Madras Agric. J., 2019; doi:10.29321/MAJ 2019.000214

RESEARCH ARTICLE

Screening of Rice Apoplast Associated Endophytic Bacterial Isolates for Moisture Stress Tolerance and Plant Growth Promoting Traits

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Received : 16th August, 2018
Revised : 20th September, 2018
Accepted : 01st October, 2018

ABSTRACT

Investigations were made to identify, screen and characterize rice apoplastic fluid endophytic bacterial strains for osmotic stress tolerance and plant growth promoting activity. These bacterial strains were identified phylogentically as *Bacillus subtilis* TSAC5, *Bacillus subtilis* TSAA2, *Bacillus* sp. R2AA10, *Bacillus cereus* R2AA7, *Bacillus marisflavi* TSAC7, *Cupriavidus alkaliphilus* TSAC1, *Delftia* sp. TSAC2, *Janibacter melonis* R2AA1, *Microbacterium oleivorans* R2AA6, *Kocuria rosea* R2AA5, *Pseudomonas aeruginosa* NAAI3 and *Sphingobium yanoikuyae* R2AI1. Of these 12 strains, eight were obtained from drought tolerant rice varieties such as Anna R4 and IR DRT 64 and four from normal rice variety CO51. These endophytes exhibited varied level of osmotic stress tolerance created using different concentrations of PEG 6000 (-1.5, -3.0, -4.0, -4.5, -5.0 and -5.5 MPa) under *in vitro* conditions in liquid and solid medium. Among the 12 strains, *Cupriavidus alkaliphilus* TSAC1, *Delftia* sp. TSAC2, *Bacillus marisflavi* TSAC7, *Janibacter melonis* R2AA1, *Kocuria rosea* R2AA5, *Pseudomonas aeruginosa* NAAI3 and *Sphingobium yanoikuyae* R2AI1 showed moisture stress tolerance up to -5.5 MPa. Further increase in moisture stress of -6.0 MPa inhibited growth of 12 tested strains. These strains also exhibited Indole acetic acid (IAA), gibberellic acid (GA3) and extracellular polysaccharide (EPS) production and also showed ACC deaminase (ACCD) activity. Maximum extracellular proline production was found in *Bacillus marisflavi* TSAC7 under both normal and water stressed conditions. Higher IAA productivity was registered in *Janibacter melonis* R2AA1 followed by *Cupriavidus alkaliphilus* TSAC1 and *Sphingobium yanoikuyae* R2AI1 in the absence of PEG 6000. In the presence of PEG 6000 *Delftia* sp. TSAC2 recorded maximum IAA productivity. *Bacillus subtilis* TSAA2 showed greater ACC deaminase activity. EPS production was in the order of *Pseudomonas aeruginosa* NAAI3 followed by *Sphingobium yanoikuyae* R2AI1. *Sphingobium yanoikuyae* R2AI1 produced maximum quantity of GA3 both under normal and water stressed condition. None of the tested strains indicated insoluble phosphate, silicate and zinc solubilisation.

Keywords*: Rice apoplast, Moisture stress, PEG 6000, GA3, IAA, ACCD*

Drought is one of the major constraints affecting agricultural productivity worldwide and is likely to increase further. Several mitigation strategies are under adaptation to cope with drought stress. One of the strategies employed in recent past is utilization of microorganisms as inoculants for imparting drought tolerance. Although several beneficial plant-microbe interactions that could enhance plant yield and health have been utilized for the benefit of agricultural productivity over past few decades, usage of microorganisms for improving abiotic stress management received little attention. Henceforth, as a relatively simple and low cost alternative strategy, the use of plant growth promoting bacteria has been highlighted as a promising broad-spectrum means to improve plant growth (Yang *et al*., 2009). Endophytes are microorganisms which invade plant tissues and cause imperceptible and asymptomatic infections and improve plant growth by enhancing plant nutrient availability and imparting biotic and abiotic stress tolerance (Mei and Flinn, 2010). In recent years, plant microbiome, the entire collection of microorganisms associated with particular plant system has been studied and reported to benefit plant in a larger way. The beneficial plant microbiome alleviates plant stress by a variety of mechanisms (Rolli et al., 2015) such as a) nitrogen fixation, b) solubilisation of insoluble mineral nutrients, c) sequestration of iron by siderophores, d) production of phytohormones *viz.*, auxins, cytokinins, and gibberellins,

