BIOSCIENCES BIOTECHNOLOGY RESEARCH ASIA, June 2018. Vol. **15**(2), p. 485-494

Isolation and Characterization of Abiotic Stress Tolerant Plant Growth Promoting *Bacillus* spp. from Different Rhizospheric Soils of Telangana

K. Damodara Chari[1], R. Subhash Reddy[1], S. Triveni[1], N. Trimurtulu[2], CH.V. Durga Rani[3] and M. Sreedhar[4]

[1]Department of Agricultural Microbiology & Bioenergy, College of Agriculture, Professor Jayashankar Telangana State Agricultural University, R'nagar, Hyderabad-500030,Telangana ,India.
[2] Biofertilizer Production Laboratory, Agricultural Research Station, Amaravathi, Guntur-522020, Andhra Pradesh. India.
[3]Department of Molecular Biology & Biotechnology, Institute of Biotechnology, Professor Jayashankar Telangana State Agricultural University, Hyd-030, India.
[4]Quality Control Laboratory, Professor Jayashankar Telangana State Agricultural University, R'nagar, Hyd-030, India.

http://dx.doi.org/10.13005/bbra/2653

(Received: 23 April 2018; accepted: 01 May 2018)

Present investigation was carried out to identify plant growth promoting rhizobacterial isolates for abiotic stress tolerance. To achieve this bacterial isolates were isolated from different rhizospheric soils of Telanagana and screened for plant growth promoting properties and tolerance to different abiotic stresses such as pH, temperature, salt, drought and heavy metals. Such PGPR will be helpful for efficient management of abiotic stresses in crop production. Rhizospheric soils from normal, salt affected, drought affected and bulk soils were collected from different places of Telangana state. From all soil samples, based on cultural, morphological and biochemical characterization it was found that forty four were of *Bacillus* spp. Among the forty four (44) *Bacillus* isolates, twenty eight (28) isolates were showing plant growth promoting properties. These positive isolates tested for abiotic stress tolerance to pH, temperature, salt, drought and heavy metals (As and Cd). Four isolates were showed growth at pH range from 4-12 (BS 1, BS 3, BS 14, BS 18), five isolates were showed tolerance to 1.5 to 20 % of NaCl concentration (BS 1, BS 3, BS 14, BS 18, BS 42, six isolates showed tolerance to temperature from 20 ºC -50 ºC (BS 10, BS 14, BS 18, BS 27, BS 37, BS 43), four isolates showed tolerance to water potential from - 0.05 Mpa to- 0.73 Mpa (BS 4, BS 10, BS 18, BS 33).

Keywords: *Bacillus*, PGPR, pH, temperature, salt, drought and heavy metal tolerance.

One of the major problems in rain fed agro-ecosystems is predominance of abiotic stresses like high temperature, salinity and drought where the applied bioinoculants survival and viability is a major issue in Indian conditions. Abiotic and biotic stresses are the limiting factors negatively affecting the crop growth and productivity worldwide. Plants responses to such factors are very complex which manifest in a range of developmental, molecular and physiological modifications that lead to either

*Corresponding author E-mail:

stress sensitivity or tolerance/resistance (Harb *et al.,* 2010.

Increasing crop productivity and enhancing resistance or tolerance against various stress factors has become major aim for modern agriculture (Farooq *et al*., 2009). In sustainable agriculture, integrated pest management is considered the most efficient strategy to manage stress causing agents, such strategy rely on combining several approaches including using resistant varieties, crop rotation, monitoring pests, biocontrol and in severe situations employing pesticides in an attempt to keep stress agents under control (Wegulo, 2012). Biological control forms an integral part of the IPM strategy (Landa *et al*. 2004).

Plant growth promoting rhizobacteria can improve plant growth and productivity by several mechanisms. Few strains from genera such as *Pseudomonas*, *Azospirillium*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Rhizobium*, *Erwinia* and *Flavobacterium* are well known PGPR. They aid in improving plant stress tolerance to drought, salinity and metal toxicity. The underlying mechanisms of plant growth promotion by PGPR have been comprehensively described in several articles (Kloepper *et al*., 2004). The variations in results from biofertilizers in a laboratory to field could be due to various abiotic stresses that prevail under farmers field conditions for microbial inoculants to establish and to show the efficient desirable effect. Such type of problems can be overcome by sound screening programmes for efficient stress tolerant plant growth promoting rhizobacteria from different rhizospheric soils for effective management of abiotic stress in crop plants.

