decline in pH level. In our study the streptomyces sp isolated from the AM spores solubilized phosphate in a range between 64.88 – 339.83 µg ml^{-1}. Highest range was observed in the *S.cinnamonensis* (339.83 µg ml^{-1}) with pH of 3.0 followed by *S.avermitilis* (162.78 µg ml^{-1}). Bacterial species like *Bacillus, Pseudomonas, Brevibacillus parabrevis,*

Providencia rettgeri have been studied for their Zinc solublization activity (Saravanan *et al.,* 2003: Panneerselvam et al, 2013), but reports on zinc solublization by Streptomyces sp are scanty, so in the present study we analyzed the zinc solublization activity of streptomyces isolated from the AM spores. Out of ten isolates, *S.cinnamonensis* showed standard activity on three zinc substrates with the pH drop to 3.5 from 7.

Hydrolytic enzymes

Chitinolytic activity is a major attribute of streptomyces sp to be used as bio control agent. Prapagdee *et al.,* (2008) have tested the antifungal activity of extracellular chitnase enzyme against pathogenic fungi *Colletotrichum gloeosporioides* and *Sclerotium rolfsii* and found maximum Chitinase activity as 5.2 U/mg $^{-1}$.

Tang-um and Niamsup, 2012 reported that the endophytic *Streptomyces* sp P4 had Chitinolytic activity of 0.050 U/mg^{-1} against *Fusarium oxysporum* f.sp. *lycopersici.* In our study, after seven days incubation the maximum production of the Chitinase enzyme with specific activity of 87.10 U mg^{-1}was observed in *S.cinnamonensis* followed by *S. avermitilis* (81.59 U mg^{-1}) and the antifungal activity of these isolates was tested by dual culture assay (Debananda *et al.,* 2009) against *Colletrotricum capsici, F lycopersicum,* and *P.capsici* (Fig. 2).

Table.1 Growth tolerance of actinomycetes at different pH and NaCl concentration

Isolates	pH-5	pH-6	pH-7	pH-8	pH-9	pH-10
S.fradiae	+	+	+	+	+	-
S.avermitilis	+	+	+	+	+	-
S.cinnamonensis	+	+	+	+	+	+
Leifsonia poea	+	+	+	+	+	+
S.canus	+	+	+	+	+	+
S.netropis	-	+	+	+	+	-
S.scabiei	+	+	+	+	+	-
S.albidoflaves	+	+	+	+	+	-
S.violarus	+	+	+	+	+	+
S.griseus	+	+	+	+	+	+

+ Positive growth, - No growth.

Table.2 Growth tolerance of actinomycetes at different NaCl concentration

Isolates	4%	8%	12%	16%
S.fradiae	+	+	+	+
S.avermitilis	+	+	+	-
S.cinnamonensis	+	+	+	+
Leifsonia poea	+	+	-	-
S.canus	+	+	+	-
S.netropis	+	+	-	-
S.scabiei	+	+	+	-
S.albidoflaves	+	-	-	-
S.violarus	+	+	+	+
S.griseus	+	+	+	+

+ Positive growth, - No growth.

Table.3 Zinc solublization by Actinomycetes

Isolates	Total solublization of ZnSo4 µg ml^{-1}	Final pH	Total solublization of ZnO2 µg ml^{-1}	Final pH	Total solublization of ZnCO3 µg ml^{-1}	Final pH
S.fradiae	565.270±0.03	5.3	79.37±0.05	6	339.22±0.02	4.5
S.avermitilis	645.21±0.03	4.5	60.47±0.02	6	422.23±0.03	4
S.cinnamonensis	820.38±0.03	3.5	668.23±0.01	4	504.15±0.04	4
Leifsonia poea	942.25±0.04	3	51.17±0.02	6	50.15±0.04	6.8
S.canus	645.15±0.04	4.8	610.29±0.02	4	405.3±0.04	4
S.netropis	540.13±0.10	5	180.16±0.04	5.4	320.25±0.02	4.5
S.scabiei	675.29±0.04	4.5	350.21±0.00	4.5	446.25±0.04	3.6
S.albidoflaves	875.23±0.03	3.8	750.21±0.00	4	175.2±0.05	6
S.violarus	675.16±0.04	4.5	239.19±0.02	6	65.23±0.02	6.8
S.griseus	618.16±0.04	4.5	62.16±0.05	6	233.23±0.02	5
Control	0	6.93	0	6.9	0	6.91
SEM	0	0.979901	0	0.986604	0	1.223857
CD at 5% (0.05)	0.099	0.204	0.067	0.252	0.065	0.337

