Biological Forum – An International Journal **15(10): 602-609(2023)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Mining of drought Tolerant Endophytic Bacteria from Weed species of Dry Regions of Karnataka

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(Received: 29 July 2023; Revised: 30 August 2023; Accepted: 25 September 2023; Published: 15 October 2023)
(Published by Research Trend)

ABSTRACT: The present study aims to isolate and characterize the drought tolerant endophytic bacteria from different weed species grown on dry regions of Karnataka. Totally fifty endophytic bacterial isolates were isolated and were screened for different drought stress concentration (PEGMW8000 concentration - 10, 15 and 20%). Among the isolates, eight isolates were able to grow at 20 percent PEGMW 8000 concentration (-0.51MPa), further these isolates characterized for drought tolerant potential and plant growth promotion potential under *in-vitro* condition. The results showed that among the isolates, two endophytic bacterial isolates *viz.*, AVS2B and CDS1A isolated from the weed species *Amaranthus viridis* and *Cynodon dactylon* respectively exhibited positive response to most of the *in vitro* PGPR characteristics and drought tolerant characteristics studied under both stressed (-0.51MPa) and unstressed condition. Therefore, these strains could have vital implications for the agricultural sector if used as biofertilizer. Two efficient isolates. AVS2B and CDS1A were them molecularly identified as *Alcaligenes faecalis and Pseudomonas taiwanensis* respectively by 16S rRNA gene sequence.

Keywords: Drought tolerance, endophytic bacteria, Polyethylene glycol and plant growth promotion.

INTRODUCTION

Plants are frequently encountered by variety of environmental stresses resulting in altered growth and metabolism (Ahmad *et al*., 2010). Among the various environmental constraints, drought is a major abiotic factor that adversely affects crop growth and productivity worldwide. Drought is expected to cause serious plant growth problems for more than 50% of the arable lands by 2050 (Vinocur and Altman 2005). Drought stress may range from moderate and short to extremely severe and prolonged duration, restricting the crop yields. Global warming will further increase the severity and frequency of drought in the future leading to a possible decrease in global food production and affecting world's food security. At the same time a steadily increasing human population which could hit 9 billion by 2050 demands an increase in food supplies (UN, 2017). In this regard, numerous approaches are used to improve drought tolerance in plants.

Modern agro-biotechnological strategies are being currently used to enhance drought stress tolerance in plants such as development of drought-tolerant varieties through genetic engineering, plant breeding and generation of transgenic plants with introduced novel genes or with altered expression levels of the existing genes (Lu *et al*., 2013). However, the complexity of drought stress tolerance mechanisms makes the task of introducing new tolerant varieties very difficult and genetically modified plants are not well accepted in many regions of the world (Wahid *et al*., 2007). Therefore, there is an immense need of a sound and environment-friendly strategy to cope with this condition.

Plants possess various strategies to adapt to or avoid the damages caused by different stresses, but on the other hand, they also interact with a variety of microorganisms that can alleviate the stress symptoms. Number of microorganisms is naturally associated with plants in several ways including mutualistic, commensal, neutral, exploitative and competitive. Some of these microbes, i.e., bacteria and fungi found in plant rhizosphere, have ability of entering the plants as endophytes (Lareen *et al*., 2016; Ullah *et al*., 2019). Endophytes are those microorganisms which colonize the internal tissue of a plant (Hardoim *et al*., 2015).

Under stress conditions, plants are more dependent on these endophytes which are able to enhance their metabolic activity to combat stress (Kavamura *et al*., 2013). Endophytic bacteria may in future be even more efficient than rhizosphere bacteria, because they escape competition with rhizosphere microorganisms and achieve more intimate contact with plant tissues (Mehta *et al*., 2014). Presently, plant–endophytic bacterial interactions received much attention due to its efficient role in increased crop production and providing enhanced drought tolerance (Finkel *et al*., 2017). This interactions result in plant growth promotion under drought stress through various mechanisms including nitrogen fixation, production of indole-3-acetic acid (IAA) and siderophore, and phosphate solubilization (Ullah *et al*., 2019). These plant growth promoting characteristics of endophytic bacteria improve root

length and density, which lead to the enhance drought tolerance. In addition, the production of drought tolerant substances such as abscisic acid, 1-aminocyclopropane-1-carboxylate (ACC) deaminase and numerous volatile chemicals by plant endophytic bacteria also aids plants in surviving drought stress.