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e) production of stress alleviating enzyme, amino-cyclopropane-1-carboxylic acid (ACC) deaminase, f) volatile growth stimulants such as acetoin and 2,3-butanediol, g) antibiosis, h) induced systemic resistance (ISR), i) hydrogen cyanide (HCN) production, j) production of cell wall degrading enzymes and k) formation of bacterial biofilm (Conrath *et al.,* 2006; Dimkpa *et al.,* 2009). Thus studies were undertaken to characterize rice leaf apoplast endophytic bacteria for their plant growth promoting activity in terms of moisture stress tolerance, enhancement of availability of nutrients, plant growth hormones and bioactive metabolites.

MATERIAL AND METHODS

Bacterial strains and medium

Twelve bacterial strains used in this study were *Bacillus subtilis* TSAC5, *B. subtilis* TSAA2, *Bacillus* sp. R2AA10, *B. cereus* R2AA7, *B. marisflavi* TSAC7, *Cupriavidus alkaliphilus* TSAC1, *Delftia* sp. TSAC2, *Janibacter melonis* R2AA1, *Microbacterium oleivorans* R2AA6, *Kocuria rosea* R2AA5, *Pseudomonas aeruginosa* NAAI3 and *Sphingobium yanoikuyae* R2AI1 and the medium Luria Bertani (LB) agar was employed.

Screening of bacterial isolates for osmotic stress tolerance

Luria Bertani (LB) medium was sterilized and infused with various concentrations of sterile PEG 6000 solution (-1.5 MPa, -3.0 MPa, -4.0 MPa, -4.5 MPa and -5.5 MPa) for 24 h to allow diffusion of the PEG into the medium as per the methodology of Michel and Kaufmann (1973). Then the cultures were streaked on the plates and incubated at room temperature for 2-3 days. The growth of the cultures was observed and scored. Various concentrations of PEG 6000 solution was prepared by using the formula given below,

Water potential (ψ) = - (1.18 x 10-2) C - (1.18 x 10-4) C2 + (2.67 x 10-4) CT + (8.39 x l0-7) C2T

where, C- concentration of PEG 6000 (g/Kg H2O); T- Temperature in °C

Osmotic stress related metabolites

Metabolites such as proline and extra-cellular polysaccharide of 12 cultures were assessed by following standard protocols. Extracellular proline content of stressed and unstressed bacterial strains was estimated quantitatively by Ninhydrin method (Bates *et al.,* 1973). Exopolysaccharide production under unstressed condition was determined as total carbohydrates content by phenol-sulphuric acid method (Dubois *et al.*, 1956). ACC deaminase activity was analysed as per the methodology of Penrose and Glick (2003)

Plant growth promoting activity of selected isolates

Plant growth promoting hormones such as indole acetic acid (IAA) and gibberellin (GA_3) were estimated by following standard protocols. Production of IAA was determined according to the method of Patten and Glick (2002). Gibberellin (GA_3) production was estimated by following the methodology of Mahadevan and Sridhar (1982). Phosphate solubilizing ability of the isolates was assessed by streaking on Pikvoskayas' and Sperbers' Hydroxy Apatite medium. Silicate solubilizing (potassium releasing) potential of the isolates was assessed by using the method of Aleksandrov *et al.* (1967). Zinc solubilization capacity of the isolates was tested by streaking isolates on Bunt and Rovira medium (Jerlin *et al.*, 2017) containing 0.1 per cent zinc oxide.

