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Exploring the Plant Growth Promoting Traits of the Actinobacteria Isolated from Compost

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Abstract

The objective of the study was to isolate and screen the Actinobacteria for their plant growth promoting activities. A total of forty one isolates were recovered from different types of compost like FYM, coir pith compost, spent mushroom compost and vermicompost. Studies on plant growth promoting activities revealed that seventeen isolates showed the abilities to produce indole acetic acid, 15 isolates produced gibberellic acid, 11 isolates produced cytokinin, 31 were able to produce ammonia, seven isolates produced siderophores, nine isolates were able to produce HCN and 18 were observed to solubilize phosphate and 16 were able to solubilize Zinc, 18 isolates were able to produce volatile compounds and nine isolates produced non-volatile compounds. Based on the plant growth promoting traits analysed the selected efficient isolates may be used for plant growth promotion.

Keywords : Actinobacteria, Plant growth promotion, Compost

Plant growth-promoting rhizobacteria (PGPR) are free-living beneficial bacteria of agricultural importance. The presence of PGPR produces beneficial effects on plant health and growth by suppressing disease-causing microbes and accelerating nutrient availability and assimilation. Hence, in the quest to improve soil fertility and crop yield and reducing the negative impact of chemical fertilizers on the environment, there is a need to exploit PGPR for beneficial agricultural uses (Glick, 2012). As like other PGPR, Actinobacteria also employ both direct and in-direct mechanisms to influence the plant growth and protection. Actinobacteria are one of the major components of microbial populations present in soil. They belong to an extensive and diverse group of Gram-positive, aerobic, mycelial bacteria that play an important ecological role in soil nutrient cycling and plant growth promotion.

The members of the phylum Actinobacteria are widely distributed in different parts of the world. Because they can live in different environments and exhibit high versatility in their nutrition, this allows them to spread and thrive in different regions across the globe and compete with other organisms in their surroundings and they can be found in a variety of habitats, they exist in the soil in significant numbers making them some of the most common microorganisms in different types of soil. Compost is rich source of microflora, which are involved in plant nutrient uptake (Yogananda *et al*., 2010).

Actinobacteria, especially thermophilic species are well known components of the microflora of composts. Composts for mushroom cultivation which were prepared from animal manures and straw have been most studied but Actinobacteria may also colonise household and green waste composts. Kesarwani and Sharanappa, 2009 reported that available nutrients uptake was higher when combination of biofertilizers and compost were added. Actinobacteria, particularly *Thermomono spora* spp., *Thermomonospora chromogena* and *Microtetraspora* spp., develop abundantly during the second phase of compost and many spores are released during spawning (Gillian *et al*., 2020).

The direct mechanisms involve the production of vital factors for crop growth such as growth hormones and the assistive actions on nitrogen fixation, phosphate solubilization and iron acquisition. PGP

Actino bacteria indirectly influence the plant growth by controlling and minimizing the deleterious effects of external stresses of either biotic or abiotic sources through competition for nutrients, production of low molecular inhibitory substances such as ammonia, alcohols, aldehydes and ketones, cell-wall degrading enzymes and secondary metabolites with biocidal properties (Tarabily and Sivasithamparam, 2006). Also different activities performed by Actinobacteria for plant growth promotion like phosphate solubilization, production of siderophores, enzymes and plant growth harmones have been studied by Marcela and Vanessa (2016). In this context, the present study was taken up to know more about the plant growth promoting abilities of Actinobacterial isolates which were recovered from different types of compost.

Material and Methods

Collection of Samples and Isolation of Actinobacteria from Compost

Different types of compost like, spent mushroom compost, coir pith compost, vermicompost and FYM were collected. A total of 41 Actinobacteria isolates were isolated from different types of compost by serial dilution pour plate method. Cycloheximide and Nalidixic acid (50 µg/ml of each) were added to media to supress the growth of bacteria and fungi, respectively. After seven days incubation, the Actinobacteria colonies were picked and pure cultures of the respective isolates were obtained by repeated streaking on starch casein agar (SCA) plates. The purified colonies were transferred to freshly prepared SCA slants and kept at 4 °C for further processing.

Sources of Sample Collection

Sample name	Location	Total sample
Spent mushroom	IIHR (Bengaluru)	10
Vermicompost	GKVK, IIHR (Bengaluru)	12
FYM	GKVK	5
Coir pith	IIHR (Bengaluru)	8
Total		35

Screening of Actinobacteria Isolates for Plant Growth Promotion Traits

Phosphate Solubilization

Screening of isolates for phosphate solubilization was done by qualitative method by using NBRI-BPB medium. Plate containing NBRI-BPB medium were inoculated with Actinobacteria cultures and incubated at 28 ^{0}C for 7 days. The colonies forming halo zone were considered as phosphate solubilizers. Characterization of isolates for phosphate solubilization was done by using Olsen and Sommers method (1982).