ND= Non detected

Table.4 Quantification of Specific enzyme activity of mycorrhizae associated Actinomycetes by using different substrate

Isolates	Chitinase activity U mg^{-1}	Amylase activity Umg^{-1}	Cellulase activity U mg^{-1}	Lipase activity U mg^{-1}	Protease activity U mg^{-1}
S.fradiae	40.19±0.00	0.39±0.03	0.4±0.04	12.79±0.07	37.84±0.0
S.avermitilis	81.59±0.00	0.32±0.00	0.45±0.04	12.33±0.02	107.09±0.07
S.cinnamonensis	87.10±0.08	3.02±0.01	0.1±0.04	17.02±0.01	117.09±0.07
Leifsonia poea	19.44±0.03	1.63±0.01	0.33±0.02	30.27±0.05	32.49±0.07
S.canus	7.12±0.01	2.5±0.20	0.53±0.02	28.42±0.01	37.11±0.0
S.netropis	6.38±0.06	4.8±0.24	0.3±0.04	20.18±0.06	36.15±0.04
S.scabiei	ND	1.15±0.04	0.25±0.04	24.67±0.05	34.52±0.01
S.albidoflaves	0.8±0.08	0.71±0.02	ND	6.89±0.07	121.9±0.04
S.violarus	9.76±0.06	2.1±0.08	0.18±0.01	ND	64.5±0.04
S.griseus	11.64±0.03	2.09±0.07	0.53±0.02	12.58±0.06	41.38±0.06
Control	0	0	0	0	0
SEM	30.965	1.351	0.169	9.096	0.168
CD at 5% p(0.05)	0.743	0.161	0.075	0.096	0.168

ND= Non detected

Table.4a Quantification of different organic acids produced by mycorrhizae associated actinomycetes for the solublization of tri-calcium phosphate

Isolates	Total P liberated µg ml^{-1}	Final pH	Gluconic acid µg ml^{-1}	2-Keto glutaric acid µg ml^{-1}	Tartaric acid µg ml^{-1}	Formic acid µg ml^{-1}	Malic acid µg ml^{-1}	Malonic acid µg ml^{-1}	Lactic acid µg ml^{-1}	Citric acid µg ml^{-1}	Succinic acid µg ml^{-1}	Propionic acid µg ml^{-1}	Total acids production µg ml^{-1}
S.fradiae	120.44±1.6	4.51	5686.59±3.4	304.51±2.3	29.35±2.3	4.13±0.5	ND	359.5±5.2	3517.3±1.7	2454.3±2.1	931.12±6.3	ND	1514.31±28.9
S.avermitilis	162.74±1.2	4.31	8096.11±3.4	421.91±1.7	ND	29.63±5.1	ND	ND	ND	2938.86±4.6	ND	235.3±2.8	11721.81±17.6
S.cinnamonensis	339.85±5.4	3.52	106545.21±2.8	703.4±1.7	65.23±2.8	71.56±3.4	ND	376.49±3.4	338.32±4.6	ND	ND	ND	109127.8±22.7
Leifsonia poea	104.64±2.3	3.82	6051.11±2.8	103.32±1.7	872.9±1.1	81.31±0.5	2467.6±4.0	1918.81±4.6	4939.38±5.1	1546.71±3.4	ND	137.12±4.0	21363.35±29.9
S.canus	126.1+2.7	4.1	239.65±2.3	100.51±2.8	826.33±3.4	113.12±1.7	7402.61±1.1	1101.83±1.7	3150.28±2.8	838.25±4.6	ND	204.51±2.3	13977.09±22.9
S.netropis	78.95±1.7	4.81	269.87±5.1	59.59±2.3	70.08±2.8	ND	ND	372.1±1.1	585.13±2.8	ND	ND	ND	1356.77±14.1
S.scabiei	115.45±3.0	4.01	91939.41±5.1	48.53±1.7	28.5±1.7	ND	ND	356.65±3.4	285.21±2.8	38.45±1.7	348.13±4.6	234.15±2.3	102094.6±26.1
S.albidoflaves	143.68±1.7	4.3	36504.16±2.3	403.2±1.7	23.18±1.7	ND	4370.2±2.8	2793.81±1.7	4476.6±3.4	2351.12±2.8	638.25±4.6	141.42±0.5	51701.94±21.5
S.violarus	127.65±4.0	4.52	47357.63±4.0	ND	818.21±4.6	56.47±3.4	3817.24±4.6	1118.92±4.6	3632.91±1.1	3192.21±1.1	ND	ND	59993.59±22.3
S.griseus	64.88±0.1	5.08	136.99±3.4	182.9±1.1	ND	ND	ND	ND	822.5±1.1	ND	ND	233.79±1.7	1376.18±7.3
Control	0	6.93	0	0	0	0	0	0	0	0	0	0	0
Total production µg/ml	1384.38±23.7	-	302826.73±34.6	2327.87±17.0	2733.78±20.4	356.22±14.6	18057.65±12.5	8398.11±25.7	21747.63±25.4	13359.9±20.3	1917.5±15.5	1186.29±13.6	-
CD at 5% P(0.05)	7.805	0.251	0.106	0.09	0.074	0.047	0.055	0.088	0.132	0.06	0.044	0.141	-