RESULTS AND DISCUSSION

Growth of endophytic bacteria in osmotic stress condition

The twelve rice apoplast endophytic bacterial strains *viz.*, *Bacillus subtilis* TSAC5, *Bacillus* sp. R2AA10, *Bacillus subtilis* TSAA2, *Bacillus cereus* R2AA7, *Bacillus marisflavi* TSAC7, *Cupriavidus alkaliphilus* TSAC1, *Delftia* sp. TSAC2, *Janibacter melonis* R2AA1, *Microbacterium oleivorans* R2AA6, *Kocuria rosea* R2AA5, *Pseudomonas aeruginosa* NAAI3 and *Sphingobium yanoikuyae* R2AI1 were screened for moisture stress by growing on LB medium with various water potential of -1.5 MPa, -3.0 MPa, -4.0 MPa, -4.5 MPa and -5.5 MPa created using PEG 6000. All the 12 isolates showed normal growth at -1.5 MPa. Except *Janibacter melonis* R2AA1 and *Bacillus* sp., remaining cultures showed normal growth up to – 4.0 MPa. Above the water potential of - 4.0 MPa, each strain behaved differently. Growth of *Janibacter melonis* R2AA1 and *Bacillus* sp reduced drastically beyond -4.0 MPa. Two strains of *Bacillus subtilis* (*Bacillus subtilis* TSAC5, *Bacillus subtilis* TSAA2) originated each one from C051 and Anna R4 respectively and one *Bacillus* sp. R2AA10 originated from Anna R4 showed poor growth at - 4.5 MPa; only small, tiny colonies appeared at - 4.5 MPa. On the other hand, *Bacillus cereus* R2AA7 exhibited no growth at - 4.5 MPa. Among twelve strains, *Cupriavidus alkaliphilus* TSAC1, *Delftia* sp. TSAC2, *Bacillus marisflavi* TSAC7, *Janibacter melonis* R2AA1, *Microbacterium oleivorans* R2AA6, *Pseudomonas aeruginosa* NAAI3 and *Sphingobium yanoikuyae* R2AI1 exhibited considerable growth at -5.5 MPa (Table.1). Increased drought due to climate change, unpredicted rainfall and depletion of ground water etc.,

has adversely affected the Indian Agriculture. To mitigate the ill effects of drought, various strategies such as selection of moisture stress tolerant cultivators, breeding for resistant varieties through classical and molecular approaches are advocated however these strategies have their own limitations in terms of time requirement and biosafety. Microorganisms with plant growth promoting activity including osmotolerant capacity could be efficient, alternate and cost effective inputs for drought management. Plant growth promoting bacteria (PGPB) of phyllosphere, rhizosphere, rhizoplane or endophytes enhance plant growth by improving plant nutrition (nitrogen fixation, mineral solubilisation), plant growth hormones (auxins, gibberellins, cytokinins), bioactive metabolites (siderophores, hydrogen cyanide) and enzymes improving plant growth and nutrition (ACCD, phosphatases) under stressed and normal conditions. Although numerous bacteria are already in use as PGPB, employment of crop specific growth promoting endophytic bacteria with osmotic stress tolerance would further enhance colonization potential and crop growth in stressed conditions.

Table 1. Growth characteristics of bacterial strains under different concentrations of PEG 6000, ACC deaminase activity and EPS production

Isolates	Source of rice variety	Maximum tolerable limit of PEG 6000	ACC deaminase activity (nmoles mg^{-1} protein h^{-1})	EPS ($\mu g\ ml^{-1}$)
Cupriavidus alkaliphilus TSAC1	CO51	-5.5 MPa	103.20 (±1.36)i	22.94 (±0.464)e
Delftia sp.TSAC2	CO51	-5.5 MPa	193.00 (±4.02)e	32.35 (± 0.673)ab
Bacillus subtilis TSAC5	CO51	-4.5 MPa	136.10 (±0.30)fg	22.25 (± 0.556)e
Bacillus marisflavi TSAC7	CO51	-5.5 MPa	96.80 (±1.60)i	30.49 (±0.778)c
Bacillus subtilis TSAA2	Anna R4	-4.5 MPa	271.60 (±5.94)a	28.04 (±0.584)d
Janibacter melonis R2AA1	Anna R4	-5.5 MPa	194.17 (±2.35)d	32.35 (±0.421)ab
Bacillus cereus R2AA7	Anna R4	-4.0 MPa	43.60 (±0.31)l	28.33 (±0.487)d
Kocuria rosea R2AA5	Anna R4	-5.5 MPa	184.00 (±4.93)c	30.39 (±0.775)c
Microbacterium oleivorans R2AA6	Anna R4	-5.0 MPa	219.20 (±4.77)b	31.27 (± 0.114)bc
Bacillus sp. R2AA10	Anna R4	-4.5 MPa	146.18 (±1.62)gh	23.63 (±0.406)e
Pseudomonas aeruginosa NAAI3	IR 64 DRT	-5.5 MPa	92.07 (±1.02)j	34.02 (±0.496)a
Sphingobium yanoikuyae R2AI1	IR 64 DRT	-5.5 MPa	210.00 (±0.34)bc	32.84 (±0.820)ab