In this regard, several bacterial endophytes associated with numerous crops have been explored, but only a few studies have focused on exploration of endophytic microflora of weeds. Weeds predominate in crop lands despite all of the unfavourable environmental conditions due to the presence of endophytes, which are known for regulating plant growth under stress condition. Numerous taxonomic genera of bacteria including those from *Sinorhizobium, Bacillus, Pseudomonas, Marinorhizobium, Sphingomonas, Sphingobium, Herbaspirillum, Micrococcus, Microbacterium* and *Rhodococcus* are linked to weed species and may be used as natural bioresources to boost agricultural productivity (Fatema *et al*., 2019). Despite having a great deal of promise for use in environment friendly sustainable agriculture, bacterial endophytes of weed species have received little research.

Thus, with all this comprehensive information, the present study focus on exploration of bacterial endophytes from different weed species grown on dry regions and studying their drought tolerance potential under in vitro condition. Then the efficient isolates were tested for drought tolerant traits as well as PGP traits under *in vitro* condition.

MATERIALS AND METHODS

Collection of plant samples. The healthy weed species *viz*., *Ageratum conyzoides, Cynodon dactylon, Digitarias anguinalis* and *Tridax procumbens* which are known for their drought tolerance were collected in polythene bags from dry regions of UAS, GKVK, Bengaluru.

Isolation of endophytic bacteria from the drought tolerant weed species. The root, stem and leaves of each weed plant were washed under tap water to remove the adhering soil particles and majority of epiphytes. The root stem and leaves were cut into approximately 1 cm length and were surface sterilized by treating with 70 % ethanol for 1 min followed by sodium hypochlorite solution (2 %) for 3 min (root) and 1min 30 sec (shoot and leaves), respectively. Later were repeatedly washed using sterile water. The surface sterilized root stem and leaves bits were impregnated on nutrient agar by giving gentle press and incubated at 30°C for 48h (Anjum and Chandra 2015).

Purification of bacterial isolates. After incubation the bacterial growth around the cut ends of root, stem and leaf bits were observed. The bacterial growth was picked up and streaked on nutrient agar medium which were incubated at 30 °C for 48 h to obtain the pure culture. Then the pure cultures were maintained in slants, broth and glycerol stock (70 percent) at 4 °C under refrigerator condition.

Screening of endophytic bacterial isolates for their drought stress tolerance and plant growth promoting ability under *invitro* condition

(A) Screening of endophytic bacterial isolates for drought stress tolerance at different PEGMW 8000 concentration. All the isolated bacterial endophytes were tested for drought tolerance at different drought stress concentration (PEGMW 8000 concentration - 10,15and20%) inoculated into nutrient broth. The elite isolates which showed tolerance at higher PEG concentration (20% PEG)were selected for further studies.

(B) 1-Aminocyclopropane-1-carboxylated eaminase assay. To determine the ACC deaminase activity, screened eight isolates were grown in tryptic soya broth in a shaker at 28ºC with 150 rpm for 24h. After centrifugation cell pellet was washed with 0.1 M Tris-HCl (pH 7.6) and resuspended in 0.1 M Tris-HCl(pH 7.6). Finally spot inoculated on DF minimal salts medium (with and without mannitol as drought osmotic stress inducer) with 3 mM ACC as sole nitrogen source (Singh and Jha 2015). Mannitol of 0.2 M induces an osmotic stress of -0.51 MPa as mentioned by Mozdzen *et al*. (2015). Bacterial growth was monitored daily and those that grew in ACC supplemented DF medium were considered putative ACC degrading bacteria.

(C) Proline production assay. Proline content accumulated in bacterial isolates was estimated in nutrient broth with (20% PEG MW 8000) and without stress. Inoculated broths were incubated for 48h and were quantified following the method by (16). Two mL of culture was centrifuged at 10,000 rpm for 10 min. Cell pellets were kept in water bath at 60 °C for 45 min with 80 % ethanol. Further ethanol added suspension was centrifuged at 8,000rpm for 15 min and 1 mL of supernatant was collected. The supernatant was mixed with 1mL of acid ninhydrin and 1 mL of glacial acetic acid. The reaction mixture tube was kept in boiling water for 1 h and transferred to ice bath for cooling. Extraction of proline from the reaction mixture was done by adding 2 mL of toluene. Then the extracted proline appears pinkish to red colour, which was separated and transferred to new tubes and the absorbance was measured using the spectrophotometer (BioMate3S,USA) at 520nm. A standard curve was prepared using the proline. The results were expressed as µg of proline perm L(µgmL^{-1}) of bacterial culture (Ceylan *et al*., 2012).