Table.4b Quantification of different organic acids produced by mycorrhizae associated actinomycetes for the solublization of Aluminium phosphate

Isolates	Total P liberated µg ml^{-1}	Final pH	Gluconic acid µg ml^{-1}	2-Keto glutaric acid µg ml^{-1}	Tartaric acid µg ml^{-1}	Formic acid µg ml^{-1}	Malic acid µg ml^{-1}	Malonic acid µg ml^{-1}	Lactic acid µg ml^{-1}	Citric acid µg ml^{-1}	Succinic acid µg ml^{-1}	Propionic acid µg ml^{-1}	Total acids production µg ml^{-1}
S.fradiae	95.47±1.6	3.7	4.7±0.0	0.05±0.0	0.03±0.0	0.005±0.0	ND	0.34±0.0	0.40±0.0	0.15±0.0	ND	0.23±0.0	5.905±0.0
S.avermitilis	132.23±0.8	3.2	13.97±0.01	0.04±0.0	0.03±0.0	ND	ND	ND	0.99±0.0	0.11±0.0	ND	0.23±0.02	15.37±0.03
S.cinnamonensis	257.90±3.3	3.7	305.39±0.0	ND	0.03±0.01	0.001±0.0	ND	4.55±0.04	ND	0.80±0.04	ND	0.23±0.01	311.001±0.1
Leifsonia poea	102.92±1.6	3.4	5.08±0.01	0.04±0.0	0.03±0.0	ND	ND	0.42±0.01	0.38±0.02	0.06±0.0	ND	0.23±0.0	6.24±0.04
S.canus	145.34±2.4	3.2	17.24±0.0	0.04±0.01	0.03±0.0	0.005±0.0	ND	1.48±0.06	0.53±0.02	0.15±0.01	ND	0.23±0.0	19.705±0.1
S.netropis	124.53±0.0	4.0	39.31±0.0	0.06±0.0	0.07±0.0	ND	ND	0.37±0.01	0.59±0.0	ND	ND	ND	40.4±0.01
S.scabiei	116.64±3.2	3.7	7.82±0.01	0.05±0.01	0.03±0.0	ND	ND	0.36±0.01	0.29±0.01	0.04±0.0	0.35±0.02	0.23±0.0	9.17±0.06
S.albidoflaves	76.98±0.0	3.5	2.35±0.04	0.04±0.0	0.03±0.0	ND	ND	ND	12.74±0.03	ND	0.05±0.0	0.23±0.01	15.44±0.08
S.violarus	278.65±2.4	3.5	380.77±0.05	ND	ND	ND	ND	0.39±0.03	5.65±0.04	ND	0.78±0.01	0.23±0.02	387.82±0.15
S.griseus	423.78±2.5	2.7	2397.69±0.03	0.18±0.01	ND	ND	ND	ND	0.82±0.01	ND	ND	0.23±0.01	2398.92±0.06
Control	0	6.85	0	0	ND	0	0	0	0	0	ND	0	0
Total production µg/ml	1754.44±17.8		3174.32±0.15	0.5±0.03	0.28±0.01	0.011±0.0	-	7.91±0.16	22.39±0.13	1.31±0.05	1.18±0.03	2.07±0.07	-
CD 5% P(0.05)	4.284	0.257	0.067	0.021	0.016	0.001	-	0.055	0.043	0.029	0.019	0.030	-