In a column, means followed by a common letter in superscript are not significantly different at 5%

In this context, 12 bacterial strains were screened for osmotic stress tolerance under in vitro condition using PEG 6000. Among these 12, five isolates with maximum osmotic tolerance (-5.5MPa) were obtained from drought tolerant rice varieties *viz.,* AnnaR4 and IR64 DRT (Table.1). All gram negative strains and certain gram positive bacteria such as Bacillus marisflavi TSAC7, *Janibacter melonis* R2AA1 and *Kocuria rosea* R2AA5 tolerated the maximum osmotic stress in liquid and solid medium. In fact, enhanced growth was evidenced for *Sphingobium yanoikuyae* R2AI1 and *Microbacterium oleivorans* R2AA6 in osmotic stress condition. The study also revealed that osmotic stress tolerance of the tested strains was greater than already reported well-established plant growth promoting bacteria such as *Azospirillum brasilense* (-1.9 MPa) and *Pseudomonas* (-0.3 MPa) (Ali et al., 2014).

Extracellular proline content

The results of proline content of 24 h old cultures grown in normal and osmotic stress conditions are given in the Table.2. Proline production by gram negative bacteria reduced drastically in elevated osmotic stress conditions. Most of the gram positive strains particularly different species of *Bacillus* accumulated more proline in normal and elevated osmotic stress conditions. In the absence of PEG 6000, *Bacillus marisflavi* TSAC7 produced more proline (35.50± 0.499 $\mu g\ ml^{-1}$). In the presence of PEG 6000, *Bacillus marisflavi* TSAC7 registered maximum value for proline (30.35± 0.158 $\mu g\ ml^{-1}$). Under high osmolarity, bacterial cells have to maintain an intracellular osmotic pressure greater than the growth medium in order to generate cell turgor, generally considered to be the driving force for cell extension, growth and division (Laszlo N Csonka, 1989). Therefore under osmotic stress, bacterial cells accumulate either inorganic osmolyte (K+) or low molecular weight organic solutes such as sugars (sucrose and trehalose), sugar alcohols (sorbitol and mannitol), amino

acids (proline and glutamate), quaternary ammonium compounds (glycine betaine and carnitine) and tetrahydro pyrimidines (ecotine and hydroxyecotine) (Takagi, 2008). Their functions include stabilization of sub-cellular structures, regulation of co-enzymes and scavenging of free radicals to prevent membrane degradation (Bhauso *et al.,* 2014). The significance of each osmolyte varies between species and within species and the environmental conditions, however, the most commonly studied are mannitol, proline and glycine betaine (Kalsoom *et al.,* 2013).

In general, under low osmotic stress, gram positive and negative bacteria accumulate more K^+ ions in cytosol as a primary response and further increase in osmotic stress triggers secondary response in which neutral organic osmolytes will accumulate.