Zinc Solubilization

Screening of isolates for zinc solubilization was done by qualitative method by using zinc minimal medium. Plates containing zinc minimal medium were inoculated with Actinobacteria cultures and incubated at 28 ^{0}C for five days. The colonies forming halo transparent zone were considered as zinc solubilizers. Characterization of isolates for zinc solubilization was done by using the procedure of Gandhi and Muralidharan, 2016.

Ammonia Production

The Actinobacteria isolates were tested for the production of ammonia using the method described by Cappucino and Sherman (1992). In this method 20 µl of seed culture was propagated in 10 ml of peptone water and incubated at 28 °C with shaking at 120 rpm for 10 days. Subsequently, 0.5 ml of Nesseler's reagent was added to the culture and the development of brown to yellow color indicated a positive test for ammonia production.

Siderophore Production

The ability of the Actinobacteria isolates to produce siderophore was assessed as per procedure given by Schwyn and Neilands (1987). A loop full of culture was inoculated on Chrome azurol S (CAS) agar medium and incubated at 28 ± 2 °C for five days. The colony with a halo zone of yellow-orange color was considered positive for siderophore production.

Production of HCN

Hydrogen cyanide production was detected as described by Bakker and Schippers (1987). Petri plates containing 10 per cent Trypticase soya agar supplemented with 4.4 g of glycine per litre were inoculated with the Actinobacteria and inverted with a lid containing filter paper, impregnated with 0.5 per cent picric acid and two per cent sodium carbonate, over each petri plate. The plates were incubated at 28 °C for three to five days. A change in color from yellow to orange-brown on the filter paper indicated cyanide production.

Production of Plant Growth Regulators

Indole Acetic Acid (IAA), Gibberellic Acid (GA3) Production and Cytokinin

Qualitative : IAA producing ability of Actinobacteria isolates determined by Cucumber root elongation bioassay and bioassay for GA3 production was determined by Starch agar Test (Loper and Schroth, 1986).

Quantitative : The specified media was inoculated with 24 hrs old culture and incubated at 37 °C for seven days in dark condition. After seven days of incubation, centrifuged at 6000 rpm for 10 minutes, to the supernatant, added 1N HCl and adjusted pH at 2.8. The total acidified supernatant taken into 250 ml conical flask and added equal volume of diethyl ether to the supernatant and incubated in dark condition for 4 hrs. Then kept the samples at 4 °C overnight in separating funnel. Later organic phase (down layer) was discarded and solvent phase (upper layer) was collected. Upper layer was allowed to evaporate and 2-3 ml of HPLC grade methanol added and IAA and GA3 were quantified by a high performance liquid chromatography (HPLC). Bioassay for cytokinin production by Actinobacteria isolates was done using cucumber cotyledon greening bioassay (Fletcher *et al.,* 1982).

Volatile and Non-Volatile Metabolites Production

The isolates of antagonistic microorganisms were evaluated in laboratory to screen out the most efficacious one, which inhibits growth of pathogen by producing volatile and non-volatile substances by following the techniques described by Dennis and Webster (1971).

Results and Discussion

Isolation of Actinobacteria from Compost

A total of forty one Actinobacteria isolates were obtained from different types of compost like FYM, coir pith compost, spent mushroom compost and vermicompost. This reveals that the compost harbour a vast density of the Actinobacteria. Earlier the Actinobacteria strains have been isolated from variety of sewage composts which may include *Nocardia* and *Promicromonospora* spp. Actino bacteria development is dependent on aerobic conditions, temperature and water content although the interrelationships of these factors and occurrence of different taxa of Actinobacteria have not been closely studied in composts. John, 1997 reported thermophilic Actinobacteria can be found in compost / manure particularly during the early stages of decomposition. Through their involvement in decomposition, the heat level is increased in manure / compost which provides a favourable living environment.

Plant Growth Promoting Traits of Actinobacteria

Phosphate Solubilization and Zinc Solubilization

Among the forty one Actinobacteria isolates, 18 (44%) isolates were able to solubilize inorganic phosphate and were identified as potential phosphate solubilizing isolates based on a clear yellow halo zone around the colony on NBRI-BPB agar medium. Quantitative estimation of phosphate solubilization by the Actinobacteria isolates ranged from 3.54 to 30.05 mg/ml (Table 2). Solubilization of zinc was observed in 16 (39%) isolates on zinc minimal media, forming a clear halo zone around the colonies.