PGPR belonging to *Bacillus* spp. are frequently isolated from the rhizosphere and most have shown favorable effects on plant growth, higher yield and disease tolerance (Vessey, 2003). *Bacillus mucilaginosus* has been observed particularly for potassium and phosphate-solubilizing abilities (Idriss *et al.,* 2002). Woitke *et al.* (2004) demonstrated the ability of *B. subtilis* to induce stress tolerance to salinity in hydroponically grown tomato plants. However, of late, the role of microbes in management of biotic and abiotic stresses is gaining importance. The subject of PGPR elicited tolerance to abiotic stresses has been reviewed (Venkateswarlu *et al*., 2008). It has been shown that certain PGPR enhance plant stress tolerance through 1- aminocyclopropane-1-carboxylate deaminase and provide significant protection to a wide range of plant species from the damage caused by various abiotic stress conditions. ACC breakdown and ethylene synthesis inhibition by ACC deaminase decreases the damage of various stress situations by enhancing homeostasis in and around the plant root, especially at early stages of stress exposure (Ali *et al*., 2009).

Increased incidence of abiotic and biotic stresses has become major cause for stagnation of productivity in principal crops. Besides high temperature, droughts, elevated CO_2, extreme rainfall events, more floods, cold waves, heat waves, and cyclones are the other important natural disasters that cause serious economic losses, are likely to be witnessed as a result of global warming. These factors are likely to cause serious negative impact on crop growth and yields and impose severe pressure on our land and water resources (Grover *et al.*, 2011). EPS-producing plant growth- promoting rhizobacteria can also bind cations including Na^+. Therefore, an increase in the population density of EPS-producing bacteria in the root zone is expected to decrease the content of Na^+ available for plant uptake, and thereby alleviate salt stress in plants growing in saline environments (Alami *et al.,* 2000). The present investigation is on the isolation of abiotic stress tolerant plant growth promoting rhizobacterial isolates followed by *in vitro* screening for tolerance to high pH, temperature, salt conditions, osmotic stress, metal toxicity etc. and molecular identification of few efficient isolates. Such PGPR will be helpful for efficient of management of abiotic stress in crop production.

MATERIALS AND METHODS

Soil samples, Bacterial isolation, and culture media

Rhizospheric soils were collected from different places of Telangana such as normal soils, salt affected, drought soils. Forty four strains of *Bacillus* were isolated form these soils. The strains were coded as BS1-BS-44. For isolation of rhizobacteria, the method proposed by Vlassak *et al.* (1992) was followed. The sample was agitated

for 15 minutes on a vortex and serial dilutions of soil suspensions were prepared. 0.1 ml of respective dilutions was spread on sterilized Petri plates containing specific media *i.e.* nutrient agar. The bacterial isolates were identified on the basis of morphological, physiological and biochemical characteristics according to the standard methods described in Bergey's manual of systematic bacteriology (Holt and Kreig, 1984).

Determination of mineral solubilization, IAA production, ACC Deaminase activity, EPS production, Siderophore production

Phosphate solubilization activity was determined using Pikovskaya's agar medium containing 0.5 % (W/V) $Ca_3(PO_4)_2$ (Pikovskaya, 1948), Potassium solubilization determined using Aleksandrov medium containing 0.2 % potassium aluminum silicate (Prajapati and Modi, 2012), Zinc solubilization determined using Tris mineral salt medium containing 0.1 % ZnO (Saravanan *et al,* 2003). IAA production (Duby & Maheswari, 2012) , EPS production at stress induced conditions were checked (Ali *et al*., 2013), bacterial utilization of ACC as sole nitrogen source was screened using qualitative assay(Jacobson *et al*.(1994). Siderophore production was determined by the Chrome Azurol S plate assay (Schwyn & Neilands, 1987).