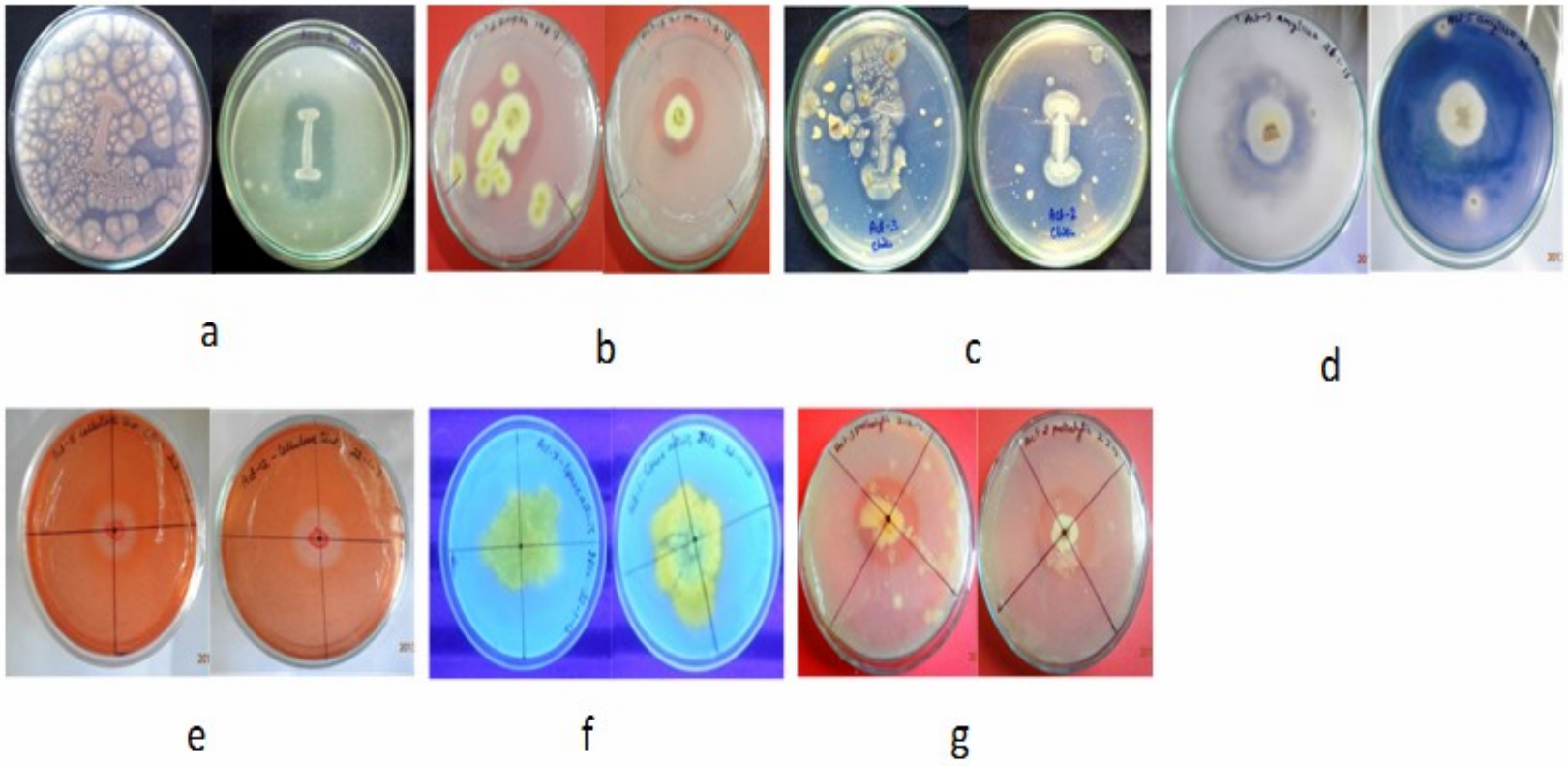
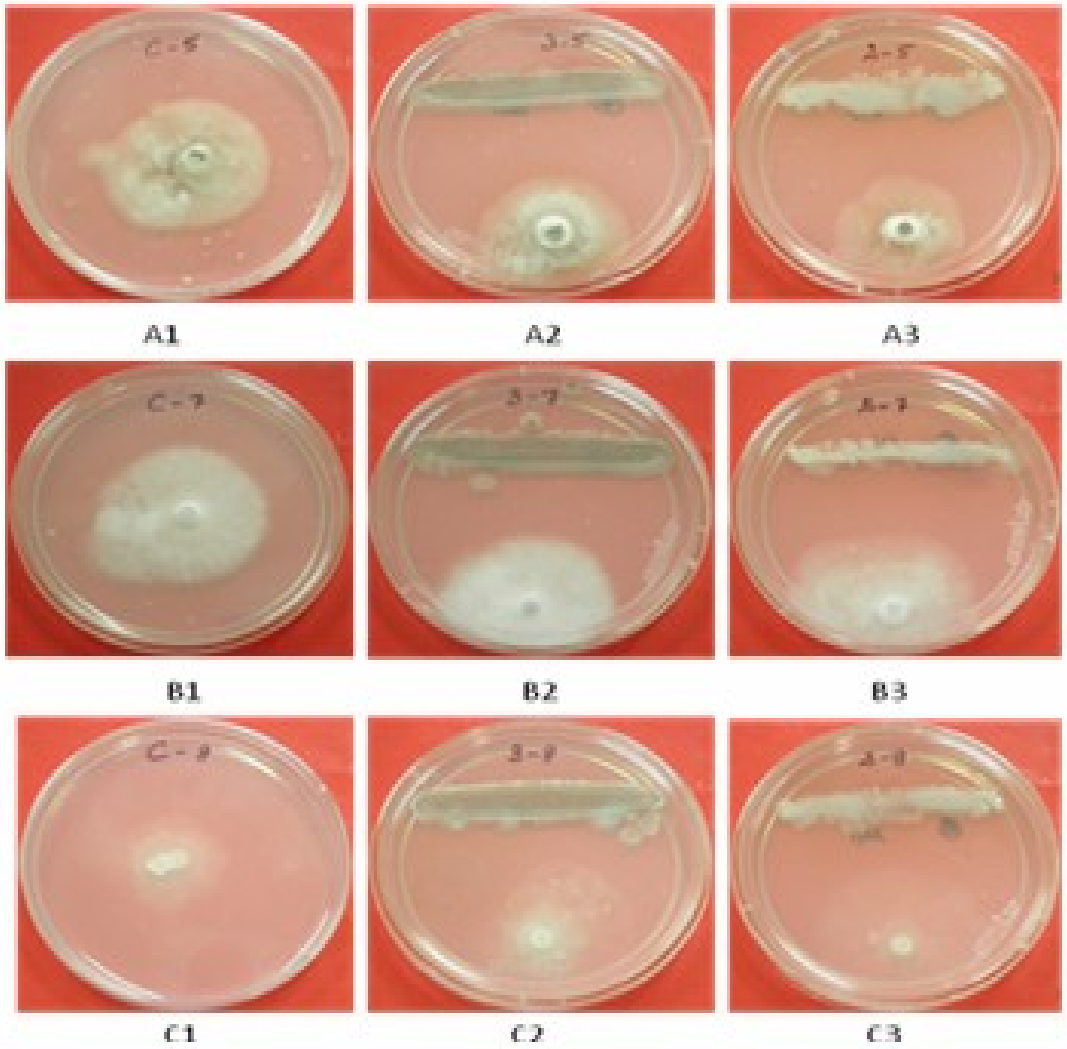