TABLE 1

Relative production of IAA, Gibberellic acid, Cytokinin, Ammonia & phosphorus and zinc solubilization by Actinobacteria isolates from compost

Isolates	Plant Growth Regulators				Nutrient solubilization		
	IAA (µg/ml)		Gibberellic acid (µg/ml)	Cytokinin (µg/ml)	Ammonia (mg/ml)	Phosphate (mg/100ml)	Zinc
	Trp (-)	Trp (+)					
CA1	16.34 f	34.33 h	-	-	-	12.26 g	+
CA2	-	-	124.66 c	-	5.52 o	-	-
CA3	-	-	-	-	8.77 j	-	-
CA4	48.47 b	143.66 b	208.36 b	9.24 a	22.35 b	28.83 b	+
CA5	4.02 n	13.25 m		2.01 i	5.63 o	-	-
CA6	13.24 h	35.54 g	-	-	-	-	-
CA7	-	-	-	-	16.30 c	18.04 c	+
CA8	-	-	-	-	10.10 gh	12.26 g	+
CA9	-	-	-	4.73 f	8.773 j	12.26 g	-
CA10	10.24 k	28.36 i	-	-	6.02 n	5.44 j	-
CA11			-	2.26 h	10.02 hi	-	-
CA12	6.24 m	18.36 l	78.00 h	-	5.52 o	-	-
CA13	-	-	-	5.50 e	-	-	-
CA14	-	-	-	-	10.20 g	-	-
CA15	-	-	-	2.00 i	12.03 e	17.24 e	+
CA16	11.54 j	26.66 j	30.01 l	-	6.63 m	6.57 h	+
CA17	-	-	-	-	10.11 gh	-	-
CA18	59.26 a	158.33 a	273.33 a	8.84 b	28.86 a	30.05 a	+
CA19	18.55 d	56.87 c	102.00 e	-	8.11 k	12.24 g	+
CA20	9.74 l	22.36 k	49.23 j	6.53 c	-	-	-
CA21	-	-	112.33 d	3.36 g	-	-	-
CA22	-	-	-	-	11.15 f	-	+
CA23	18.25 e	53 f	-	-	12.02 e	17.87 d	+
CA24	-	-	-	-	-	-	-
CA25	-	-	-	-	15.85 d	-	-
CA26	-	-	-	-	11.15 f	6.59 h	+
CA27	3.86 o	11.36 n	94.56 f	-	9.92 i	3.54 k	-
CA28	22.47 c	72.60 d	42.45 k	1.73 j	11.15 f	3.54 k	-
CA29	-	-	82.64 g	-	4.76 p	-	+
CA30	13.84 g	34.89 gh	-	-	-	-	-
CA31	11.54 j	28.86 i	78.00 h	-	3.26 r	-	+
CA32	12.74 i	29.24 i	-	-	10.15 gh	-	+
CA33	-	-		-	6.91 l	13.27 f	-
CA34	-	-	-	-	3.67 q	-	-
CA35	-	-	12.03 n	-	-	-	-
CA36	22.47 c	79.22 c	65.16 i	-	6.63 m	-	+
CA37	-	-	13.85 m	-	6.85 l	-	-
CA38	-	-	-	-	-	5.55 i	-
CA39	-	-	-	-	12.02 e	5.45 j	-
CA40	-	-	-	-	-	18.06 c	-
CA41	-	-	-	6.02 d	5.53 o	-	+

Note : Means with same superscript, in a column do not differ significantly at P=<0.05 as per Duncan Multiple Range Test (DMRT)

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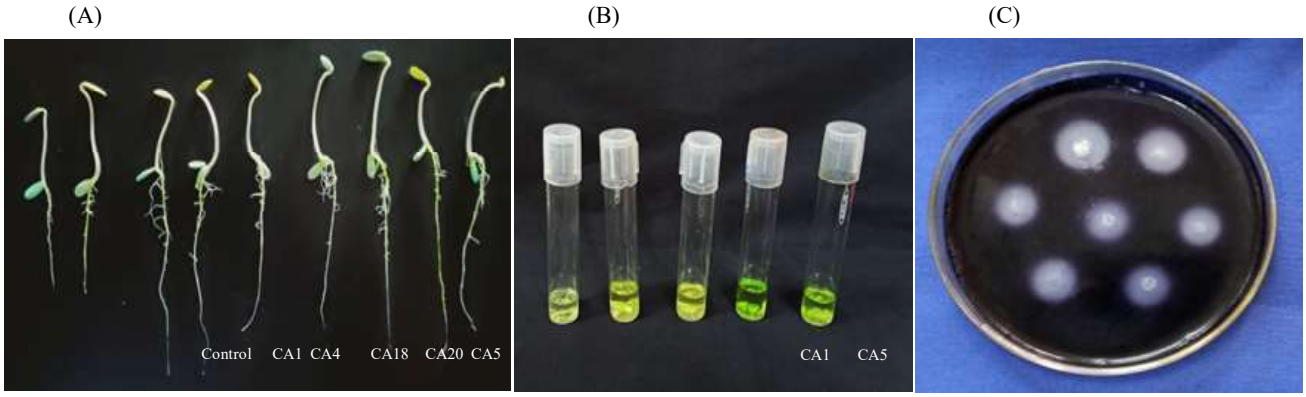
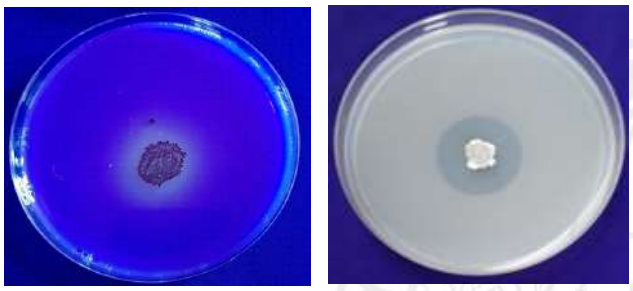