Screening for abiotic stress tolerance

Influence of pH

The pH of the culture medium was adjusted to 4, 6, 7, 8, 10 and 12 using sterile using buffers. 5 ml TSB (Trypticase soya broth) culture medium having different pH ,and 0.1 ml bacterial suspension (10^8 -10^9 cells ml^{-1}) was poured in sterile culture tubes in three replicates for each pH and incubated in shaker incubator at 120 r/m was measured at 600 nm.

Screening for salinity tolerance of isolated bacterial isolates by the inducing of different % of NaCl concentrations from 1.5 %, 5%, 10%, 15% and 20% were checked.

Influence of temperatures

0.1 ml of bacterial suspension (10^8 -10^9 cells ml^{-1}) was poured into the vials containing 5 ml TSB culture medium and culture in incubators at 20°C, 30°C, 40°C,45°C and 50°C in three replicates for each. After 24 hrs of culture, their absorbance was measured at 600 nm.

Influence of drought

Trypticase soya broth (TSB) with different water potentials ("0.05, "0.15, "0.30, "0.49, and "0.73 MPa) was prepared by adding appropriate concentrations of polyethylene glycol (PEG 6000) (Sandhya *et al*. 2009) and was inoculated with 1% of overnight raised bacterial cultures in TSB. Osmotic potential of broth media was measured by osmometer Three replicates of each isolate with each concentration were prepared. After incubation at 28°C under shaking conditions (120 rpm) for 24 h, growth was estimated by measuring the optical density at 600 nm using a spectrophotometer.

Influence of Heavy metals

Freshly prepared agar plates were amended with various soluble heavy metal salts namely As, Cd, Hg and Mn at concentration of 50 and 100 ¼g ml^{-1} were inoculated with overnight grown cultures. Heavy metal tolerance was determined by appearance of bacterial growth after incubating the plates at room temperature for 24-48 hours.

RESULTS AND DISCUSSION

Growth and colony morphology of isolates

Forty four of the total isolates showed off white, irregular, non - spreading smooth, flat, opaque, viscid colonies and dull white, irregular, spreading, smooth, flat, opaque, viscid colony characteristics on nutrient agar medium plates. All the 44 isolates showed $gram^+$ve reaction, rods with endospore formation, when observed under microscope. Among forty four Gram positive bacterial isolates, all the isolates showed positive results for starch hydrolysis, citrate utilization, oxidase test, catalase test. Twenty isolates were positive for gelatin hydrolysis, all the isolates showed negative result for casein hydrolysis, twenty nine isolates positive for indole production, twenty eight isolates were positive for methyl red test, twenty three isolates positive for Voges-praskauer test and almost all isolates showed positive reaction for acid production capability.

Plant growth promoting properties

Table 1 shows the Plant growth promoting properties of *Bacillus* isolates. Out of twenty eight isolates, BJRB-18 showed highest phosphate solublized zone (44.00 ± 1.52 mm), followed by BS 26 (16.00 ± 1.155), BS 31(16.00 ± 1.52 mm).

Table 1. Physico-chemical properties of soil samples

Sampling site		pH	Electrical conductivity (dS m^{-1})	Organic carbon (%)	Heavy metal concentrations (µg ml^{-1})			
					Arsenic	Cadmium	Mercury	Manganese
Rangareddy	Koheda	8.1	0.30	0.40	3.08	3.80	17.00	11.20
	Ibrahimpatnm	8.0	0.41	0.42	3.11	2.80	12.00	9.20
	Choutuppal	7.9	0.50	0.43	3.00	2.00	17.00	12.20
	College farm, PJTSAU	7.9	0.35	0.39	-	-	-	3.21
Mahabubnagar	Kalvakurthy	7.8	0.40	0.41	-	-	-	-
	Bijenpaally	7.2	0.24	0.36	3.08	3.80	-	-
Wanaparthi	Wanaparthi	7.5	0.28	0.42	-	-	-	
	Pebbair	8.0	0.45	0.37	-	-	-	-
Nagarkurnool	Kollapur	7.4	0.24	0.50	-	2.00	-	-
Shamshabad	Maheswaram	7.6	0.30	0.39	3.08	3.80	12.00	12.20
	Kandhukur	7.5	0.26	0.38	3.08	3.80	8.00	-
Yadagirigutta	Bhongir	7.8	0.30	0.43	-	8.00	-	-
	Yadagirigutta	8.0	0.34	0.40	-	-	3.80	-

