Studies on Non-Symbiotic Diazotrophic Bacterial Populations of Coastal Arable Saline Soils of India

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Abstract The effect of fluctuations of salinity in three different seasons on diazotrophic populations and N_2 fixation in six mono cropped rice field soils of the coastal region of the Gangetic delta of West Bengal, India, was studied. The average pH, E_{Ce}, organic carbon and total nitrogen of the soils ranged from 4.99–7.08, 2.02–19.58 dSm⁻¹, 4.68–12.03 g kg⁻¹ and 0.44–1.70 g kg⁻¹, respectively. The average log colony forming units of the bacterial populations and N_2 -fixation in the soils varied from 4.61 to 5.86 and 2.74 to 4.52 mg N_2 fixed 50 ml⁻¹ culture media respectively, with the lowest value recorded in summer. Recovery of microorganisms and N_2 -fixation gradually decreased with extraneous addition of NaCl in the culture media. All the eight isolates were Gram positive, spore and capsule formers. They could utilize glucose, sucrose, mannitol, starch, citrate and nitrate, and were catalase and gelatinase positive, but indole, methyl red and Vogues Proskauer reaction negative. The organisms produced alkaline reaction on TSI agar slant. The acetylene reduction assay of the isolates at 0 and 1% NaCl in the culture media were 4.51–164.52 and 1.72–100.6 nmole C₂H₄ ml⁻¹ culture media in 72 h, respectively. The isolates could fix 2.42–4.45 and 2.04–4.08 mg N_2 fixed 50 ml⁻¹

culture media at 0 and 1% NaCl in the culture media respectively. 16S rDNA sequences of the isolates were similar to the species: *Bacillus* sp. isolate 28A, *Bacillus* sp. MOLA 87, *Bacillus* sp. By113 (B)Ydz-dh, *Bacillus* sp. PN13, *Bacillus licheniformis* strain RH101, *Bacterium Antarctica* 14, *Bacillus* sp. PN13 and *Bacillus megaterium*.

Keywords Salinity · Diazotrophs · Dinitrogen fixation · Coastal arable soils · DNA sequencing

Introduction

Salinity is a serious threat to world agriculture. In India, out of an estimated area of 187.7 mha of total degraded land, 8.1 mha are salt affected, and out of which 3.1 mha are in the coastal regions [1]. The soil salinity in coastal regions of Gangetic delta in West Bengal shows wide spatial and seasonal variability, being minimum during monsoon and maximum during summer. This in turn seriously interferes with microbial and biochemical parameters of coastal soils [2, 3]. Saline habitats are nitrogen poor [4] and have their own microbial population adapted to their environment [5]. Therefore N-input is very important in this environment. One of the sources of N-input in saline habitats is N_2 -fixation [6]. Biological nitrogen fixation offers non-polluting source of nitrogen and could improve crop production and decrease the use of synthetic fertilizers [7]. In view of the impending cost of fertilizers, the biological N_2 -fixation in terrestrial ecosystems has, therefore, received vast attention in recent time. Agriculture in the coastal region is restricted to rice monoculture with poor yield. The resource poor farmers of the region cannot afford costly input like fertilizers. Efficient strains that are area specific with selective traits to perform well under

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adverse conditions like soil acidity, salinity and moisture stress need to be developed and tested [8]. Therefore, harnessing the potential of biological N₂-fixation through use of N-fixing biofertilizer specific to the coastal areas seem prudent. With this background, present study deals with (1) seasonal dynamics of non-symbiotic diazotrophic (N₂ fixing) bacterial populations and N₂ fixation and (2) isolation and identification of salt tolerant bacterial diazotrophs and their potentiality for adoption as biofertilizer.

Materials and Methods

Study Site and Soil Sampling

Soils of this study were collected from six different plots of the Central Soil Salinity Research Institute, Canning Town (20°15' N and 80°40' E), West Bengal, India during summer, monsoon and winter seasons of 2007. The soils were collected from different sites having variable salinity and history of rice (*Oryza sativa*) cultivation, the major land use of the region, with different organic and inorganic inputs either exclusively or in combination. After collection of three replicate soil samples randomly (0–15 cm depth) from each site, plant roots, visible faunas, stones, rubbles etc. were removed. About 1 kg soil from each site was brought to the laboratory in labeled sterile containers. The samples were kept at 4°C before microbial analyses of the field moist soils.

