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SALINITY TOLERANCE IN FREE LIVING PLANT GROWTH PROMOTING RHIZOBACTERIA

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ABSTRACT

Free living rhizospheric bacteria from wheat rhizosphere have capability to produced plant growth promoting attributes under saline condition. The electrical conductivity (ECe) of saline soil samples from four different districts viz. Varanasi, Mau, Ballia and Ghazipur of Uttar Pradesh- India was 10.47 to 5.69 dSm^{-1} and maximum bacterial population (6.9 X 10^6 cfu g^{-1} soil) was found on NA media as compared to rest of the media like King's B, Jensen's N free, TSA and SEA. About 33% of bacterial isolates were survive up to more then 8% NaCl (w/v) and only 19% showed PGP attributes at higher NaCl concentration. Ten identified PGPRs were used in the present study for their osmotolerence mechanism. Proline content was increased with NaCl stress and maximum production was recorded with Isolate SU8- *Bacillus aquimaris viz.* 2.73 and 11.95 g mg^{-1} protein at 0% and 10% NaCl (w/v) respectively. The tendency RS and TSS production in rhizobacterial isolates were reverse proportional to the salt (NaCl) concentration. *Bacillus* and *bacillus* derived genera were dominant under high saline condition along with PGP attributes, which could be mitigate salinity levels and improve agriculture crops under saline condition.

KEY WORDS: Osmolytes, PGPR, Proline, Reducing Sugars, Total soluble sugars, Rhizobacteria

An understanding of ecological conditions affecting bacterial inoculants is important when introducing microbes for increasing plant growth and productivity under saline condition. Agricultural crops and soil microorganisms are affected with salinity. Beneficial soil microorganisms such as PGPRs (plant growth promoting rhizobacteria) have received in agriculture attention of scientists throughout the world (Berg, 2009). PGPRs have been reported for the plant growth under saline condition (Tripathi et al., 2002; Yue, 2007; Upadhyay et al., 2011and 2012), so that the osmotolerance mechanisms of these PGPRs are quite important to hyper osmotic injury. Osmoregulation in bacteria has captured major interest, not only to understand cell response and adaptation to varying environmental condition, but also because of the applied aspect of this field (such as interaction between microbe and plant). Most of the bacteria adopted universal mechanisms of osmoadaptation, which consist of accumulation of potassium and/or small molecular weight organic solutes, designated compatible solutes (Miller, 1996; Paul and Nair 2008; Upadhyay et al., 2011and 2012). Compatible solutes are known to protect cells and biological macromolecules against denatured effect of not only hyper osmotic stress, but also other stresses such as heating, freezing and desiccation (Welsh, 2000; Paul and Nair, 2008). Accumulation of these solutes, in response to the osmotic constraint, is carried out by *de novo* synthesis and/or by active transport from the surrounding environment and they consequently stimulate the endogenous capacities of osmoprotection in this bacterium (Miller and Woods, 1996; Welsh, 2000). The long-term goal of improving plantmicrobe interactions for salinity affected fields and crop productivity can be met with an understanding of the mechanism of osmoadaptation in *Azospirillum* sp. . Tripathi et al. (2002) reported that in *Azospirillum* sp. there is an accumulation of compatible solutes such as glutamate, proline, glycine betaine and trehalose in response to salinity/osmolarity, proline plays a major role in osmoadaptation through increase in osmotic stress that shifts the dominant osmolyte from glutamate to proline in *A. brasilense*. Bacteria from the genera *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. Spore-forming bacteria, typically *Bacillus* species, are one of the major types of soil bacteria. Therefore, the objectives of the present study were to analyze *in vitro*

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salinity stress tolerance mechanisms in PGPRs through osmolytes production.

