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Studies on drought tolerate mediated plant growth-promotion by the application of actinobacteria isolated from marine sources

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Abstract
The use of plant growth-promoting (PGAB) action bacteria for improving and mange drought condition this study aimed to evaluate the effectiveness of salt loving actinobacteria isolated from Cochin port kerala. Actinomycetes from marine sediments isolated by conventional method and totally 16 strains fall in five different genera were selected. All the strain subjected to plant growth attribution. actinomycetes isolates tested for production of plant growth promoting (PGP) traits like IAA, Hydrogen cyanide and phosphate solubilization. About 25-36% of strains only positive on plant growth promotion characters. Studies shows three Streptomyces sp isolated from sample sites respectively 1, 3, 7, two Micromonospora recovered from site 2 and 3 as well as one *Nocardia* sp from sample 3 were found to have plant growth stimulant activity. Further growth effect under drought of V. mungo shows effectively balanced growth among Streptomyces sp with 70-80% seed germination and significant increases in the growth parameters with high vigor index. Results conclude that these isolates capable of being developed into bio-inoculants for drought management.

Keywords: vigor index, actinomycetes, drought, IAA, germination

Introduction
Actinomycetes adopted from halophilic are attracted the researchers because of their less explored applications in the agricultural and biotechnological fields. Marine sediment, soil, water, contaminated regions on the seashore, salt lakes, saline soils, alkaline–saline habitats, brines, and other regions are good sources for the selection of novel halophilic actinomycetes (Magarvey *et al.*, 2004) [12]. Recent literature claimed that the saline regions contain many significant uncultured actinomycetes. actinomycetes such as *Streptomyces pharmamarensis, Prauserella halophila, Pseudonocardia* sp., *Salinispora arenicola, Aeromicrobium* sp., *Micromonospora* sp., *Marinactinospora thermotolerans, Microbacterium* sp., *Nocardiopsis xinjiangensis, Salinibacterium* sp., *Salinactinospora qingdaonensis, Rhodococcus* sp., *Actinomadura* sp., *Saccharopolyspora* sp., *Streptosporangium* sp., *Actinopolyspora algeriensis, Marinophilus* sp., *Streptomonospora halophila, Verrucosispora* sp., Gordonia sp., and Nonomuraea species were recovered and characterized from hypersaline regions(Mincer *et al.*, 2005). halophiles have been used in the food and nutraceutical industries for the fermentation of soy and fish sauces and β-carotene production; also they have been recently used in many novel and unique molecules such as compatible solutes, biopolymers or carotenoids, enzymes, biodegradable plastics, biosurfactants, bioemulsifiers, and bacteriorhodopsins for molecular biotechnology applications (DasSarma *et al.*, 2010) [5]. Halophilic actinomycetes isolated from the oil-polluted soil in Russia were identified as Rhodococcus erythropolis. It was grown in a medium containing crude oil as a unique source of carbon. Actinomyces strain that belongs to Actinopolyspora sp. was isolated from saline exhibited the capability of Degrading alkanes (Al-Mueini *et al.*, 2007) [2]. The improvement of leguminous crop production is the use of a category of microbes called Plant Growth-Promoting Rhizobacteria. Legumes, through symbiotic nitrogen fixation, meet a major part of their own N demand and partially benefit the following crops of the system by enriching soil. Entophytic actinobacteria like Arthrobacter and Streptomyces also exhibited N-fixing ability among different non leguminous plants (Gopalakrishnan *et al.* 2015) [9]. Plant growth-promoting (PGP) bacteria for improving soil and plant health has become one of the attractive strategies for developing sustainable agricultural systems (Bhattacharyya and Jha 2012) [4]. Phytohormone like indole acetic acid (IAA), cytokinins, and gibberellins producing capacity of actinobacteria also reported by many studies (Vijayabharathi *et al.* 2016) [14] studies on actinomyces and its pant growth promotion are limited. This work highlights to find PGP traits of marine actinomycetes. Inoculation of Actinobacteria tolerance towards temperature, salinity, and metals have promote plant growth especially on maize (Aly *et al.* 2012) [3]

Materials and Method
Isolation of Actinomycetes
7 different marine sediments were collected from Cochin port kerala and subjected to isolation of actinomycetes by crowded plate method. Sediments were serially diluted up to 107 and one ml of 105 was plated on actinomycetes isolation agar prepared in 50% seawater. All the plates kept under incubation for 15 days at 28° C. Spore ornamentation was observed by a Nikon photo microscope followed by simple stain. Mycelia production was identified by Slide culture method and the nature of mycelium was determined by staining with Sudan black.