Determination of Physico-chemical Properties of Soil

The pH of the soils was determined in 1:2.5 soil water suspension using glass electrodes. Electrical conductivity of the soil saturation extract (ECe) was determined by measuring the electrical conductance of soil water saturation extract with the help of conductivity meter [9]. Organic carbon and total nitrogen of the soils were determined by the methods as described by dichromate oxidation method [10] and macro-Kjeldahl method [11], respectively.

Enumeration of Culturable Aerobic Non-symbiotic Diazotrophic Bacterial Count of Soil

It was carried out by soil-dilution plate technique using nitrogen free mineral salt-yeast extract-agar medium. The composition of the media in g l⁻¹ of water was as follows: Sucrose 20 g, K₂HPO₄ 0.05 g, KH₂PO₄ 0.15 g, CaCl₂ 0.01 g, MgSO₄·7H₂O 0.2 g, Na₂MoO₄·2H₂O 0.002 g, FeSO₄ 0.01 g, yeast extract 0.1 g, agar 18 g, pH adjusted at 7.2. To prepare salt containing media requisite quantities of NaCl was added to the medium to obtain final concentrations of 1, 2 and 3% NaCl. Colony count was taken after three days of incubation at 28–30°C.

Determination of Nitrogen Fixation

One gram of each soil sample was added to duplicate flasks containing 50 ml nitrogen free mineral salt-yeast extract broth with or without added NaCl, and then sterilized. These served as soil blanks. To other duplicate flasks containing 50 ml sterilized medium, 1 g of each soil sample was added aseptically. The flasks were incubated at 28–30°C for 7 days. Total nitrogen in the media was determined by macro-Kjeldahl method [12]. Nitrogen fixation in soil, 50 ml⁻¹ of culture media, was determined by subtracting the value of nitrogen in the blank from the nitrogen in the sample.

Isolation and Purification of Non Symbiotic Diazotrophic Bacteria

It was carried out by enrichment culture technique in nitrogen free mineral salt-yeast extract-broth followed by streaking in the same agar plates to obtain single isolated colony. In this way, 40 pure cultures were obtained. Out of which eight pure cultures were selected, based on their N₂ fixing potentiality or nitrogen fixing ability, for detailed characterization. The cultures were maintained in same agar slants.

Phenotypic and Biochemical Characterization of the Isolates

Colony characteristics of the isolates on nitrogen free mineral salt-yeast extract- agar slants and cellular morphology by negative staining, Gram character, spore and capsule formation were determined by standard procedures [13]. Different biochemical characteristics of the isolates were also determined by the standard procedures [13].

Determination of Salinity Tolerance of the Isolates

The degrees of salt tolerance of the isolates were determined by measuring the optical density of the broth cultures containing 0–3% NaCl at 595 nm after 72 h of incubation. Salinity tolerance was expressed as percentage increase/decrease in OD of the cultures containing NaCl with respect to the culture without NaCl. Their growth was compared to that of a reference *Azotobacter vinelandii* strain isolated from non saline soil.

Determination of Nitrogen Fixing Potentiality of the Isolates

It was carried out by acetylene reduction assay (ARA). The cultures were incubated in nitrogen free mineral salt-yeast extract-broth with or without extraneous addition of NaCl,

for 72 h at 29°C in 7 ml Becton–Dickinson Vacutainer tubes stoppered with cotton plugs. After visible growth was observed, cotton plugs were aseptically exchanged with rubber stoppers and the headspace air was replaced with 10% (by volume) of high purity C_2H_2 gas by hypodermic syringe. The C_2H_4 production was measured after 72 h incubation of the tubes in dark at 29°C [14]. Tubes without C_2H_2 served as control. For the determination of C_2H_4 , 0.5 ml of the gas phase from each tube was injected into gas chromatograph fitted with flame ionization detector. The nitrogen fixing potentiality was expressed in C_2H_4 formed ml^{-1} culture in 72 h.

Determination of Nitrogen Fixing Efficacy of the Isolates

Nitrogen fixing efficacy of the isolates were estimated by growing the isolates in nitrogen free mineral salt–yeast extract-broth (composition as described above) with or without extraneous addition of NaCl, for seven days at 28–30°C, followed by the determination of nitrogen in the cultures as well as blanks by macro-Kjeldahl method [12]. Nitrogen fixing efficacy was expressed as mg N fixed 50 ml^{-1} culture media.

Isolation of Genomic DNA

The DNA was prepared from all the eight isolates by the sodium dodecyl sulfate proteinase K-cetyltrimethylammonium bromide (CTAB) method [15]. All the DNA preparations were treated with RNase A, and the DNA concentrations were estimated by visual examination of ethidium bromide stained agarose gels as well as by spectrophotometric examination.