Table 2. Screening of *Bacillus* isolates for plant growth promoting properties

Isolates	P Solubilization (mm)	K Solubilization (mm)	Zn Solublization (mm)	IAA production (µg ml^{-1})	HCN production	Siderophore production	ACC deaminase activity	Exo polysaccharides production
BS 1	8.33 ± 1.45	11.66±0.33	10.33±0.88	11.50 ± 1.32	++	+	+++	+
BS 3	14.67 ± 1.45	12.66±0.33	23.66±1.45	12.33 ± 1.33	++	+	++	+++
BS 4	4.00 ± 1.00	5.00±0.57	14.66±1.33	8.00 ± 2.00	++	+	++	-
BS 7	11.66±1.24	13.66±0.88	24.00±0.57	15.22 ± 1.15	++	+	-	-
BS9	7.00±0.00	7.00±1.00	17.33±0.88	9.23±0.007	++	+	+	+
BS 10	13.00±1.73	3.33±0.33	3.33 ±0.88	8.20 ± 0.100	+	+	-	-
BS-12	7.33±1.15	14.66±0.88	15.00±1.73	9.68 ± 0.16	++	+	-	-
BS 14	5.33±0.33	6.00±1.52	28.00±0.57	6.52 ± 0.00	+	+	+++	+++
BS 15	4.00±1.52	1.00±0.50	10.66±0.88	5.97 ± 0.013	++	+	+	+
BS 17	4.33±0.33	8.00±1.52	7.66± 0.33	8.70 ± 0.147	+	+	-	-
BS 18	44.00±1.52	16.66±0.33	26.00±1.15	4.23 ± 0.57	+	+	++	+
BS 19	4.00±1.33	6.00±0.57	5.66± 0.88	5.210 ±0.57	+	+	-	-
BS 21	13.00±1.52	15.00±1.52	15.00±0.57	18.46 ± 0.26	+	+	+	++
BS 22	12.00±1.52	4.00±0.57	23.33±0.33	9.25 ± 0.57	++	+	-	-
BS 23	4.00±1.155	11.66±0.33	10.00±1.00	5.57 ± 0.21	+	+	++	++
BS 24	5.00±0.00	3.00±0.57	3.33± 0.33	2.23 ± 0.57	+	+	++	-
BS 26	16.00±1.155	3.00±0.57	23.00±0.57	5.57 ± 0.21	+	+	-	+
BS 27	7.00±1.52	13.00±1.52	12.66±0.33	1.50 ± 0.25	+	+	-	-
BS 28	8.00±2.30	3.33±0.33	23.33±1.45	8.25 ±1.15	++	+	+	-
BS 30	11.33±0.33	8.00±1.00	18.33±0.33	14.50±0.25	+	+	+++	++
BS 31	16.00±1.52	15.00±1.52	19.66±0.33	13.00 ±1.00	+	+	-	++
BS 33	5.00±1.52	3.66±0.33	14.00±1.52	8.320 ± 0.57	+	+	-	-
BS 35	12.66±0.33	11.66±0.33	12.00±1.52	16.32 ± 0.00	++	+	-	-
BS 37	7.00±0.57	7.00±1.52	4.66±0.88	8.30 ± 0.57	+	+	+++	+
BS 38	14.00±1.52	13.00±0.57	13.66±0.66	14.26 ± 0.57	+	+	-	-
BS 40	14.66±0.33	7.33±0.33	25.66±0.33	5.32 ± 0.57	+	+	+	+
BS 42	3.00±1.00	5.33±0.33	10.33±0.33	5.34 ± 0.57	+	+	-	+
BS 43	11.66±0.33	3.66±0.33	5.33 ± 0.88	3.45 ± 0.57	++	+	+	-
SE(m) ±	1.218	0.867	0.928	0.687				
CD	3.461	2.462	2.636	1.951				

Out of twenty eight isolates, BS 18 showed highest K solubilization zone (16.66 ± 0.33 mm), followed by BS 31(15.00 ± 1.52 mm), BS 21 (15.00 ± 1.52 mm). Among twenty eight isolates, BS 14 showed highest Zinc solubilization zone (28.00 ± 0.57 mm), followed by BS-18 (26.00 ± 1.15 mm). The indole acetic acid production results revealed that, out of twenty eight isolates, BS 21 showed highest IAA production (18.46 ± 0.26 µg ml^{-1}), followed by BS 35(16.32 ± 0.00 µg ml^{-1}).