Phosphate solubilization activity

The ability of actinomycetes to solubilize phosphate, Pikovskayas Aga was used. Freshly grown 5 days old culture was spot inoculated on Pikovskaya's agar plates containing 2% tri-calcium phosphate. Inoculated plates were incubated at 37°C for 7 days, plates were observed for the appearance of a clear zone around the actinomycetes colonies.

Hydrogen cyanide activity

Cultures were separately streaked on yeast glucose agar amended with 0.5% (w/v) of glycine. A Whatman no. 1 filter paper soaked in 0.5% (w/v) picric acid in 2% (w/v) sodium carbonate was placed on the lid of the Petri dish. Plates were incubated for seven days. The change in color of the filter paper from yellow to deep orange indicates a positive result.

Indole-3-acetic acid production

Freshly prepared bacterial cultures (20 µl) were inoculated in LB broth (20 ml) amended with 5 mmol L-tryptophan (Merck, SA) and incubated at 25°C for 7 days. After incubation, 5 ml of bacterial culture was transferred into sterile tubes and centrifuged at 5000 g for 15 min. The supernatant was collected in a 15 ml centrifuge tube. Subsequently, 2 ml of the supernatant and 2–3 drops of orthophosphoric acid was added to 4 ml of Salkowsky reagent (50 ml of 35% perchloric acid in 1 ml of 0.5 M FeCl3). The contents in the tubes were incubated at room temperature under dark conditions for 20 min, the development of a pink color indicated IAA production.

Plant growth and drought management study (Ghorbanpour and Hatami, 2014). 7 days old actinoculture Pelleted cells were resuspended in phosphate buffer at pH 7 and adjusted to an absorbance of 1.4 at 600 nm with a UV spectrophotometer. Seed germination tests were conducted in the presence of 5% polyethylene glycol (PEG) 8000. Prior to the test, *V.radiata* seeds were surface sterilized using 70% ethanol for 5 min followed by 2% sodium hypochlorite (NaClO2) solution for 15 min and severally rinsed with sterile distilled water to remove the remains of the disinfectant. Petri plates were prepared by placing two filter papers at the bottom of each plate and subsequently 10 ml of each bacterial suspension in 5% PEG 8000 was transferred. In untreated water is used as control. Sterile seeds were immersed in 10 ml of culture suspension containing 2% PEG 8000 for 3 h in a rotary shaker at 150 rpm after which 20 seeds were placed in each petri plate and incubated at 25°C for 10 days. Germinated seeds in each Petri plate were counted and 5 seedlings per plate were randomly selected for growth parameter measurements (shoot length, root length and dry seedling weight). Percentage germination and vigor index were estimated as follows:

Germination % = number of germinated seed/ total seeds tested

Vigor index= % germination × total length of seedling (shoot length + root length)

Results and Discussion

Totally 16 actinomycetes were isolated and identified as *Streptomyces* spp, *Micromonospora* spp, *Micropolyspora* sp, *Nocardia* sp, Microbispora sp from 7 different sample. The frequency of Streptomyces is dominate and found to be 7 out of 16 followed by Micromonospora recorded as 4 out of 16. Rest of five the isolates were rare isolates. All the 16 were subjected to phosphate solubilization, cyanide production, IAA production test and data were given in table 1. Of which 31% were found to be produce cyaninde and indole aceteic acid and only 25% were phosphate solublizer (PS) showing clear zones around colonies on petri dishes. Among the 25% PS two were comes under Streptomyces sp and each one from Nocardia and *Micromonospora* sp. similarly three Streptomyces were found to be IAA and cyanide producer and rest are Micromonospora sp. none of the rare isolates found to be positive on IAA and cyanide. *Micromonospora* and Streptomyces were frequently been reported for Po_4 solubilizer (Jog *et al*., 2014) [11]. Similarly IAA producing *Streptomyces* also reported by many researchers. Recently *Streptomyces olivaceoviridis*, *Streptomyces rimosus*, *Streptomyces rochei*, *and Streptomyces viridis* showed IAA production, improved seed germination and acted as biocontrol agent *(*El-Tarabily 2008*;* Abd-Alla *et al*., 2013) [6, 1]. Increasing the availability of phosphorus (P) to plants by actinomycetes mediated by production of phosphatase and the production of organic acids. Plants' developmental processes are being regulated by the production of phytohormones in their various parts. The phytohormone, indole-3-acetic acid, plays a major role in plant development and its supply is capable of supporting its host during stress conditions like drought and pathogenic attack (Sathya *et al*. 2017) [15]