Amplification of 16S rDNA

Partial amplification of the 16S rRNA gene was performed with the thermal cycler ABI 2700 (ABI, Foster City, USA). The PCR of the genomic DNA of the eight isolates were conducted in a final volume of 50 μ l. The reaction mixture included 20–50 ng of isolated genomic DNA, 2U Taq polymerase (Promega, USA), 1 $\times$ PCR buffer with 1.5 mM $MgCl_2$, 200 μ M each dNTP, and 10 pmol of each primer (IDT, USA). The primers were chosen to amplify partial 16S rDNA sequence. The forward primer 515F used was (5′–3′) GTGCCAGCAGCCGCGGTAA and the reverse primer 1492R was (5′–3′) TACGGYTACCTTGTTACGACTT. Before amplification cycle, DNA was denatured for 2 min at 94°C and after amplification an extension step (7 min at 72°C) was performed. The cycling parameters consisted of 28 cycles at: denaturation at 94°C for 30 s, primer annealing at 45°C for 1 min, extension at 72°C for

1 min. The samples were held at 4°C until analysis by agarose gel electrophoresis. All the amplified PCR products were agarose-gel-eluted using Promega gel elution kit.

Sequencing of the 16S rDNA Fragment and BLAST Search

The amplified and gel-eluted PCR fragments of the rDNA were sequenced in ABI 3100 Genetic Analyzer with primers 515F and 1492R. Sequencing reaction was performed by using the Big Dye terminator cycle sequencing Kit V3.1 (Applied Biosystems, Foster City, USA) following the manufacturer's protocol. The partial 16S rDNA sequences of the isolated strains were compared with those available in the public databases. Identification to the species level was determined as a 16S rDNA sequence similarity of >99% with that of the prototype strain sequence in the GenBank.

Statistical Analysis

Season and soil were considered as two treatment factors. The factor season had three levels, i.e., monsoon, summer and winter. The factor soil had six levels depending on soil properties and is given in Table 1. The upper series in Table 1 represents the data of each season averaged over six soils collected from different sites. The lower series represents the data for each soil averaged over three seasons. Analysis of variance (ANOVA) was carried out by completely randomized design (CRD).

Table 1 Physico-chemical properties of studied soil

	pH 1:2.5	ECe (dSm ⁻¹)	Organic carbon (g kg ⁻¹)	Total nitrogen (g kg ⁻¹)
Season				
Monsoon	6.12c	3.44c	10.17a	1.12b
Winter	6.19b	9.87b	9.78b	1.25a
Summer	6.31a	11.32a	9.38c	0.80c
Soil code				
S1	7.08b	2.02f	12.05a	0.94b
S2	5.67d	3.89e	8.81d	0.8c
S3	5.66d	19.58a	9.86c	0.78c
S4	6.65c	14.45b	4.68e	0.44e
S5	7.22a	4.31d	11.24b	1.68a
S6	4.99e	5.00c	12.03a	1.70a

Figures denoted by same alphabets are statistically similar at 5% probability level by DMRT

Result and Discussion

Physico-Chemical Properties of Soils

The soils, selected on the basis of salinity, were collected from six different plots with four different salinity (ECe) levels, viz., very low (S1), low (S2, S5 and S6), medium (S3) and high (S4). The soils S1, S2, S5 and S6 were silty clay, while S3 and S4 were loam and clay loam, respectively. The pH of the soils varied significantly both with respect to season and site (Table 1). The average pH of the soils varied from 6.12 in monsoon to 6.31 in summer. The soils, averaged over the three seasons, were generally acidic (pH 4.99–6.65) with the exception of S1 (7.08) and S5 (7.22). The ECe (dSm⁻¹) of the soils also varied significantly with respect to season and site. The mean ECe of the soils, averaged over three seasons, from different locations, could be ranked in descending order as: S3 (19.58), S4 (14.45), S6 (5.00), S5 (4.31), S2 (3.89) and S1 (2.02) (Table 1). The overall ECe value of the soils (Table 1) was lowest during the monsoon season (3.44), which increased to 9.87 during the winter season and then increased further to 11.32 in the summer season. Seasonal effect on organic carbon status of the soils was prominent. The highest average organic carbon (g kg⁻¹) content of the soils was observed in monsoon, followed by winter and summer. The mean organic carbon content of soils (Table 1) from different locations, averaged over three seasons, could be ranked as: S1 (12.05) > S6 (12.03) > S5 (11.24) > S3 (9.86) > S2 (8.81) > S4 (4.68). Among the seasons, the winter season soil recorded the highest total nitrogen content (g kg⁻¹) of 1.12, while during the summer season the soil registered the lowest value of 0.80 (Table 1). Statistical variation in the total nitrogen content of soils from different sites was evident. The highest total nitrogen could be registered in the S6 (1.70) soil followed in decreasing order as S5 (1.68), S1 (0.94), S2 (0.80), S3 (0.78) and S4 (0.44).