MATERIALS AND METHODS

Source of Salt Tolerant Plant Growth Promoting Rhizobacteria

Ten identified bacterial strains were used in the present study and they were earlier isolated from the rhizospheric soil of wheat crop growing on saline soil from different locations of the four districts *viz*., Varanasi, Mau, Ballia and Ghazipur of Uttar Pradesh- India. (Upadhyay et al., 2009). These strains are having plant growth promoting (PGP) attributes like, Indole Acetic Acid (IAA) production, phosphate solubilization, gibberellins and siderophore production at higher salt concentration [NaCl: 8% (w/v)] (Upadhyay et al., 2009). These PGPRs were identified through 16S rDNA sequensing. All the sequences were submitted to NCBI GenBank (Upadhyay et al., 2009).

In Vitro Screening of Osmolytes Production From Salt Tolerant PGPR

The proline content (µg mg^{-1} protein) and total soluble sugars (TSS; g mg^{-1} protein) were analyzed as described earlier by (Upadhyay et al., 2011and 2012) in the supernatant of the growth medium. *Protein content was measured by Bradford method (*Bradford, 1976). *Screening of* Exo-polysaccharides (EPS) production was earlier described by (Upadhyay et al., 2011). Strains were plated on RCV mineral medium enriched with glucose ($40gL^{-1}$). The bacterial colonies showing EPS production on sugar media were randomly selected on Trypticase Soya Agar (TSA) medium.

Analyses of variance were performed with the SAS (statistical analysis system) software (Version 9.1). Duncan's test was used for multiple range analyses to determine the significant difference between groups of data. The results were considered to be significant at $P < 0.05$.

RESULTS

The color of soil field were whitish and texture were sandy loam of all sampled sites among four districts of Uttar Pradesh, most of the rhizospheric soils have electrical conductivity (ECe) with range 10.47 to 5.69 dSm^{-1}, pH ranging from 8.0 to 9.5, and organic carbon from 0.27 to 2.29%, Available N, PO_4 and K_2O content was examined over all the soil samples, However, maximum N and K_2O content was recorded in region of Varanasi district (219.5 and 159.3 kg ha^{-1}) respectively, while maximum PO_4 content (13.34 kg ha^{-1}) was recorded in Ballia district (Table ,1).

Salt Tolerant Plant Growth Promoting Rhizobacteria

A total of 130 bacteria were isolated from the rhizosphere of wheat growing in the salt affected soils of Varanasi, Mau, Ballia and Gorakhpur. The number of isolates obtained on different media: Nutrient agar, King's B, Jensen's N-free, Soil extract agar and Trypticase soy agar were shown in Table-1. Bacterial population in rhizospheric soils was examined on the basis of average viable bacterial counts (cfu: Colony forming units) g^{-1} of soil. Maximum bacterial population was found (6.9×10^6 cfu g^{-1} soil) on Nutrient Agar media followed by Kings'B medium for almost all the samples (Table,1). The All the isolates were screened for salt tolerance at graded concentrations of NaCl (w/v). Of the 130 isolates, 42 isolates were able to tolerate NaCl stress of 8% (w/v), while only two isolates showed tolerance to 12% NaCl (w/v). Out of 42 only 24 isolates were positive for PGP attributes upto higher NaCl concentrations, *viz.,* all isolates were IAA (Indole acetic acid) producers (Upadhyay et al., 2009), ten isolates solubilized phosphorus, eight produced siderophore, six gibberellins producers and two isolates ACC deaminase activity (Upadhyay et al., 2009), among of 24 isolates only ten isolates were potent salt tolerance plant growth promoting rhizobacteria were identified through 16S rDNA sequencing and sequences were submitted to NCBI GenBank. Identified bacterial isolates with NCBI Gene Bank Accession No. were SU3 (*Bacillus pumilus*: EU927407), SU8 (*Bacillus aquimaris*:EU927408), SU10 (*Bacillus pumilus*:EU430990), SU13 (*Bacillus arsenicus*: EU927409), SU16 (*Bacillus sporothermodurances*: Eu430991), SU18 (*Arthrobacter* sp.:EU927410), SU24(*Bacillus cereus*:EU927411), SU40 (*Pseudomonas medicona*: EU927412), SU44 (*Bacillus aquimaris*: EU927415), SU47 (*Bacillus subtilis*:EU927413)