Table 2 shows the impact of seeds treated with actinobactrial colonies. Under PEG induced drought stress conditions(image 1), better growth was observed in seed inoculated with *Streptomyces* sp3 showed maximum percentage of germination and vigor index recorded as 80/1856 followed by *Streptomyces* sp7 gave 70 % germination with 1512 VI. The vigor index of treated plant showed 1856≥1104≥1512≥952.2≥988.8≥267≥291 respectively for *Streptomyces* sp 3, *Streptomyces* sp 6, *Streptomyces* sp 7, *Micromonospora* sp 2, *Micromonospora* sp 3, *Nocardia* sp 3 and Control. The maximum root and shoot length were 8.4±/14.8 cm for *Streptomyces* sp3 and minimum was recorded against Nocardia sp found to be 2.68/4 cm. Except *Nocardia* sp all other strains showed better survival with moderate germination and vigor index than the un-inoculated plants. Application of drought tolerant endophytic actinobacteria, Streptomyces among wheat was found to enhanced tolerance and highest yield (Hasegawa *et al*. 2005) [10]. Similarly *Streptomyces filipinensis* found to reduce ACC levels and thus promoted both roots and shoots reported by El-Tarabily (2008) [7].

Conclusion

Plant growth promoting actinobacteria from marine facilitated germination and growth under drought condition of *Vigna radiata* was achieved with actinobacterial colonies. The strains Streptomyces 3 and 7 and *Micromonospora* sp provide resistant mechanism to the seed under dry environmental conditions (drought), and produced major plant growth promoting hormones. Further co inoculation and ACC deaminse activity need to be studied for field application.

Table 1: Plant growth trait of marine action isolates

Strain	Phosphate solubilization	Cyanide production	IAA
Streptomyces sp *1*	-	-	+
Streptomyces sp *2*	-	-	-
Streptomyces sp *3*	+	+	+
Streptomyces sp *4*	-	-	-
Streptomyces sp *5*	-	-	-
Streptomyces sp *6*	-	+	+
Streptomyces sp *7*	+	+	-
Micromonospora1	-	-	-
Micromonospora2	-	+	+
Micromonospora3	+	+	+
Micromonospora4	-	-	-
Microbispora sp *3*	-	-	-
Micropolyspora sp2	-	-	-
Micropolyspora sp *2*	-	-	-
Nocardia sp *3*	+	-	-
Nocardia sp *5*	-	--	-

Table 2: Seed germination and vigor index under drought condition

Strain	seed germination %	root length (cm)	shoot length (cm)	Vigor index
Streptomyces sp 3	80	8.4±0.001	14.8±0.003	1856
Streptomyces sp 6	60	6.2±0.002	12.2±0.002	1104
Streptomyces sp 7	70	7.8±0.002	13.8±0.021	1512
Micromonospora sp2	60	5.88±0.001	10±0.002	952.2
Micromonospora sp 3	60	5.68±0.001	10.8±0.0002	988.8
Nocardia sp 3	40	2.68±0.02	4±0.003	267
Control	40	2.88±0.02	4.4±0.003	291

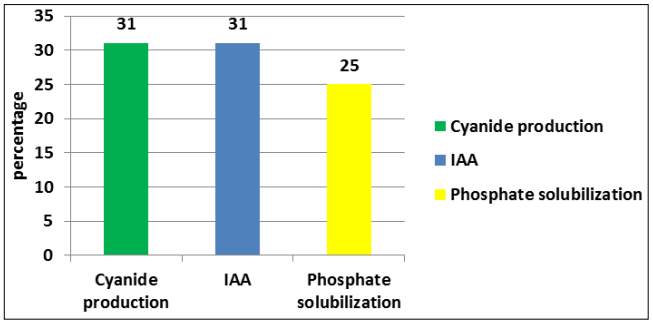

Fig 1: Percentage of plant growth trait among isolates

Image 1: Seed germination (*V.radiata*) under drought

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