(D) Ammonia production assay. The selected bacterial isolates were tested for ammonia production by the method described by Cappuccino and Sherman (1992). The bacterial cultures were inoculated into 10 mL peptone water broth provided with (20 % PEG MW 8000) and without stress, which were incubated at 30 ± 0.1 °C for 72 h. After incubation 0.5 mL Nessler's reagent was added into each tube. The development of brown to yellow colouration is positive for ammonia production.

(E) Phosphate solubilization assay. The qualitative evaluation of tricalcium phosphate solubilization in the isolated strains was done using Pikovskaya's agar. Selected bacterial isolates were spot inoculated on the surface of Pikovskaya agar medium with (0.2 M mannitol) and without osmotic stress. The phosphate

solubilizing activity was estimated after 72 h of incubation at 28 °C. The formation of aclearzone around the colony indicated in organic phosphate solubilization (Gour, 1980). The phosphorous solubilization index was calculated in mm using the formula mentioned (Sadiq *et al*., 2013).

SI=(Z+C)/C

Where, SI = Solubilization Index

Z = Halo zone diameter in mm

C=Colony diameter inmm

(F) Potassium solubilization assay. The selected bacterial isolates were spot inoculated on the Aleksandrov's medium provided with(0.20Mmannitol) and without osmotic stress whichwereincubatedat28 °C for 72 h. The formation of clear zone around the growth of colony is positive for potassium solubilization. Further potassium solubilization index was calculated in millimeter using the formula mentioned (Sadiq *et al*., 2013).

SI=(Z+C)/C

Where, SI = Solubilization Index

Z = Halozone diameter in mm

C=Colony diameter in mm

(G) Hydrogen cyanide production assay. Bacterial isolates were streaked on nutrient agar medium with (0.20 M mannitol) and without stress containing glycine at the rate of 4.4 g L^{-1}. Filter papers were cut into pieces as per the size of a petri plate lid and soaked in picric acid solution (2.5 g of picric acid and 12.5 g of Na_2CO_3 in 100 mL of sterile distilled water). After soaking filter paper was placed in the inner section of the lid of a petri dish containing inoculum and allowed for incubation at 30 ˚C for 48 h. The positive results were noted by the colour change of the disc from yellow to brown or reddish brown (Millar and Higgins 1970).

(H) Siderophore production assay. The bacterial isolates were tested for their ability to produce siderophores with (20% PEG MW 8000) and without stress. Supernatant was extracted from 3 d old culture grown in nutrient broth. One mL of culture was centrifuged at 10,000 rpm for 10 min. The supernatant and the universal Chrome Azurol S(CAS) reagent was taken in the ratio of 1:1 as described by Schwyn and Neilands (1987) and kept under room temperature for 1 h in order to detect the siderophore production by the bacterial isolates. The absorbance was recorded at 630 nm using the spectrophotometer (BioMate 3S, USA)against a reference consisting of uninoculated broth.

% Siderophore units=[(Ar–As)/ Ar] × 100

Where,

Ar = Absorbance of reference at 630 nm (CAS reagent)

As = Absorbance of sample at 630 nm

(I) Indole-3-acetic acid production assay. Estimation of IAA in the nutrient broth was done using colorimetric assay. The endophytes cultured in 20 mL of nutrient broth with (0.1 g/ L) and without L – tryptophan and incubated at 30 ± 2 ºC with (20 % PEG MW 8000) and without stress at 200 rpm for 7 d without the interference of light. Then they were centrifuged at 10,000 rpm for 10 min at 4 ºC and the cell free cultures were filtered through 0.45 µm cellulose acetate filter. The filtrates were acidified top H 2.8 with 1N of HCl and extracted 3 times with 20 mL of ethyl acetate. The ethyl acetate fractions were combined and evaporated under vacuum at 45 ºC in a rotary evaporator. The residue was re-suspended in 3 mL 50% methanol. Two mL of Salkowski reagent (12 g of $FeCl_3$ L^{-1}, 7.9 M of H_2SO_4) was added to 1 mL of supernatant and kept in dark for 30 min (Latifkhan *et al*., 2016). The resultant reddish colour was read with spectrophotometer (Bio Mate 3S, USA) after 30 min at 530 nm (Patten and Glick 2002).