The rhizobacterial strains were screened for their ability to utilize ACC as a sole source of nitrogen and for this purpose a qualitative ACC metabolism assay was performed. The results of the bioassay showed that all the rhizobacterial strains had the ability to utilize ACC as a sole source of nitrogen but with variable degree of efficacy. So, all these strains possessed ACC-deaminase activity. On the basis of growth [optical density values at 540 nm (OD540)], these strains were grouped into low, medium and high ACC utilizing strains. Among twenty eight isolates, fourteen isolates (50%) were positive for ACC deaminase production by utilization of ACC as the sole nitrogen source. Among fourteen isolates, four isolates showed strong (+++) ACCd production (BS 1, BS 14, BS 30, BS 37), five isolates showed moderate (++) ACCd production (BS 3, BS 4, BS 18, BS 23, BS 24), remaining six isolates showed weak (+) ACCd production (BS 9, BS 15, BS 21, BS 28, BS 40, BS 43).

These microorganisms produce siderophores and inhibit the root pathogens by creating iron limiting conditions in the

Table 3. *In-vitro* stress tolerance ability of the *Bacillus* isolates

Isolates	pH range	NaCl concentration (%)	Temperature (ºC)	Drought (Mpa)
BS 1	4-12	1.5 - 20	30-50	- 0.05 to- 0.30
BS 3	4-12	1.5 - 20	20-30	-
BS 4	4-7	1.5 -5	30-45	- 0.05 to- 0.73
BS 7	7	-	30	-
BS9	4-8	1.5 - 15	30	- 0.05 to- 0.30
BS 10	4-7	1.5 -5	20-50	- 0.05 to- 0.73
BS-12	7	-	30	- 0.05 to- 0.15
BS 14	4-12	1.5 - 20	20-50	-
BS 15	4-8	1.5 - 15	30-45	- 0.05 to- 0.30
BS 17	7	-	30	-
BS 18	4-12	1.5 - 20	20-50	- 0.05 to- 0.30
BS 19	4-8	1.5 - 15	30	- 0.05
BS 21	4-8	1.5 - 15	30-45	-
BS 22	7	-	30	-
BS 23	4-8	1.5 - 15	20-45	- 0.05 to- 0.30
BS 24	4-8	1.5 - 15	30	- 0.05 to- 0.30
BS 26	7-8	-	20-45	-
BS 27	4-8	1.5 - 15	20-50	- 0.05
BS 28	4	-	30	- 0.05
BS 30	4-8	1.5 - 15	20-45	-
BS 31	4-8	1.5 - 15	30-45	- 0.05 to- 0.15
BS 33	4-8	1.5 - 15	30-45	- 0.05 to- 0.73
BS 35	7	-	30	-
BS 37	4-10	1.5 -5	20-50	- 0.05 to - 0.30
BS 38	4-7	1.5 - 10	30-40	- 0.05 to - 0.15
BS 40	4-8	1.5 - 15	20-45	-
BS 42	7	1.5 - 20	30	- 0.05 to - 0.30
BS 43	4-8	1.5 - 15	20-50	- 0.05 to - 0.15

rhizosphere and reduces probability of plant disease. Some siderophores are low molecular weight biomolecules secreted by microorganisms in response to iron starvation for acquisition of iron from insoluble forms by mineralization and sequestration. All the *Bacillus* isolates showed less amount (+) of siderophores.

Among the twenty eight isolates, two isolates showed strong (+++) EPS production (BS 3, BS 14), four isolates showed moderate (++) EPS production (BS 21, BS 23, BS 30, BS-31) and eight isolates were weak (+) in EPS production (BS1, BS 9, BS 15, BS 18, BS 26, BS 37, BS 40, BS 42).