Non-Symbiotic Heterotrophic Diazotrophic Bacterial Counts of Soil

The diazotrophic population varied during different periods of sampling, with much higher counts in the winter (5.87) and the monsoon seasons (5.87) than that observed in the summer season (4.38) (Table 2). Significant decrease in the diazotrophic count in the summer season corresponded to rise in salinity and depletion of available soil moisture [16, 17]. The soils, collected from different sites, showed considerable variation in their diazotrophic populations. The S4 soil had the highest population (5.86) followed by S5 (5.67), S2 (5.55), S1 (5.47), S6 (5.08), and S3 (4.61). These variations seemed to be related to the physico-chemical characteristics of soils, the inherent type and

Table 2 Seasonal dynamics and effect of sodium chloride addition on nitrogen fixing population and nitrogen fixation of studied soil

	Population (log colony forming unit)	Nitrogen fixation (mg nitrogen fixed 50 ml ⁻¹ culture media)
Season		
Monsoon	5.87a	4.75a
Winter	5.87a	4.17b
Summer	4.38b	3.55c
Soil code		
S1	5.47c	4.52b
S2	5.55c	3.76d
S3	4.61e	2.74e
S4	5.86a	3.77d
S5	5.67b	5.86a
S6	5.08d	4.29c
Salt concentration (%)		
0	6.28a	8.35a
1	5.84b	5.06b
2	5.16c	2.54c
3	4.2d	0.68d

Figures denoted by same alphabets are statistically similar at 5% probability level by DMRT

diversity of microorganisms occurring in these soils [18, 19, 20]. It was interesting to note that inspite of rise in salinity, even up to the levels of 26 and 28 dSm⁻¹ in S3 and S4 soils, respectively, during the summer season, the soils supported considerable diazotrophic populations. The total count of bacteria is usually negatively correlated with the total soluble salts of saline soils [21], but positively correlated with organic carbon contents [21, 22]. However, these soils might have their own bacterial communities, which have adapted to saline environments. Highly saline S3 and S4 soils might be containing microbial communities that were adapted to high salt stress. When the soil dilutions were plated with the addition of graded doses of NaCl (0–3%) in the media, there was significant statistical variation on the recovery of salt tolerant microorganisms. In all the soils, the populations substantially decreased with the increase of salt concentration in the medium. The highest population (6.28) was observed at 0% NaCl concentration and the lowest (4.20) at 3% NaCl. Higher supplement of NaCl in the culture media seemed to be detrimental. This indicated that nitrogen-fixing microorganisms of the soils were basically salt tolerant, requiring certain amount of salts for their optimal growth. It was opined that within the halotolerants, a distinction can be made between those, for which growth rate is decreased by the addition of any salt, and those for which growth rate reaches an optimum with the addition of some salt, but then declines at higher salinities [23]. The N₂ fixing microorganisms recovered

Table 3 Phenotypic characteristics of the isolates from the studied soils

Strain isolated and code	Colony characteristics on nitrogen free mineral salt yeast extract agar slants	Morphology of cells by negative staining	Gram character	Spore formation	Capsule formation
Can1	Dry, non acid producers	Long rod shaped bacterium, in chain	Gram Positive	+	+
Can2	Dry, weak acid producers	Short rod shaped bacterium, isolated	Gram Positive	+	+
Can3	Dry, weak acid producers	Rod shaped bacterium, isolated	Gram Positive	+	+
Can4	Dry, weak growers non acid producers	Long rod shaped bacterium, in chain	Gram Positive	+	+
Can5	Dry, weak growers non acid producers	Long rod shaped bacterium, in chain	Gram Positive	+	+
Can6	Dry, white powdery colony	Rod shaped bacterium, in chains	Gram Positive	+	+
Can7	Dry, non acid producers	Rod shaped bacterium, in chains	Gram Positive	+	+
Can8	Dry, strong acid producers	Short rod shaped bacterium, isolated	Gram Positive	+	+

from the studied soils seemed to be halotolerant belonging to the earlier category.