Figure 1: a- Phosphate solublization activity of Act2&3 on tri-calcium phosphate medium. b- Zinc solublization activity of Act3&5 on media enriched with ZnPo4. C- Chitinolytic activity of Act2&3 on colloidal chitin media d- Amylase activity by Act 3&5 on starch agar media. e- Cellulase enzyme activity by Act5&12 on Congo-red agar media. f-Lipase enzyme activity by Act4&7 on olive oil enriched media. g- Proteolytic enzyme activity by Act 3&2 on casein agar media.

Figure.2 Antifungal activity of S.cinnamonensis and S.avermitilis against C.capsici, F.lycopersicum and P.capsici



A1 - Control *Coletrotricum capsici*, **A2, A3-Antifulgal activity of** *S.cinnamonensis* **and** *S.avermitilis* **against** *C.capsici.*

B1 - control *Fusarium lycopersicum*, **B2, B3-Antifulgal activity of** *S.cinnamonensis* **and** *S.avermitilis* **against** *F.lycopersicum.*

C1 - control *Phytopthora capsici*, **C2, C3-Antifulgal activity of** *S.cinnamonensis* **and** *S.avermitilis* **against** *P.capsici*

Enzymes which are industrially important are produced by *streptomyces* sp in large scale. Ragunathan and Padhmadas (2013) recorded 56U/mL amylase activity after 4 days incubation from the *streptomyces* sp isolated from the Western Ghats.