Osmolytes Production From Salt Tolerant PGPR

All the 24 rhizobacterial isolates were tolerate at higher salt concentration [≥ 8% NaCl;(w/v)] were examined for their osmolytes production at different salt (NaCl) concentrations (0, 2, 4, 6, 8 and 10%) (w/v). Production of proline content for all the isolates was proportional to concentration of NaCl. The isolates SU3, SU8, SU10, SU13, SU16, SU44 and SU47 produce higher concentration of proline upto 10% NaCl shown in table,2 (data shown only ten identified isolates). Isolate SU8 was produce maximum proline concentration 11.95g mg^{-1} protein at 10% NaCl concentration while 2.73 g mg^{-1} protein at 0% NaCl. The tendency of reducing sugar (RS) production in rhizobacterial isolates were reverse proportional to the salt (NaCl) concentration. Isolate SU3, SU8, SU13, SU18, SU40, SU44 and SU47 produce higher concentration of RS upto 10% NaCl (Table,3). Isolate SU18 was sowing maximum production of RS over all range of salt (NaCl) concentrations (52.49 µg mg^{-1} protein) at 10 % NaCl (w/v) and 83.48 µg mg^{-1} protein at 0%. Isolate no SU16 and SU24 were not produced RS at 10 % NaCl (w/v) concentration. Total soluble sugar (TSS) of bacterial isolates was given same pattern as a RS production with salt (NaCl) concentration. All ten isolates were able to produced sufficient amount of TSS up to 10% NaCl (w/v) concentration (Table,4), except isolates SU 10 and SU 24. Isolates SUS 8 produces maximum amount of TSS (62.44 µg mg^{-1} protein) at 10% and 210.40 µg mg^{-1} protein at 0 % NaCl concentration.

DISCUSSION

Salinity of the soil plays a prominent role in the microbial selection process as environmental stress has been shown to reduce bacterial diversity (Borneman et al., 1996). A detailed screening of the natural population in the present study was carried out to identify salt tolerant rhizobacteria that could not only tolerate salt stress but could express its PGP traits at high salt concentration. This study was carried out to investigate the coping behavior of rhizobacteria under salinity stress due to osmotolerant mechanism through production osmoprotectents, the salt (NaCl) tolerant PGPR colonized with wheat rhizosphere, it shows their rizoadaptation (Upadhyay et al., 2012). While increasing the salinity in the soil decreases the plant growth and nutrient uptake (Yue et al., 2007; Upadhyay et al., 2012). These salt-tolerant plant growth-promoting bacteria are free-living bacteria, and their population and activity are greatly influenced by the soil conditions (Borneman *et al*., 1996). Beneficial free-living rhizobacteria that improve plant growth or increase yield are referred to as PGPR. PGPR have ability to mitigate the salinity effect in wheat plant (Upadhyay et al., 2011). In the present study the population density of rhizobacteria (Table,1) were varies with media, maximum population was recorded on the Nutrient media. Different media was earlier used by Upadhyay et al., (2009 and 2011) for isolation of diverse bacterial form. In the present study the concentration of proline content of all PGPRs were proportional to the concentration of NaCl stress (Table-2). Similar finding was reported in my previous paper (Upadhyay et al., 2011and 2012). The proline content could maintain the growth of bacterial isolates upto higher salinity level because it may act as a mediator of osmotic adjustment protects macromolecules during dehydration and serve as a hydroxyl radical scavenger (Csonka, 1982; Miller, 1996). RS and TSS content of bacterial isolates were reduced with increasing concentration of salinity, while both are osmolytes and protect against bacteria to osmotic injury (Upadhyay et al., 2011). EPS production by PGPRs (data not shown) may help the plants to overcome salinity stress by reducing the availability of Na^+ ions to roots. A decrease in Na^+ availability may alleviate salinity stress for wheat plants, as suggested earlier (Upadhyay et al., 2011). From the present study, we conclude that(i) *Bacillus and bacillus* derieved genera were dominant salt tolerant plant growth promoting rhizobacteria, which could serve as a suitable bioinoculant for crops growth under saline soils.

