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Isolation and Characterization of Desiccation Tolerant Rhizobacteria from Arid Regions of Karnataka, India

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A B S T R A C T

An attempt was made to isolate and characterize the desiccation tolerant rhizobacteria from arid regions of Karnataka. A total of nine desiccation tolerant rhizobacteria were isolated from soil samples collected from arid regions of Chitradurga, Gadag and Koppal districts. These isolates were further subjected to morphological and biochemical studies. The Gram staining study of the nine isolates revealed thatCHI-1, CHI-2, CHI-3, and CHI-5 are Gram negative. The other isolates *viz.*, CHI-4, GAD-1, GAD-2, KOP-1 and KOP-2 are Gram positive. The isolates CHI-1, GAD-1, KOP-1, 2 were positive for H_2S production. CHI-2, 3, 4, 5, GAD-2, KOP-1, 2 were positive for casein hydrolysis. Bacterial isolates were screened for ammonia production, ACC deaminase activity, siderophore production. Among the isolates, isolate KOP-2, GAD-1, and CHI-4 showed better results for above parameters compared to other isolates.

Introduction

Drought is one of the major constraints on agricultural productivity worldwide and is likely to increase further. Several adaptations and mitigation strategies are required to cope with drought stress. Drought is the most destructive stress among abiotic stresses that increased in intensity over the past decades affecting world's food security. Drought stress may range from moderate and short to extremely severe and prolonged duration, restricting the crop yields. Desiccation tolerance refers to the ability of an organism to withstand or endure extreme dryness or drought like conditions.

In recent times, the use of rhizobacteria for alleviation of drought stress in plants has gained momentum. Inoculation of plants with beneficial microorganisms promotes plant growth and increases drought tolerance in arid or semiarid areas (Marulanda *et al.*, 2007). These rhizobacteria should possess stress alleviation properties, such as ammonia production, Siderophore production and ACC-

deaminase activity. Inoculation of plants with beneficial microorganisms promotes plant growth and increases drought tolerance in arid or semiarid areas (Marulanda *et al.,* 2007). The term 'Induced Systemic Tolerance' (IST) has been proposed for PGPR-induced physical and chemical changes that result in enhanced tolerance of plants to abiotic stress (Yang *et al.,* 2009). Plant growth promoting rhizobacteria (PGPR) are a group of free-living saprophytic bacteria that can be found in the rhizosphere in association with root system and enhance the growth and development of plant either directly or indirectly (Kloepper and Beauchamp 1992; Liu *et al.,* 1995).

Interestingly these PGPR strains also possess the enzyme ACC deaminase (Jacobson *et al.,* 1994; Glick *et al.,* 1998; Shah *et al.,* 1997) and this enzyme can cleave the plant ethylene precursor ACC to ammonia and a-ketobutyrate thereby lowers the level of ethylene under various biotic and abiotic stresses (Glick *et al.,* 1998)

Materials and Methods

Isolation of desiccation tolerant rhizobacteria

The soil samples were collected from arid and irrigated regions of Karnataka were further used for isolation of desiccation tolerant rhizobacteria on Poly ethylene glycol (PEG 8000) amended medium by serially diluting up to 10^6 and from this dilution transferred one ml of aliquot into the each flask containing 25 per cent of PEG (8000) amended medium like trypticase soya broth, nutrient broth and half strength nutrient broth then incubated at 30°C for 48hrs.

Purified by four way streak plate method and were preserved on agar slants at 4°C for further study.

Morphological studies of desiccation tolerant rhizobacterial isolates

All the bacterial isolates were subjected to study their morphological characters based on the colony characters, Gram reaction, cell shape as per the standard procedures given by Barthalomew and Mittewer (1950).

Biochemical characterization

The biochemical characterization of the isolates was essentially carried out as per the procedures outlined by Cappuccino and Sherman (1992). The tests conducted are detailed below.

Hydrogen sulphide production

Sulfide in dole motility (SIM) agar stabs were inoculated with the bacterial isolates and incubated at 30°C for 48 hrs. Black coloration along the line of stab inoculation indicated H_2S production.

Casein hydrolysis (Seeley and Vandemark, 1970)

The plates containing skim milk agar was streaked with test cultures and incubated at 30°C for one week. The clear zones around the colony against a black background after incubation were taken as positive for casein hydrolysis.

Catalase test (Blezevic and Ederer, 1975)

The nutrient agar slants were inoculated with test organisms and incubated at 30°C for 24 hours.

After incubation the tubes were flooded with one ml of three per cent hydrogen peroxide and observed for production of gas bubbles. The occurrence of gas bubbles was scored positive for catalase activity.

Starch hydrolysis (Eckford, 1927)

The ability of the isolates to hydrolyse starch was examined by the petri plates containing two per cent starch agar. Inoculated with test cultures and incubated at 30°C for three days. After incubation the plates were flooded with Lugolsiodine solution and allowed to stand for 15-20 minutes. The clear halo zone around the colony was considered as positive for the test.