Stress tolerance of the selected bacterial isolates

Table 2 shows the abiotic stress tolerant selected *Bacillus* isolates. The results of different *in vitro* abiotic stress tolerance of *Bacillus* isolates, four isolates were showed growth at pH range from 4-12 (BS 1, BS 3, BS 14, BS 18), single isolate was showed tolerance to pH range from 4-10 (BS 37), twelve isolates showed tolerance to pH range from 4-8 (BS 9, BS 15, BS 19,BS 21, BS 23, BS 24, BS 27, BS 30, BS 31, BS 33, BS 40, BS 43).

The results of salt tolerance ability reveals that, five isolates were showed tolerance to 1.5 to 20 % of NaCl concentration (BS 1, BS 3, BS 14, BS 18, BS 42), twelve isolates were showed tolerance from 1.5 to 15 % of NaCl concentration (BS 9 BS 15, BS 19, BS 19, BS 21, BS 23, BS 24, BS 27, BS 30, ,BS 31, BS 33, BS 40, BS 43), only one isolate was showed tolerance from 1.5 to 10 % of NaCl concentration (BS 38), three isolates were showed

Table 4. Tolerance of *Bacillus* isolates at different concentrations of heavy metals

Isolates	Heavy metal tolerance ($\times 10^7$ cfu ml^{-1})							
	As		Cd		Hg		Mn	
	50 µg ml^{-1}	100 µg ml^{-1}	50 µg ml^{-1}	100 µg ml^{-1}	50 µg ml^{-1}	100 µg ml^{-1}	50 µg ml^{-1}	100 µg ml^{-1}
BS 1	71	31	112	50	-	-	-	-
BS 3	-	-	-	-	-	-	56	33
BS 4	112	32	53	-	-	-	-	-
BS 7	79	32	112	50	58	-	1	43
BS9	120	30	112	40	-	-	102	43
BS 10	112	32	112	30	-	-	-	-
BS-12	-	-	-	-	-	-	-	-
BS 14	-	-	-	-	-	-	-	-
BS 15	122	37	98	30	-	-	-	-
BS 17	86	31	70	30	46	-	123	33
BS 18	120	32	56	-	-	-	-	-
BS 19	-	-	-	-	-	-	-	-
BS 21	120	32	112	50	-	-	103	-
BS 22	42	-	112	63	42	-	113	30
BS 23	-	-	-	-	-	-	-	-
BS 24	120	32	112	50	-	-	93	33
BS 26	180	32	98	35	-	-	75	
BS 27	130	32	102	86	70	-	65	-
BS 28	98	123	43	50	-	-	56	-
BS 30	-	-	-	-	-	-	-	-
BS 31	-	-	-	-	-	-	-	-
BS 33	101	32	112	50	53	30	123	43
BS 35	122	32	112	62	-	-	98	43
BS 37	-	-	-	-	-	-	-	-
BS 38	-	-	-	-	-	-	-	-
BS 40	85	32	-	-	42	32	-	-
BS 42	-	-	78	50	-	-	123	-
BS 43	-	-	-	-	-	-	-	-

tolerance from 1.5 to 5 % of NaCl concentration (BS 4, BS 10, BS 37).

The results of temperature tolerance ability of *Bacillis* isolates revealed that, six isolates showed tolerance to temperature from 20 ºC -50 ºC (BS 10, BS 14, BS 18, BS 27, BS 37, BS 43), four isolates were showed tolerance to temperature from 20 ºC - 45 ºC (BS 23, BS 26, BS 30, BS 40), one isolate was showed tolerance to temperature from 30 ºC -50 ºC (BS 1), five isolates were showed tolerance to temperature from 30 ºC - 45 ºC (BS 4, BS 15, BS 21, BS 31, BS 33), one isolate was showed tolerance to temperature from 30 ºC - 40 ºC(BS 38).