(J) Quantification of Gibberellic acid, Abscisic acid and Salicylic acid (HPLC). The 24 hold cultures were inoculated in 20 mL nutrient broth and incubated at 30 °C for 7 d. After incubation they were centrifuged at 6000 rpm for 10 min and the supernatant was collected, which was adjusted to the pH of 2.8 using 1 N HCl solution. The acidified supernatant was taken in 100 mL conical flask and equal volume of diethyl ether was added and incubated for 4h at 4°C. The solvent phase(upper layer) formed was collected and allowed to evaporate. To the evaporated samples 2 - 3 mL of HPLC grade methanol was added and stored at-20°C after membrane sterilization to perform high performance liquid chromatography (Shimadzu, Japan).

Molecular identification of effective isolates using 16Sr RNA gene sequence

Isolation of genomic DNA of entophytic bacteria

The genomic DNA was isolated by alkali lysis method and further used for PCR

Polymerase chain reaction. The fragment of 16S rRNA gene was amplified by 16S rRNA-F and 16S rRNA-R primers. PCR was performed in 20 µL volume reaction mixture containing the following PCR components. PCR components (20 µL) contained: 2.0µl of 1XPCR Taq. Buffer with $MgCl_2$ (1.5mM), 2.0µl of 10Mm dNTP's mix(200 µM), 0.5µl of 16S rRNA primers (forward and reverse 0.5 Mm each), 0.3µl of Taq DNA Polymerase (1Ugenei Bengaluru), 1.0µl of Template DNA(~50ng/µL) and 13.7µL of Sterile distilled water.

PCR amplification was carried out with an initial denaturation at 96 °C for 4 min followed by 35 amplification cycles consisting of 94 °C for 1 min, 55 °C for 30 sec and 72°C for 1min and a final extension at 72 °C for 12 min. Controls for PCR reactions were carried out with the same primers without providing template DNA. Then the amplified product of DNA was electrophoresed using 1 % agarose gel as described earlier. The DNA band was visualized under UV light and documented using gel documentation unit and got sequenced by Barcode Biosciences, Bangalore, Karnataka. The sequence data received from the company was analysed for homology using NCBIGenBank.

Sequence analysis and homology search. Sequence results were analysed using National Centre for Biotechnology Information (NCBI), USA. The BLAST (Basic Local Alignment Search Tool) search gave the partial length sequence homology with NCBI data (Altschul *et al*., 1997).

Phylogenetic analysis. Phylogenetic analysis was performed to know the relationship between identified species with the species deposited in the NCBI

GenBank. Preliminary pairwise and multiple alignments were performed using Cluster W for the two entophytes sequenced independently. Phylogenetic analysis was performed using MEGAX software and phylogenetic trees were generated using the maximum likelihood joining algorithm (Kumar *et al*., 2016).

Statistical analysis. The data obtained from experimentation were statistically analyzed using completely randomized design (CRD).The statistical analysis was done by using WASP: 2.0 (Web Agri Stat Package 2) statistical tool (https://ccari.res.in/waspnew.html) and means were separated by Duncan Multiple Range Test (DMRT). The obtained data were the mean of independent experiments with three replicates. p values<0.05 were considered as statistically significant.

RESULT AND DISCUSSION

Drought is a stress condition where the plants suffer from water shortages and plants will depend on the groundwater to carry out their metabolic activities. Plants that grow under adverse environments, such as dry land plants, will undergo water and nutritional deficiencies but have several mechanisms to overcome the deleterious effects. One of the mechanisms is by microorganisms help, particularly plant–endophytic interactions. Weed plants are native and pioneering, they are very abundant in nature, grow in dense populations and do not undergo selection or breeding. Only a few studies have focused on characterization of endophytic microflora of weeds, therefore selection, screening and application of drought-stress-tolerant endophytic microbes from weeds can help to overcome productivity limits in drylands.

In this study, a total of fifty endophytic bacterial isolates were isolated from the root, stem and leaves of drought tolerant weed plants *viz*., *Ageratum conyzoides, Cynodon dactylon, Digitaria sanguinalis, Amaranthus viridis* and *Tridax procumbens.* Almost all collected weed plants have harbored bacterial endophytes. Out of total isolates, 46% of bacterial isolates were isolated from the roots of the weed species followed by stem (29%) and leaves (22%).