Fermentation test

Bacterial isolates were tested for acid production by inoculating 10ml of the sterile glucose and sucrose broth in test Durham´s tubes. The tubes were incubated for 7 days at 30°C. Accumulation of gas in these Durham´s tube was taken positive for gas production and change in color of the medium to yellow was taken as positive for acid production.

Screening of desiccation tolerant rhizobacterial isolates for their plant growth promotion under in vitro osmotic condition

Ammonia production

Actively grown 48 hrs old cultures of 100µl were added to 10ml of the peptone water with and without PEG separately into individual tubes. Inoculated tubes were kept for 72hrs of incubation. After 72hrs of incubation 0.5ml of Nessler´s reagent was added and observed for colour development. Indication of brown to yellow colour indicates the positive test for ammonia (Cappuccino and Sherman 1992).

ACC-deaminase activity

Screening for ACC deaminase activity of bacterial isolates was done by using 1-aminocyclopropane-1-carboxylate (ACC) as a sole nitrogen source. The bacterial isolates were grown in 5 ml of TSB medium incubated at 28°C at 120 rpm for 24 hrs. The cells were harvested by centrifugation at 5000 rpm for 5 minutes and washed twice with sterile 0.1 M Tris- HCL (pH 7.5) and resuspended in 1 ml of 0.1 M Tris-HCL (pH 7.5) and spot inoculated on petriplates containing DF (Dworkin and Foster) salts minimal medium (Dworkin and Foster 1958); supplemented with 3mM ACC as sole nitrogen source. Plates containing only DF salts minimal medium without ACC kept as negative control and with $(NH_4)SO_4$ as positive control. The plates were incubated at 28°C for 72hrs. Growth of the isolate on ACC supplemented plates was compared to negative and positive controls (Glick *et al.*, 2005).

Siderophore production

CAS agar used to estimate the production of siderophore. One single colony of culture was spot inoculated on the CAS agar plates with and without mannitol and incubated at 30°C for 3-4 days.

Orange halo zone around the colony is indicated as positive and no zone as negative. Nutrient agar was prepared with and without mannitol and autoclave it separately and the 20ml CAS dye (Chromoazural) was prepared (Schwyn and Neilands, 1987).

Preparation of chrome azurol S (CAS) solution

Dehydrated chrome azurol S (CAS) solution was prepared by dissolving 60.5 mg dehydrated chrome azurol S in 50 ml double distilled water and further mixing with 10 ml of iron solution (1 mM $FeCl_3.6H_2O$ in 10 mMHCl). This was then slowly added to 40 ml aqueous solution containing 72.9 mg hexadecyltrimethyl ammonium bromide (HDTMA) by continuous stirring and the final solution was autoclaved.

Results and Discussion

A total of nine desiccation tolerant rhizobacteria were isolated from soil samples collected from arid regions of Chitradurga, Gadag and Koppal districts (Table 1). The isolates from Chitradurga were denoted as CHI-1, 2, 3, 4, 5, while isolates from Gadag district were denoted as GAD-1 and 2, while isolates from Koppal district were designated as KOP-1 and 2 respectively. The morphology of desiccation tolerant rhizobacterial isolates was studied and is presented in Table 2. The desiccation tolerant rhizobacterial isolates colonies were observed as creamy- dense white in colour, slimy, mucoid with opaque, circular to irregular, smooth, tiny in texture or dry in texture on the agar medium. The Gram staining study of the nine isolates revealed thatCHI-1, CHI-2, CHI-3, and CHI-5 are Gram negative. The other isolates *viz.*, CHI-4, GAD-1, GAD-2, KOP-1 and KOP-2 are Gram positive.

The observations are in agreement with the studies carried out by Mukherjee *et al.*, (2011), Saravanan and Raj (2004) and Desai *et al.*, (2012), who isolated several Gram positive and Gram negative bacterial species. The biochemical characterization of desiccation tolerant rhizobacterial isolates was done and the results are presented in Table 3. The results revealed that among the isolates CHI-3, CHI-4, CHI-5, GAD-1, GAD-2, KOP-1 and KOP-2 were positive for starch hydrolysis except isolate CHI-1 and CHI-2. The isolates CHI-1, GAD-1, KOP-1, 2 were positive for H_2S production and other isolates were not able to produce H_2S. Isolate CHI-2, 3, 4, 5, GAD-2, KOP-1, 2 were positive for casein hydrolysis. The isolates CHI-2, 4, 5, and GAD-1, 2 and KOP-1, 2 utilized glucose. All the isolates showed a negative reaction for catalase enzyme production. All the results of biochemical characterization were in confirmation with Raza *et al.*, (2015), who isolated and characterize the desiccation tolerant rhizobacteria from Cholistan desert, Pakistan. The plant growth promotion attributes of the desiccation tolerant isolates was determined under normal and osmotically stressed conditions. All the isolates with exception of GAD-1 produced ammonia under normal conditions, but failed to produce ammonia under osmotically stressed conditions (Table 4). The production of ammonia (Wani *et al.*, 2007) is an important attribute of PGPR that influences plant growth indirectly and strengthen the host disease resistance mechanism respectively (Joseph *et al.*, 2007).