The results of drought tolerance ability of *Bacillus* isolates revealed that, four isolates showed tolerance to water potential from - 0.05 Mpa to- 0.73 Mpa (BS 4, BS 10, BS 18, BS 33), seven isolates were showed tolerance to water potential from - 0.05 Mpa to- 0.30 Mpa (BS 1,BS 9, BS 15, BS 23, BS 24, BS 37, BS 42), four isolates were showed tolerance to water potential from - 0.05 Mpa to- 0.15 Mpa (BS 12, BS 31, BS 38, BS 43).

The results of heavy metals tolerance of *Bacillus* revealed that, out of 28 isolates, seventeen isolates (61%) showed growth on 50 µg ml^{-1}(BS-26 (180× 10^7 cfu ml^{-1}) > BS-27 (130× 107 cfu ml^{-1}) > BS-15 (122× 10^7 cfu ml^{-1}), BS-35 (122× 10^7 cfu ml^{-1}) > BS-9 (120× 107 cfu ml-1), BS-18 (120× 10^7 cfu ml^{-1}), BS-21 (120× 10^7 cfu ml^{-1}), BS-24 (120× 10^7 cfu ml^{-1}) > BS-4 (112× 10^7 cfu ml^{-1}), MFSB-10 (112× 10^7 cfu ml^{-1}) and 100µg ml^{-1}(PRB-28 (123× 10^7 cfu ml^{-1}) BS-15 (37× 10^7 cfu ml^{-1}) on arsenic (As) enriched trypticase soy agar.

Seventeen isolates (64%) showed cfu on 50µg ml^{-1}(BS 7 (112×10^7 cfu ml^{-1}), BS 9 (112×10^7 cfu ml^{-1}), BS 10 (112×10^7 cfu ml^{-1}), BS 1 (112× cfu ml^{-1}), BS 21 (112×10^7 cfu ml^{-1}), BS 22 (112× 10^7 cfu ml^{-1}), BS 24 (112× cfu ml^{-1}), BS 33 (112× cfu ml^{-1}), BS 35 (112×10^7 cfu ml^{-1}> BS 27 (102×10^7 cfu ml^{-1}) > BS 26 (98×10^7 cfu ml^{-1}), BS-15 (98×10^7 cfu ml^{-1}) and fifteen isolates (61%) showed growth at 100µg ml-1(BS 27 (86 ×10^7 cfu ml^{-1}) > BS 22 (63× 10^7 cfu ml^{-1}> BS-35 (62 × 10^7 cfu ml^{-1}) on cadmium (Cd) enriched trypticase soy agar respectively . Six isolates (21%) showed cfu on 50µg ml^{-1}(PPB-27 (70X 10^7 cfu ml^{-1}) > IPB-7 (58 × 10^7 cfu ml^{-1}) > BHPB-33 (53× 10^7 cfu ml^{-1}) > BJMZB-17 (46 × 10^7 cfu ml^{-1}) and two isolates (7 %)showed cfu on 100µg ml-1 (KVBB2-40 (32× 10^7 cfu ml^{-1}) > (BHPB-33 (30× 10^7 cfu ml^{-1}) on mercury (Hg) enriched trypticase soy agar respectively. Thirteen isolates (46 %) showed cfu on 50µg ml-1(BJMZB-17 (123× 10^7 cfu ml^{-1}), BHPB-33 (123 × 10^7 cfu ml^{-1}) > CAGB-42 (123 × 10^7 cfu ml^{-1}) > KLMZB-22 (113× 10^7 cfu ml^{-1}) > KLPrB-21 (103 × 10^7 cfu ml^{-1}) > IGB-9 (102× 107 cfu ml-1)) and eight isolates (29 %) showed cfu on 100µg ml-1(IPB-7 (43× 10^7 cfu ml^{-1}), IGB-9 (43× 10^7 cfu ml^{-1}), BHPB-33 (43× 10^7 cfu ml^{-1}), BHMZB-35 (43× 10^7 cfu ml^{-1}), KTB-3 (43× 10^7 cfu ml^{-1}), BJMZB-17 (33× 10^7 cfu ml^{-1}), WNRB-24 (33× 10^7 cfu ml^{-1}), KLMZB-22 (30× 10^7 cfu ml^{-1}) on manganese (Mn) enriched trypticase soy agar respectively.