In light of above, we have analysed the neutral osmolyte like proline production in osmotic stressed bacteria. Extracellular proline production in osmotic stressed strains was lower than normal cultures. Among gram positive and gram negative strains, proline content of the later was very low at a water potential of -5.5 MPa. However, gram positive strains such as *Bacillus marisflavi* TSAC7, *Bacillus subtilis* TSAC5, *Bacillus cereus* R2AA7 and *Kocuria rosea* R2AA5 recorded almost similar quantity in stressed and normal conditions. Many species of gram-positive bacteria have been shown to increase their internal proline pool size by increased synthesis (Cayley *et al*., 1992); while gram-negative bacteria generally achieve high intracellular concentrations of proline during osmotic stress as a consequence of enhanced transport (László N Csonka, 1981). Majority of the bacteria synthesize proline from glutamate catalysed by γ-glutamyl kinase, γ-glutamyl phosphate reductase and Δ1-pyrroline-5-carboxylate reductase (Sleator and Hill, 2002). Kunst et al. (1997) has uncovered an additional proline biosynthesis pathway in *B. subtilis* which may be responsible for the high-level accumulation of proline under hyper-osmotic growth conditions (Bremer, 2000). Among gram-positive bacteria, osmoprotection by exogenous proline uptake has been most extensively studied in *Staphylococcus aureus, Lactobacillus lactis* and *Bacillus subtilis* (Sleator *et al.,* 2002).

Table.2. IAA, GA3 and Proline content of bacterial cultures in the presence and absence of PEG 6000

Isolates	IAA (µg ml^{-1})		Gibberellic acid (µg ml^{-1})		Proline content (µg ml^{-1})	
	Without PEG	With PEG	Without PEG	With PEG	Without PEG	With PEG
Cupriavidus alkaliphilus TSAC1	31.10 (±0.176)b	7.62 (±0.121)b [-5.5]	361.50 (±5.61)e	1351.57 (±33.200)d[-5.5]	31.00 (±0.408)c	4.05 (±0.062)j [-5.5]
Delftia sp.TSAC2	7.62 (±0.016)fg	9.19 (±0.225)a [5.5]	193.00 (±3.21)h	1411.10 (±19.093)d [-5.5]	31.00 (±0.758)c	8.62 (±0.188)h [5.5]
Bacillus subtilis TSAC5	16.38 (±0.350)c	8.97 (±0.009)a [4.5]	450.50 (±10.08)b	4329.67 (±47.318)c [-4.5]	28.00 (±0.219)ef	26.78 (±0.237)c[4.5]
Bacillus marisflavi TSAC7	8.18 (±0.153)f	0.65(±0.015)I [-5.5]	261.50 (±2.72)g	5692.52 (±59.250)b [-5.5]	35.50 (±0.499)a	30.35 (±0.158)a[5.5]
Bacillus subtilis TSAA2	13.01 (± 0.074)d	5.48 (±0.123)e [4.5]	346.50 (±6.85)e	4187.29 (±41.404)c [-4.5]	31.00 (±0.065)cd	14.97 (±0.218)f[4.5]
Janibacter melonis R2AA1	64.70 (±0.135)a	5.82 (±0.045)d [5.5]	392.50 (±2.04)d	1420.62 (±7.393)d [-5.5]	33.00 (±0.653)b	21.81 (±0.488)d[5.5]
Bacillus cereus R2AA7	15.37 (±0.264)c	7.39 (±0.181)b [4.0]	295.50 (±2.31)f	6121.10 (±70.081)a [-4.0]	26.00 (±0.622)h	20.76 (±0.248)e[4.0]
Kocuria rosea R2AA5	11.55 (±0.054)e	1.78 (±0.004)g [5.5]	351.00 (±5.48)e	5611.57 (±96.372)b [-5.5]	29.50 (±0.476)ce	28.30 (±0.412)b[5.5]
Microbacterium oleivorans R2AA6	15.03 (±0.336)c	6.72 (±0.161)c [5.0]	418.50 (±1.52)c	4349.19 (±99.589)c [-5.0]	26.50 (±0.621)h	6.46 (±0.047)I [-5.0]
Bacillus sp. R2AA10	6.38 (± 0.106)g	4.25 (±0.009)f [-4.5]	154.00 (±1.12)i	1383.48 (±21.600)d [-4.5]	28.00(±0.073)efg	12.05 (±0.082)g[4.5]
Pseudomonas aeruginosa NAAI3	7.51 (±0.117)fg	5.37 (±0.008)e [5.5]	461.00 (±2.64)b	690.62 (±6.110)e [-5.5]	21.50 (±0.470)i	9.00 (±0.164)h [5.5]
Sphingobium yanoikuyae R2AI1	15.15 (±0.142)c	1.33 (±0.032)h [5.5]	549.50 (±8.87)a	5775.38 (±123.230)b[5.5]	21.00 (±0.415)i	12.65 (±0.171)g[5.5]