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Table 1: Physico-chemical properties of the saline soils and rhizobacterial population

Physico-chemcal properties of soil									Population of rhizobacteria (*Cfu X 10^5 g^{-1} of soil)				
Place	Soil color	pH	EC[a]	SAR[b]	OC[c]	Available N Content (kg ha^{-1})	Available PO_4 Content (kg ha^{-1})	Available K_2O Content (kg ha^{-1})	NA[d]	King's B	Jensen's N free	TSA[e]	SEA[f]
Varanasi	Whitish	8.63	5.69	41.80	0.62	219.50	11.50	159.3	69* (18)	54* (3)	41* (8)	36* (1)	32* (2)
Mau	Whitish	8.93	10.47	392.80	0.4	154.05	10.48	102.62	64* (20)	54* (5)	39* (9)	26* (9)	35* (9)
Ballia	Whitish	8.71	6.6	151.30	0.67	146.90	13.34	114.6	62* (8)	41* (4)	38* (3)	34* (4)	40* (2)
Ghazipur	Whitish	8.85	8.13	515.80	0.64	139.05	12.56	125.97	69* (9)	42* (4)	36* (6)	32* (3)	35* (3)

Data are representing average mean of ten sites from each district and distance from each sites were about 8 to 10 Km, and each sites containing five samples, Total soil samples from each district were n=50 (10 X 5), ([a])=Sodium absorption ratio, ([b])= Electrical conductivity dSm^{-1}, ([c])= Organic Carbon (%), ([d])= Nutrient Agar , ([e])= Trypticase soya Agar , ([f])= Soil Extract Agar, *=Colony forming Unit (Cfu X 10^5 g^{-1} of soil) and ()=Total number of rhizobacterial isolates who morphological differ . Texture of soils was sandy loam.

Table 2: Production of proline (µg mg^{-1} protein) by rhizobacteria at different NaCl concentration. All observations are in triplicate form (Data shown- only ten identified salt tolerant plant growth promoting rhizobacteria)

Identified rhizobacteria	Proline content (µg mg^{-1} protein) at different NaCl concentration [0 to 10% (w/v)]					
	0%	2%	4%	6%	8%	10%
SU3, *Bacillus pumilus*	0.54±0.2	0.76±0.1	1.31±0.1	2.22±0.2	4.45±0.8	8.92±1.3
SU8, *Bacillus aquimaris*	2.73±0.8	2.79±0.3	2.96±0.2	11.71± 2.5	13.09±2.0	11.95±1.8
SU10, *Bacillus pumilus*	1.86±0.3	4.03±0.5	4.23±0.2	5.23±1.2	6.46±1.2	6.68±0.7
SU13 *Bacillu Arsinicus*	0.45±0.1	0.70±0.1	3.43±0.4	4.52±1.0	6.10±1.1	3.80±0.2
SU16, *B. sporothermodurances*	1.22±0.3	4.12±0.3	4.45±0.2	5.85±1.8	6.22±0.8	6.00±0.9
SU18, *Arthrobacter sp*	1.92±0.4	2.80±0.2	3.30±0.8	3.23±0.5	3.43±0.6	2.56±0.3
SU24, *Bacillus cereus*	1.09±0.2	12.92±2.3	7.95±1.4	7.11±1.5	9.29±1.3	2.45±0.2
SU40, *Pseudomonas mendocina*	2.01±0.2	4.86±0.3	3.68±0.4	3.45±0.6	3.06±0.2	0.47±0.1
SU44, *Bacillus aquimaris*	1.45±0.5	1.95±0.2	2.79±0.3	4.23±0.9	6.64±1.2	4.12±1.2
SU47, *Bacillus subtilis*	0.65±0.3	0.85±0.2	1.45±0.2	2.36±0.5	3.64±0.8	3.53±0.7

Table 3: Production of reducing sugars (µg mg^{-1} protein) by rhizobacteria at different NaCl concentration