Siderophore production was exhibited by six of the isolates with the exception of CHI-1, CHI-3 and KOP-1, under normal conditions. Under osmotically stressed situations siderophore production was marginally impaired in some isolates, while other isolate KOP-2 failed to produce siderophore under osmotically stressed conditions. Siderophore chelates iron and other metals contributing to disease suppression and acquisition of Fe2+ to plants for increasing the crop growth under stressed conditions (Hofte *et al.*, 1992). Our study shows that three isolates with siderophore production ability which could be a productive PGPR trait for selection.

The ACC deaminase activity of the isolates was detrmined on DF minimal medium with ACC and Ammonium sulphate as the sole sources of nitrogen. All the isolates were able to grow on ACC amended medium, while some isolates showed a marginal reduction in growth in the presence of osmotic stress, some isolates were not affected by the osmotic stress. The results were confirmation with the Palika *et al.*, (2013) and Lin Chen *et al.*, (2013), who isolated and examined bacterial isolates for ACC deaminase activity and reported that isolates were able to utilize the ACC as sole source of nitrogen.

Table.1 The details of soil survey locations along with latitude and longitude

SL. No	District	Location	Latitude	Longitude
1	Gadag	Meyundi	15.2674º N	75.8373º E
2	Koppal	Raghunatahnhalli	15.2245º N	75.9516º E
3	Chitradurga	Gouramanahalli	14.2152º N	76.5481º E

Table.2 Morphological characteristics of desiccation tolerant rhizobacterial isolates

SL.NO	Isolates	Cell shape	Colony characteristics	Gram reaction
1	CHI-1	Rod	White, creamy,	-
2	CHI-2	Rod	Whitish Yellow	-
3	CHI-3	Rod	White, slimy, tiny	-
4	CHI-4	Rod	Yellowish, slimy, tiny	+
5	CHI-5	Rod	White slimy, tiny	-
6	GAD-1	Rod	White, slimy, transparent, mucoid, tiny	+
7	GAD-2	Rod	Creamy white, slimy, mucoid, tiny	+
8	KOP-1	Rod	Creamy white, opaque, dried, medium	+
9	KOP-2	Rod	Creamy white, opaque, dried, medium	+

NOTE: CHI-Chitradurga, GAD- Gadag and KOP- Koppal. (+) – positive, (-)- negative

Table.3 Biochemical characteristics of desiccation tolerant rhizobacterial isolates

Isolates	Starch Hydrolysis	H_2S production	Casein Hydrolysis	Catalase Activity	Fermentation of Glucose
Control	-	-	-	-	-
CHI-1	-	+	-	-	-
CHI-2	-	-	+	-	+
CHI-3	+	-	+	-	-
CHI-4	+	-	+	-	+
CHI-5	+	-	+	-	+
GAD-1	+	+	-	-	+
GAD-2	-	+	+	-	+
KOP-1	+	+	+	-	+
KOP-2	+	+	+	-	+

Note: (+) Positive, (-) Negative, CHI-Chitradurga, GAD- Gadag and KOP- Koppal

Table.4 Plant growth promotion and drought stress alleviation traits of the isolates under *in-vitro* osmotic conditions

Isolates	Ammonia production		Siderophore production		ACC- deaminase activity			
	(+)PEG	(-)PEG	+M	-M	+ACC		$(NH_4)SO_4$	
					+M	-M	+M	-M
CHI-1	-	+++	-	-	+	+	+	+
CHI-2	-	+++	++	++	+	+	+	+
CHI-3	-	+++	-	-	+++	+	+++	+++
CHI-4	-	+++	+	+++	+++	++	+++	+++
CHI-5	-	+++	++	++	+++	++	+++	+++
GAD-1	-	_	+	++	+++	++	+++	+++
GAD-2	-	+	+	+	+	+	+	+
KOP-1	-	++	-	-	++	+	++	+++
KOP-2	-	++	-	++	+++	+++	+++	+++

Note: CHI-Chitradurga, GAD- Gadag and KOP- Koppal, PEG-Polyethelyne glycol, PEG(without PEG), +PEG(with PEG) (+M)-With mannitol, (-M)- Without mannitol, (+)- good, (++)- very good, (+++) – excellent, ACC- 1-amino cyclopropane carboxylate

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