Screening of isolated endophytes for drought tolerance efficacy. Popular approach like use of high molecular weight osmotic substance like polyethylene glycol (PEG) was carried for induction of drought stress (Landjeva *et al*., 2008). This compound can reduce the osmotic potential of the solution through the ethylene oxide sub unit matrix activity, which can bind water molecules with hydrogen bonds. Hence PEG solution *in vitro* media is expected to create a potential osmotic equivalent to soil conditions in the field capacity and critical humidity point so that bacteria responded similarly when they were stressed in the field. All the isolated bacterial entophytes were examined at different drought stress concentration (PEGMW8000 concentration - 10,15and20%). Among fifty isolates, eight isolates were able to grow at 20 percent PEGMW 8000 concentration (-0.51MPa).

The screened eight isolates were then selected for further *in vitro* studies and 20% PEG MW 8000 (-0.51MPa) stress condition was used along with non-stress condition. Efficient isolates were screened for drought tolerant character slike1-Aminocyclopropane-1-carboxylate deaminase, ammonia and proline production. Among them, AVS2BandCDS1Aisolates were able to produce ammonia and1-Aminocyclopropane-1-carboxylatedeaminase. This microbial enzyme, ACC deaminase, is responsible for dissociation of stress induced ACC (secreted as root exudates) into ammonia and α-ketobutyrate, which otherwise forwarded to produce ethylene, that has a drastic impact on physiology, growth and development of plants. Therefore, these endophytic bacterial isolates having ACC deaminase activity help plants to withstand stress (biotic and abiotic) by reducing the level of stress ethylene.

All the tested bacterial isolates possess hydrogen cyanide (HCN) production ability under unstressed condition except AVR1A isolate. The results are similar to those of Nandhini *et al*. (2012) who reported HCN production under stressful conditions. The tested strains produced HCN that may help in balanced antioxidant activities. Bacterial production of HCN acts as a biocontrol agent against a variety of pathogens, reducing disease spread.

Proline is an osmoprotectant produced when a plant subjected to a stress conditions like drought, high temperature and salinity and also an adaptive mechanism provided to the plant to withstand the harsh stress conditions. Similarly when microorganisms subjected to induced drought in a solute by PEG, it also produces osmo protectant like proline, glycine betaine, trehalose *etc*., provides stress tolerance to the microbes. Estimation of internally produced proline concentration of rhizobacteria during moisture stress conditions comparing with non-stress condition give insight about the potentiality of the microorganisms to counteract the effect of abiotic stress. In the current study, maximum production of proline was observed by the isolate AVS2B (37.28 µg mL^{-1}) followed byCDS1A (32.40 µg mL^{-1})without stress condition. Under the drought stress(-0.51MPa) maximum proline was produced by the isolate AVS2B (29.96µgmL^{-1}) followed by CDS1A (27.11 µg mL^{-1}), TPS1B (19.96 µg mL^{-1}) and AVR2B (13.62 µg mL^{-1}) (Table 1-3).

In terms of siderophore producing ability, bacterial isolate DSL1A (55.61%)was the most potent isolate in terms of producing siderophore, followed by isolates ACS1A(54.35%)andCDS1A(47.93%) respectively. Siderophores are low molecular weight iron chelating compounds which possess high affinity to iron, secreted by microorganisms aids in chelation of few essential elements for plants. Our results indicated that all tested bacterial isolates were able to produce siderophores under unstressed and stressed conditions.

The capacity to synthesize IAA is widespread among soil and plant-associated bacteria. Under osmotic stress condition (-0.51 MPa), the maximum IAA production with L-tryptophan supplementation was noticed in the isolate CDS1A (44.26 µg mL^{-1}) followed by isolate AVS2B (40.21 µgmL^{-1}) and CDR1C (26.61 µg mL^{-1}). Further, under the condition of without L-tryptophan

supplementation the highest IAA production was recorded from the bacterial isolate AVS2B (7.62 µg mL^{-1}) followed by CDS1A (4.06 µgmL^{-1}) and TPS1B (3.15 µg mL^{-1}). L-tryptophan acts as a precurs or for IAA production and many bacteria utilizing L-tryptophan present in root exudates have produced IAA as secondary metabolite and few other PGPR have synthesized IAA without the use of external precursor molecule. Our results were in line with earlier study, in which seven of 10 endophytic isolates were positive for IAA production (Jha and Kumar 2007). The ability to produce IAA is considered responsible for plant growth promotion by beneficial bacteria such as *Azospirillum* spp., *Alcaligens facalise*, *Klebsiella*, *Enterobacter*, *Acetobacter diazotrophicus*, and *Herbaspirillum seropedicae* (Costacurta and Vanderleyden 1995).