Identified rhizobacteria	Reducing sugars (µg mg^{-1} protein) at different NaCl concentration [0 to 10% (w/v)]					
	0%	**2%**	**4%**	**6%**	**8%**	**10%**
SU 3, *Bacillus pumilus*	89.50±5.3	46.98±3.5	36.81±2.4	35.34±4.7	26.48±2.5	22.59±1.5
SU 8, *Bacillus aquimaris*	115.97±12.3	75.66±5.2	53.96±5.8	52.95±4.2	49.33±4.6	33.79±4.5
SU 10, *Bacillus pumilus*	123.04±10.5	70.52±6.8	33.59±3.9	30.54±2.8	32.55±3.2	8.45±0.8
SU 13 *Bacillu Arsinicus*	71.25±4.5	60.06±9.1	50.94±4.5	51.29±6.8	44.60±5.1	41.93±3.5
SU 16, *B. sporothermodurances*	100.00±8.5	74.55±7.5	55.00±6.2	40.54±9.2	33.55±2.1	0.00
SU 18, *Arthrobacter sp*	83.48±5.2	78.27±7.2	63.74±3.9	61.86±5.7	53.28±2.0	52.49±6.2
SU 24, *Bacillus cereus*	105.61±11	75.56±9.2	49.41±5.2	45.78±2.6	33.18±3.8	0.00
SU 40, *Pseudomonas mendocina*	75.96±4.8	39.01±2.5	34.94±2.8	33.76±5.3	33.43±2.0	17.16±1.5
SU 44, *Bacillus aquimaris*	98.03±8.0	77.85±5.6	65.95±4.3	33.66±7.1	34.69±5.1	26.46±2.0
SU 47, *Bacillus subtilis*	88.08±9.3	38.16±6.5	37.50±3.0	41.54±4.8	38.47±3.2	26.17±2.9

All observations are in triplicate form
(Data shown- only ten identified salt tolerant plant growth promoting rhizobacteria)

Table 4: Production of total soluble sugars (TSS) (µg mg^{-1} protein) by rhizobacteria at different NaCl concentration

Identified rhizobacteria	TSS (µg mg^{-1} protein) at different NaCl concentration [0 to 10% (w/v)]					
	0%	**2%**	**4%**	**6%**	**8%**	**10%**
SU 3, *Bacillus pumilus*	135.87±11.25	98.22±8.5	68.60±9.56	48.49± 3.55	33.80±2.88	31.25±4.25
SU 8, *Bacillus aquimaris*	210.40±18.92	194.08±12.95	146.62±12.69	129.02±13.45	72.46±6.50	62.44±6.37
SU 10, *Bacillus pumilus*	141.39±13.04	112.31±10.63	79.33±8.55	69.12±5.63	55.76±4.25	3.82±0.90
SU 13 *Bacillu Arsinicus*	113.21± 10.50	95.53±11.25	77.35±6.29	65.29±7.28	61.75±7.21	48.02± 2.50
SU 16, *B. sporothermodurances*	124.40±14.22	100.35±14.22	80.25±5.62	71.46±5.50	54.78±6.55	12.25±1.60
SU 18, *Arthrobacter sp*	134.64±10.50	109.99±8.85	84.59±9.53	79.12±6.15	73.36±4.91	40.61±3.90
SU 24, *Bacillus cereus*	123.34±12.59	90.65±7.29	61.26±5.29	63.22±4.56	51.48±4.47	9.00±1.00
SU 40, *Pseudomonas mendocina*	194.03±10.65	151.23±11.47	121.26±10.50	69.41±7.51	49.12±5.22	32.26±3.50
SU 44, *Bacillus aquimaris*	212.47±15.62	152.89±10.22	121.25±11.26	65.37±5.50	41.58±3.25	35.95±4.80
SU 47, *Bacillus subtilis*	183.45±14.10	134.00±12.54	101.76±9.58	78.12±6.21	76.52±3.71	40.12±3.62

All observations are in triplicate form
(Data shown- only ten identified salt tolerant plant growth promoting rhizobacteria)

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