In a column, means followed by a common letter in superscript are not significantly different at 5%

Values within square bracket indicate the maximum moisture stress in MPa at which IAA, GA3 and Proline were estimated

Two modes of stress abatement by proline were suggested. In one of the modes it was suggested that bacteria accumulate proline via up-regulation of proline biosynthesis with proline serving as an osmolyte, a chemical chaperone; another strategy depends on active proline metabolic flux and linkages to other metabolic pathways. Proline metabolic flux leads to cell protection by maintaining cellular energy and NADP+/NADPH balance, activating signaling pathways that promote cell survival, and contributing to other pathways such as the tricarboxylic acid cycle and GSH biosynthesis (Liang *et al.,* 2013). Henceforth, whether the proline content enhances or a decrease depends on the strategy operates in particular bacteria. The tendency for exogenous proline to accumulate in the cytoplasm of bacteria exposed to osmotic stress would, however, be countered by increased proline catabolism (Milner *et al.,* 1987). Thus free extracellular proline pool will decrease under stressed conditions.

Extra-cellular polysaccharide

The amount of extra-cellular polysaccharide (EPS) produced by different endophytic bacteria under non stressed conditon was studied and the results are given in Table.1. *Pseudomonas aeruginosa* NAAI3 produced more EPS (34.02± 0.496 µg ml^{-1}). EPS production in stressed condition could not be detected due to poor settlement of polysaccharides. Another macromolecule associated with drought tolerance is extra-cellular polysaccharide (EPS). EPS has unique water retention and cementing property and thereby play a vital role in regulation of nutrients and water flow across plant roots through biofilm formation (Roberson and Firestone, 1992). Relatively high EPS production was noticed in the strains of *Pseudomonas aeruginosa* NAAI3, *Sphingobium yanoikuyae* R2AI1, *Janibacter melonis* R2AA1 and *Delftia* sp. TSAC2. EPS production in microorganisms occurs due to response towards the stress (Roberson and Firestone, 1992). A function for EPS substance in the fortification of *A. brasilense* Sp245 cells against dehydration was reported by Hartel and Alexander (1986). Konnova *et al.* (2001) reported a significant association between the total EPS formed by cowpea *Bradyrhizobium* strains and drought tolerance. It is expected that EPS can afford a microenvironment that absorb water and dries out more gradually than the neighbouring microenvironment, thus defending bacteria from drought and fluctuations in water potential (Hepper, 1975; Wilkinson, 1958). *Pseudomonas putida* strain GAP-P45 having the ability to produce exopolyschharides (EPS) was reported to alleviate drought stress in sunflower (*Helianthus annuus* L.) seedlings by activating the host plant's antioxidant enzyme machinery (Saikia *et al.,* 2018).

ACC deaminase activity

The ACC deaminase (ACCD) of 24h old cultures was assessed under non stressed condition and the results are shown in Table.1. *Bacillus subtilis* showed maximum activity (271.60± 5.94 nmoles mg^{-1} protein h^{-1}) and the lowest activity was found in *Bacillus cereus* R2AA7 (43.60± 0.31 nmoles mg^{-1} protein h^{-1}). Under different types of environmental stresses *viz.,* cold, drought, flooding, pathogens and heavy metals, plants respond by synthesizing 1-amino cyclopropane-1-carboxylate (ACC), which is the precursor for ethylene which has adverse effects on plant growth and leads to senescence in plants (Ali *et al.,* 2014). Some of the endophytic bacteria containing the enzyme ACCD are found to lower the level of ACC in stressed plants and thus limiting the amount of stress related ethylene synthesis and thereby damage to the plants. In the current study, all the 12 cultures exhibited substantial activity of ACCD in the absence of osmotic stress. Greater ACCD activity was evidenced with *Bacillus subtilis* TSAA2, *Microbacterium olerivorans* R2AA6 and *Sphingobium yanoikuyae* R2AI1 which were isolated from drought tolerant rice varieties.