Plants respond to environmental perturbations by modifying their levels of various hormones, including JA, ABA and ethylene. Production of ABA may be an advantageous trait for species growing in restrictive soil conditions, such as drought and salinity; *i.e.*, synthesis of these hormones could help alleviate stress on the plants. The isolate AVS2B (5.87 µg mL^{-1}) produced maximum quantity of ABA followed by CDS1A (5.75 µg mL^{-1}) and TPS1B (4.88 µg mL^{-1}) isolate. Abscisic acid generally known as stress hormone regulates drought stress by mediating osmotic stress tolerance minimizes transpirational water loss through closure of stomata. Similarly, endophytic bacterial isolate CDS1A (43.65 µg mL^{-1}) produced maximum quantity of salicylic acid followed by the isolate AVS2B (40.22 µg mL^{-1}) andAVR1A (38.60 µg mL^{-1}). Salicylic acid plays an essential role in the activation and regulation of multiple responses to abiotic stresses and also regulates systemically acquired resistance in plants against diseases.

In the gibberelic acid production assay, endophytic bacterial strain AVS2B was found to produce the highest concentration of gibberellic acid (77.61 g mL^{-1}), followed by CDS1A (64.77 gmL^{-1}), AVR1A (59.74 g mL^{-1}) and TPS1B (58.47 g mL^{-1}). The comparable results have been reported by Oteino *et al.* (2015) where three endophytic strains isolated from *Pisum sativum* L produced GA at the concentrations of (14–169gmL^{-1}) and displayed beneficial plant growth promotional effects.

In this study, the potential of endophytic isolates for plant growth promotion was determined by assessing the factors such as proline production, phosphorus solubilization, IAA production, and siderophore secretion. Although isolates exhibiting all the plant growth-promoting features simultaneously were rare, isolates AVS2B and CDS1A were positive for most of these characteristics, indicating their role in plant growth promotion.

The efficient endophytic bacterial isolates were identified based on the homology of their sequence. The 16S rRNA gene sequence of the *Amaranthus viridis* isolate (AVS2B) showed 99 % homology with *Alcaligenes faecalis* available in the NCBI database. Therefore, the endophytic bacterial isolate was identified as *Alcaligenes faecalis*. The phylogenetic tree constructed with 11nucleotide sequences available in the NCBI software (Fig. 2). The accession number of the endophytic bacterial isolate *Alcaligenes faecalis* AVS2BisOP090405.

The phylogenetic tree constructed with 11 nucleotide sequences available in the NCBI (Fig. 3). The 16S rRNA gene sequence of the isolate *Cynodon dactylon* (CDS1A) showed 98 % homology with *Pseudomonas taiwanensis* available in the NCBI database. Thus, the endophytic bacterial isolate was identified as *Pseudomonas taiwanensis*. The accession number of the endophytic bacterialisolate *Pseudomonas taiwanensis* CDS1Ais OP482260.

Table 1: Plant growth promoting activities of drought tolerant bacterial endophytes.