Selvam, *et al.,* (2011) isolated the marine *Streptomyces* sp from the south Indian costal region and studied the amylase production and recorded the maximum activity of 6.48 U/mL In the present study *S.cinnamonensis* and *S.canus* showed the maximum specific activity 3.02U mg^{-1}, 2.50U $mg^{-1}$of amylase with soluble starch. Synthesis of cellulase from *streptomyces* sp has been reported by many workers. Prasad *et al.,* (2013) have analyzed the cellulase in a *Streptomyces* sp by using filter paper and Carboxyl methyl cellulose (CMC) as substrate and recorded maximum activity of 5.6 mg/mL. Selvam, *et al.,* (2011) has also recorded the maximum activity of cellulase by *streptomyces* sp as 8.93 U/mL followed by 3.24 U/mL by using CMC substrate. In our study, out of ten isolates *S.canus* and *S.gresius* recorded maximum activity (0.53 U mg^{-1}) followed by *S.avermitilis* (0.45 U mg^{-1}) but compared with other enzymes cellulase production is considerably less in all the isolates. Lipase is one of the most important enzymes produced by the microbes especially bacteria.

There are many reports on production of lipase by streptomyces. Vishnupriya *et al.,* (2010) investigated *Streptomyces griseus* for the production of lipase with different substrate. Maximum activity was recorded with Sunflower oil and Palm oil at pH 6-9 in 48 hr incubation period. Selvam, *et al.,* (2011) recorded the maximum activity of lipase (700 U/mL) with olive oil as substrate. In our study the maximum production of lipase enzyme was observed with *L.poea* with the specific activity of 30.27 U mg^{-1} followed by *S.scabiei* (24.67 U mg^{-1}) using olive oil. Protease from the bacterial origin is most significant than the animal, or fungal proteases, *S*treptomyces sp scoring above all. Jayasree, *et al.,* (2009) reported for the first time alkaline protease production by *Streptomyces pulvereceus* when different carbon sources were used.

The maximum activity of 252 U/mL was attained with 0.3% starch, 1% casein and 1% NaCl concentration. In the present study the protease activity of *S.cinnamonensis* (117.09 U mg^{-1}) and *S.avermitilis* (107.09 U mg^{-1}) was observed by using skimmed milk powder as substrate. Based on the substrate selection the protease production varied gradually among the isolates.

Organic acids production

Phosphates solublizing bacteria play a key role in plant growth enhancement. The phosphate solublizing bacteria actinomycetes are well known for the solublization of calcium bound phosphates in soil (Shau *et al.,* 2007: Balakrishna *et al.,* 2012: Dastager and Damare, 2013).The organic acid production from the phosphate solublization bacteria have been reported by many workers but the reports for actinomycetes are very less. In the present study, the ten isolates were studied for the production of organic acids which solubilized the Tri-calcium and Aluminium phosphates. Out of ten isolates, the maximum phosphate solublization activity was observed in *S.cinnamonensis* with total organic acids production (109127.8±22.7 µg ml^{-1}) followed by *S.avermitilis* (11721.81±17.6 µg ml^{-1}) and *S.albidoflaves* (51701.94±21.5 µg ml^{-1}). When using with aluminum phosphate, the Gluconic acid production was found maximum in *S.gresius* (2397.69±0.03 µg ml^{-1}) followed by

S.violarus (387.82±0.15µg ml^{-1}) and *S.cinnamonensis* (311.001±0.1µg ml^{-1}).
Compared with tricalcium phosphate, the overall production of organic acids was less when aluminum phosphate was used as substrate. The level of phosphate solublization is highly dependent on the quantity of major organic acids secreted by the organisms. Vyas and Gulati, (2009) reported the role of gluconic and succinic acids (by *Pseudomonas* sp) in solublization of calcium bound phosphates for the crop improvement and yield but in the present study we found the secretion of succinic acid from only few actinomycetes strains which also produced gluconic acid in enough quantity, but the level of phosphate liberated greatly varied with the presence of either single or both the acids. Out of 10 acids the Gluconic acid (which plays major role in phosphate solublization) production was found common in all the strains with both the substrates, remaining were optional. But the selection priority for the appropriate acids by the microbial cells for the solublization of bound phosphates is still unclear.

In conclusion, from the above study the application of these actinobacteria as bio-inoculum will enhance the disease resistance and plant growth in less nutrient soil and also will help to produce disease free seedlings at nursery level. Mixed form of these cultures can be used as bio-inoculums for the large scale field application.

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