Plant growth promoting traits of bacterial strains

Indole acetic acid

Quantity of IAA produced by 12 endophytic bacterial strains is represented in Table.2. IAA productivity of all the cultures decreased due to osmotic stress. In the absence of PEG 6000, *Janibacter melonis* R2AA1 produced higher IAA (64.70± 0.135 µg ml^{-1}). In the presence of PEG 6000, *Delftia* sp. TSAC2 registered maximum value (9.19± 0.225 µg ml^{-1}). IAA content of the bacterial strains reduced greatly when grown in maximum concentration of PEG 6000 compared to unstressed condition. Similar observations were made by Marulanda *et al*. (2009) who have reported reduction in IAA production in moisture stressed *Pseudomonas* sp. (40 and 60 % PEG). Similarly Saikia *et al.*, 2018 reported decrease in IAA production by ACCD activity containing rhizobacteria under osmotic stress condition (0.73 MPa). The optimal functioning of ACCD containing bacteria includes the synergistic interaction between ACC deaminase and indole-3-acetic acid (IAA) (Glick, 2014). IAA induces ethylene level in bacteria. Increased ethylene levels in turn have feedback inhibitory effects on IAA signal transduction, which thereby limits IAA production (Glick, 2014). Thus, IAA production may be reduced in moisture stressed and ACCD containing strains.

Gibberellic acid

Gibberellic acid (GA_3) produced by different endophytic bacteria was studied and the results are indicated in Table.2. The maximum production of GA_3 without PEG 6000 was found in *Sphingobium yanoikuyae* R2AI1 (549.50± 8.8 µg ml^{-1}) and in the presence of PEG 6000 the maximum production of GA_3 was recorded in *Bacillus cereus* R2AA7 (6121.1± 70.01 µg ml^{-1}. Studies also revealed that osmotic stressed axenic cultures produce more GA_3 compared to cultures grown in the absence of PEG 6000. The increase ranged from minimum of 4 to maximum of 20 folds. However, the GA_3 content of strains used in current study was lesser than already reported strains such as *Pseudomonas* sp. (285 mg/L) (Rangaswamy, 2012) and *Gibberella fujikuroi* (20 to 200 mg/L) (Rademacher, 1994). Gibberellic acid was found to stimulate plant growth and development under various abiotic stress conditions (Ahmad, 2010). In addition, gibberellic acid enhanced antioxidant enzyme

activity by lowering the levels of reactive oxygen species (ROS) which contributed to better growth under stress (Manjili *et al.,* 2012).

Mineral solubilization

Selected isolates were characterized for their ability to enhance the availability of macro (P&K) and micronutrients (Zn). These strains could not solubilize phosphate, zinc and silicate. However, these results are to be confirmed.

CONCLUSION

Apoplast endophytic bacteria have the ability to tolerate various levels of osmotic stress induced by PEG 6000. It is noteworthy that most of the bacterial strains tolerated more than - 4.0 MPa which is greater than earlier records. The results are also quite interesting, since moisture stress tolerance of endophytic bacteria of drought tolerant rice varieties were greater than normal variety. These strains were also shown to produce substantial quantity of plant growth hormones such as IAA and GA_3 in stressed and normal conditions. The activity of the enzyme (ACCD) involved in reducing ethylene level and indirectly mitigating drought was quite greater. Even though, plant growth promoting activity evaluated in terms of enhancement of nutrient availability was found to be futile, these strains could serve as potential inoculants as stress alleviator in addition to improvement of plant growth through production of growth hormones and bioactive metabolites However, further studies are imperative to assess drought alleviation and plant growth promotion potential of these bacteria under field conditions.

ACKNOWLEDGEMENT

I gratefully acknowledge the financial support extended by MHRD-GOI Scheme, in the form of Junior Research Fellowship to carry out the present work.

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