Isolates	ACC Deaminase		Ammonia		HCN		Proline (µgmL^{-1})		Siderophore (%)		Phosphate (SI)		Potassium (SI)	
	Without stress	With stress	Without stress	With stress	Without stress	With stress	Without stress	With stress	Without stress	With stress	Without stress	With stress	Without stress	With stress
AVR1A	+	+	+	-	-	+	12.62^{e}	3.96^{g}	68.31^{a}	46.64^{d}	2.62^{g}	1.00^{d}	1.10^{f}	1.00^{d}
AVR2B	-	-	+	-	+	-	21.29^{c}	13.62^{d}	40.26^{f}	43.78^{g}	3.26^{f}	1.10^{bc}	1.10^{f}	1.00^{d}
AVS2B	+	+	+	-	+	+	37.28^{a}	29.96^{a}	49.64^{e}	45.18^{f}	5.32^{b}	5.12^{a}	4.01^{b}	3.76^{b}
ACS1A	-	-	+	-	+	-	11.29^{f}	6.84^{f}	15.09^{g}	54.35^{b}	2.35^{h}	1.00^{d}	1.01^{g}	1.00^{d}
DSL1A	-	-	+	-	+	-	4.84^{h}	9.96^{e}	12.59^{h}	55.61^{a}	4.31^{b}	1.00^{d}	2.32^{d}	3.20^{c}
CDR1C	+	+	+	-	+	+	15.07^{d}	2.84^{h}	50.48^{d}	45.24^{e}	4.24^{c}	1.00^{cd}	3.43^{c}	1.00^{d}
CDS1A	+	+	+	-	+	+	32.40^{b}	27.11^{b}	59.75^{c}	47.93^{c}	5.43^{a}	1.17^{b}	4.22^{a}	4.13^{a}
TPS1B	-	-	+	-	+	-	9.73^{g}	19.96^{c}	67.95^{b}	41.04^{h}	3.46^{d}	1.00^{d}	1.02^{g}	1.00^{d}
CD(p= 0.05)							1.89	1.65	4.36	4.57	0.35	0.26	0.24	0.21

Note: *ACC deaminase:(+) bacterial growth (-) no bacterial growth
*Ammonia production:(+) yellow colour formation (-) absence of yellow colour
*HCN production:(+) reddish brown colour (-) no reddish brown colour
* Mean values followed by the same superscript in each column do not differ significantly at p≤0.05 level by DMRT.

Table 2: Plant hormones production by drought tolerance endophytic bacterial isolates.

Isolates	Without stress		With stress		Gibberellic acid (µgmL^{-1})	Abscisic acid (µgmL^{-1})	Salicylic acid (µgmL^{-1})
	Tryptophan (-)	Tryptophan (+)	Tryptophan (-)	Tryptophan (+)			
AVR1A	5.47^{efg}	25.46^{i}	1.51^{i}	5.93^{h}	59.74^{c}	0.97^{g}	38.60^{c}
AVR2B	8.42^{de}	56.98^{g}	2.12^{f}	14.93^{f}	37.44^{g}	1.55^{f}	35.66^{e}
AVS2B	20.89^{a}	95.56^{b}	7.62^{a}	40.21^{b}	77.61^{a}	5.87^{a}	40.22^{b}
ACS1A	3.02^{g}	12.35^{j}	1.56^{h}	5.38^{i}	29.67^{h}	0.85^{h}	36.85^{d}
DSL1A	3.92^{fg}	54.86^{h}	2.01^{g}	18.98^{e}	29.66^{h}	0.34^{j}	27.41^{i}
CDR1C	6.28^{ef}	87.65^{c}	3.02^{e}	26.61^{d}	14.77^{i}	0.67^{i}	21.33^{j}
CDS1A	15.72^{bc}	98.54^{a}	4.06^{b}	44.26^{a}	64.77^{b}	5.75^{b}	43.65^{a}
TPS1B	13.47^{b}	78.64^{f}	3.15^{c}	7.06^{g}	58.47^{d}	4.88^{c}	34.05^{f}
Azotobacter chroococcum	11.84^{bc}	79.42^{e}	2.10^{f}	26.60^{d}	49.34^{e}	3.28^{d}	32.58^{g}
Pseudomonas fluorescens	10.25^{cd}	87.46^{d}	3.08^{d}	31.12^{c}	42.34^{f}	2.34^{e}	30.65^{h}
CD(p= 0.05)	1.14	7.35	0.47	2.30	4.86	0.34	3.45

**Note: ***Mean values followed by the same superscript in each column do not differ significantly at p ≤0.05 level by DMRT.

Table 3: Morphological and biochemical characterization of screened drought tolerant bacterial endophytes.