DISCUSSION

Present investigation was carried out to isolate Plant growth promoting Bacillus isolates for abiotic stress tolerance. To achieve this, bacterial isolates were isolated from different rhizospheric soils of Telangana and screened for plant growth promoting properties and different abiotic stresses such as pH, temperature, salt, drought and heavy metals. Such PGPR will be helpful for the management of abiotic stress in crop improvement during unfavorable conditions.

Rhizospheric soils such as normal, salt affected, drought affected and bulk soils were collected from different places of Telangana state. From all soil samples forty four Bacillus spp isolates were isolated and identified.For plant growth promoting properties and abiotic stress BJRB-18 showed higher solubilization of Phosphate, Zn & K and ACC deaminase activity. It was able to grow well by tolerating the pH stress (4, 6, 8, 10), temperature stress (40ºC, 45ºC, 50ºC), salt stress (5%, 10%, 15%, 20%), drought stress (-0.05 MPa,-0.15 MPa,-0.3 MPa) and As & Cd heavy metal toxicity. Identification of bacterial strains based on 16S rRNA gene sequence of effective bacterial isolate was matched with the available sequences in the GenBank database. BLAST Search results through NCBI showed 97% similarity of BJRB-18 with *Paenibacillus lautus.*

Our results were agreement with Damodaran *et al*. (2013), they screened for *in-vitro* NaCl tolerance and Na+ uptake pattern, wherein two stress tolerant *Bacillus* spp. (*Bacillus*

pumilus and *Bacillus subtilis*) which showed all PGPR traits with tolerance to salinity. Kannika and Maneewan *et al*. (2012) reported Bacillus licheniformis B2r which showed high ACC deaminase activity at 0.6 M NaCl salinity. Tomato plants inoculated with the selected bacterium under various saline conditions (0, 30, 60, 90 and 120 mM NaCl) revealed a significant increase in the germination percentage, germination index, root length, and seedling dry weight especially at salinity levels ranging from 30-90 mM NaCl.

Sandhya *et al*. (2011) reported similar results with three Bacillus spp. They were studied for the ability to tolerate matric stress and produce EPS under different water potentials. EPS production in all the three Bacillus spp strains increased with increasing water stress indicating correlation between drought stress tolerance and EPS production. Among the isolates, strain HYD-17 showed highest production of EPS. Banerjee *et al*. (2015) tested the isolated strains for their tolerance against six different types of heavy metals dominant in the ash samples viz. Pb, Hg, Ni, Co, Cu, Mn. Their maximum resistance existed up to 0.6 mM ml-1 of the above mentioned different metals under lab standard conditions. Three isolates were found suitable for the multiple metal resistance ability viz SM2, SM3, and SM12. They were categorized as Bacillus cereus (SM2, SM3), and *Bacillus subtilis* (SM12) after performing 16S rDNA sequencing.

This study revealed that using of these isolates as bioinoculants in this area may be benefit the crop yields by mitigating the abiotic stress. These two isolates show potential as plant growth beneficial inoculants in problematic soil regions suggesting further studies on rhizocompetence in commercial crops grown under stressed conditions. Upadhyay *et al.* (2009) reported that P-solubilizing bacteria from the genus *Bacillus* have evolved highly sophisticated regulatory networks for protection against sudden unfavorable environmental changes, including nutrient starvation, changes in temperature and humidity, oxidative stress, sudden elevation in medium salinity. So it may be the reason for the occurrence of Bacillus cereus in adverse saline conditions.

CONCLUSIONS

Selection of microorganisms both metal tolerant and efficient in producing PGP compounds can be useful to speed up the recolonization of the plant rhizosphere in polluted soils. The potent heavy-metal tolerant *Bacillus* spp species obtained in this study can potentially be used in the field of phytoremediation due to their PGPR activity like production of phytohormones and nitrogen sources, mineral solubilization simultaneously. Performing environmental parameters for bacterial growth is also showing that bacteria can easy to survive in different environmental condition. Soil fertility management by using microbial fertilizers is one of the basic components of sustainable agriculture production. Hence, proper formulation of the abiotic stress tolerant bacterial bio-inoculants is very much essential for problematic areas in the country.

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