Fig.1: Phytohormone production by Actinobacteria A) IAA production, B) Cytokinin production and C) Gibberellic acid production



(A) Phosphorus solubilization (B) Zinc solubilization

Fig. 2 : Solubilization of nutrients by Actinobacteria

(A) HCN production (B) Siderophore production

Fig. 3: Production of HCN and siderophore by Actinobacteria

Production of Plant Growth Regulators

Seventeen Actinobacteria isolates (41.46%) were positive for IAA production; quantitative range of IAA production was found from 3.86 to 59.26 µg/ml (Table 2) with the highest production by CA18 isolate. The production of GA3 was observed in 15 isolates with maximum production of 273.33 µg/ml by isolate CA18. Production of cytokinin was observed in only 11 isolates ranged from 2.00 to 9.24 µg/ml with the highest production by CA4 isolate.

Ammonia, Siderophore and HCN Production by Actinobacteria Isolates

Thirty-one isolates were positive for the production of ammonia at levels ranging from 3.26 to 28.86 mg/ml. Isolate CA18 produced the maximum amount of ammonia (28.86 mg/ml) followed by CA4 (22.35 mg/ml). Siderophore production was detected in seven (17%) isolates on CAS agar media, forming clear orange halo zone around the colonies. Studies performed by Gopalakrishnan *et al.* (2011) evidenced a higher performance of siderophores production by genus Streptomyces, including its own characteristic types such as hydroxamates siderophores. Hydrogen cyanide (HCN) is a broad-spectrum antimicrobial compound involved in biological control of root diseases in plant. The change in colour from yellow to brown indicating production of HCN was observed in nine isolates.

Volatile and Non-volatile Production by Actinobacteria Isolates

Eighteen isolates were positive for the production of volatile compounds and nine isolates were tested positive for the production of non-volatile compounds. The study of Batra and Bajaj, 1966 evidenced that volatile and non-volatile antibiotic substances produced by Actinobacteria inhibit the growth of many pathogenic soil fungi. Mallory *et al.,* 2019 measured the VOCs produced by a broad diversity of soil and dust-dwelling Actinobacteria *in vitro*. They have detected a total of 126 unique volatile compounds and each strain produced a unique

TABLE 2

Elucidation of mechanisms of inhibition of pathogens by Actinobacteria isolates from compost

Isolates	HCN	Sidero phore	Volatile compound	Non volatile compound
CA1	+	-	+	-
CA2	-	+	+	-
CA4	+	+	+	+
CA5	-	-	+	-
CA9	-	-	+	+
CA11	+	+	+	-
CA14	-	+	+	+
CA16	-	-	+	-
CA17	-	-	+	+
CA18	+	-	+	+
CA19	+	+	+	+
CA22	+	-	+	-
CA26	-	+	+	+
CA29	+	-	+	-
CA31	-	-	+	-
CA34	+	+	+	-
CA7	+	-	+	+
CA40	-	-	+	+

combination of VOCs. While some of the compounds were produced by many strains, most were strain specific. Importantly, VOC profiles were more similar between closely related strains, indicating that evolutionary and ecological processes generate predictable patterns of VOC production. Finally, they have reported that Actinobacteria VOC's had both stimulatory and inhibitory effects on the growth of bacteria that represent a plant-beneficial symbiont and a plant-pathogenic strain, information that may lead to the development of novel strategies for plant disease prevention.

Actinobacteria commonly inhabit the rhizosphere and various types of natural ecosystem like forest soil, different types of compost makes them an essential part of this environment due to their interactions with plants. Such interactions have made possible to characterize them as plant growth-promoting rhizobacteria (PGPR). As PGPR, they possess direct or indirect mechanisms that favour plant growth. Actinobacteria improve the availability of nutrients and minerals, synthesized plant growth regulators and specially, they are capable of inhibiting phytopathogens. Hence, the activities performed by Actinobacteria like phosphate solubilization, production of side rophores, enzymes and plant growth hormones makes the plant healthy by supressing the diseases indirectly and improving the soil fertility.

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