Sr. No.	Isolates	Cell shape	Colony characteristics	Gram reaction	Starch hydrolysis	H_2S production	Citrate utilization	Indole production	Catalase activity
1.	AVR1A	Rod	Creamy Transparent	+	+	+	-	+	+
2.	AVR2B	Rod	Yellow Opaque	-	+	+	+	+	+
3.	AVS2B	Rod	Creamy Translucent	-	+	+	+	+	+
4.	ACS1A	Cocci	Creamy Yellow Opaque	+	+	+	+	+	+
5.	DSL1A	Cocci	Creamy Transparent	-	-	-	+	+	+
6.	CDR1C	Cocci	Light Yellow Opaque	-	+	+	+	-	+
7.	CDS1A	Rod	Light Yellow Opaque	-	+	+	+	+	+
8.	TPS1B	Rod	Creamy Yellow Opaque	-	+	-	+	+	+

Note:(+) Positive, (-) Negative

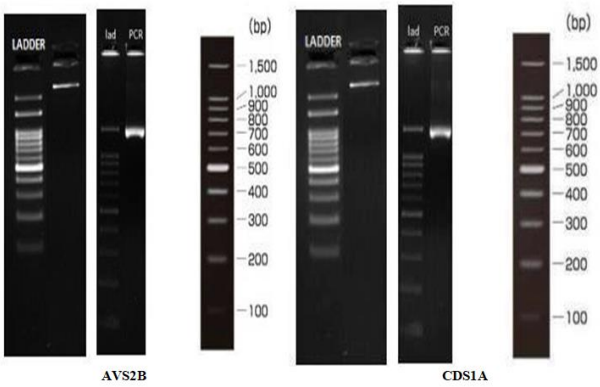

Fig. 1. Amplified DNA of endophytic bacterial isolates.

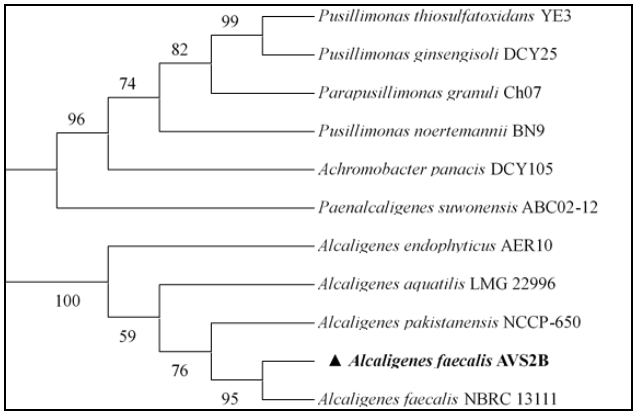

Fig. 2. Phylogenetic tree of endophytic bacteria *Alcaligenes faecalis* isolate AVS2B.

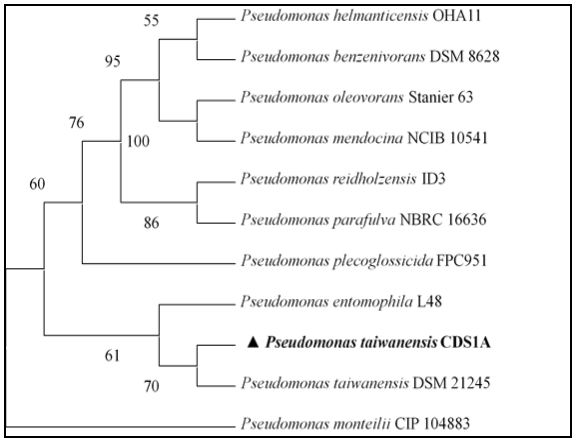

Fig. 3. Phylogenetic tree of endophytic bacteria *Pseudomonas taiwanensis* isolate CDS1A.

CONCLUSIONS

To meet current and future demand of food for a rapidly growing population, novel and sustainable agricultural systems are needed to effectively use land and water resources. This is particularly important with the current climate change challenges where abiotic stress is a limiting factor to agronomic production. The present study explored several bacteria endophytes harbored by weeds species growing under drought stress. Two bacterial strains, *Alcaligenes faecalis* and *Pseudomonas taiwanensis* showed improved drought tolerance and also exhibited positive response to all the *in-vitro* PGPR characteristics studied. Therefore these strains could have vital implications for the agricultural sector if used as biofertilizer. These findings represent a promising environmentally friendly agricultural application to mitigate the effects of climatic change on crop productivity.

Acknowledgement. Authors wants to acknowledge Department of Agricultural Microbiology, University of Agricultural sciences, Bangalore, India, for the facility provided.

Conflict of Interest. None.

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How to cite this article: Divya S.K., Abhinandana K.R., Arati, Shashank S., Lohithkumar N. and Krishna Naik L. (2023). Mining of drought Tolerant Endophytic Bacteria from Weed species of Dry Regions of Karnataka. *Biological Forum – An International Journal*, *15*(10): 602-609.