Non-symbiotic Nitrogen Fixation in Soil (mg Nitrogen Fixed 50 ml⁻¹ Culture Media)

Seasonal variation of nitrogen fixation in soil was evident. The highest nitrogen fixation occurred in the monsoon season (4.75), which gradually decreased in the winter season (4.17) and then to 3.55 during the summer season (Table 2). Although the diazotrophic populations of soils during the monsoon and winter seasons were statistically similar, the soils varied with respect to their nitrogen fixation. This indicated that rise in soil salinity during winter had a negative effect on nitrogen fixation in soil. The effect was aggravated with further rise in soil salinity during the summer season when both diazotrophic populations and nitrogen fixation were seriously impaired, which corresponds to previous reports [17, 24]. The S5 soil recorded significantly the highest value of nitrogen fixation (5.86) followed by S1 (4.52), S6 (4.29), S4 (3.77), S2 (3.76) and S3 (2.74) soils. Notably, the nitrogen fixation in the soils did not correspond to their population count (Table 2). Data indicated that the soils with high salinity harboured microorganisms with lower nitrogen fixation capacity. Nitrogen fixation in soils diminished with the increase in NaCl (0–3%) concentration in the culture medium. Highest nitrogen fixation (8.35) took place at 0% NaCl and the lowest (0.68) at 3% NaCl. In this case, however, nitrogen fixation in soils corresponded to the respective population count of the soils. These findings are in conformity with the earlier observations [17, 24].

Phenotypic and Biochemical Characteristics of the Isolates

Phenotypic characteristics of the eight isolated pure cultures from different sites (Table 3) revealed that all the colonies were dry in appearance on agar slant. The

morphology of the cells varied from small to big rods (Fig. 1). All the eight isolates were Gram (+ve), spore and capsule formers. Biochemical characteristics (Table 4) revealed that all the isolates could utilize glucose, sucrose, mannitol, citrate and nitrate and could hydrolyse starch. They were catalase and gelatinase positive but indole, MR and VP negative. The isolates showed alkaline reaction on TSI agar slants.

Nitrogen Fixation and Nitrogen Fixing Potentiality of the Isolates

The aim of this study was to identify the strains that fix nitrogen best at 1% NaCl concentration (approximately 10dSm⁻¹), so that such strains could be used as potent biofertilizer for agriculture in coastal areas. The isolates could fix 2.42–4.45 mg of nitrogen per 50 ml culture media at 0% NaCl in the culture media (Table 5). The same values at 1% NaCl were 2.04–4.08 mg of nitrogen per 50 ml culture media. The ARA of the isolates at 0% NaCl in the culture media ranged from 4.51 to 164.52 nmol of C₂H₄ formed per ml culture, and at 1% NaCl those were 1.72–100.6 nmol of C₂H₄ formed per ml culture (Table 5).

The nitrogen fixing potentiality of the isolates as measured by ARA, confirmed their potentiality for fixing nitrogen in media containing 0 and 1% NaCl. Reduction in ARA of the isolates grown in nitrogen free medium containing 1% NaCl may be the consequence of ionic component [25]. It is also established that microorganisms exposed to salinity stress deviates cellular energy towards osmoregulation, especially Na⁺ efflux and hence loss of nitrogenase activity by the isolates [25].

Salt Tolerance of the Isolates

The isolates showed negative growth as determined by the O.D, with the graded addition of NaCl (1, 2 and 3%) with respect to 0% NaCl in the culture medium (Table 5). The negative growth of the isolates in media amended with

Fig. 1 Light microscopic appearance of the isolates by negative staining. *Can1* *Bacillus* sp. isolate 28A (EU831291), *Can2* *Bacillus* sp. MOLA 87 (EU831292), *Can3* *Bacillus* sp. By113(B)Ydz-dh (EU831293), *Can4* *Bacillus* sp. PN13 (EU831294), *Can5* *Bacillus* licheniformis strain RH101 (EU831295), *Can6* *Bacterium* Antarctica-14 (EU831296), *Can7* *Bacillus* sp. PN13 (EU831297), *Can8* *Bacillus* megaterium (EU831298)

Table 4 Biochemical characteristics of the isolates from the studied soils

Strain isolated and code	Sugar utilization			Starch hydrolysis	NO ₃ ⁻ utilization	Catalase	Gelatinase	Indole	Methyl red	VP	Citrate	Growth on TSI agar slant
	Glucose	Sucrose	Mannitol									
Can1	+++	+++	+++	++	++	+	+	–	–	–	+	Shows alkaline reaction
Can2	+++	+++	+++	++	++	+	+	–	–	–	+	Shows alkaline reaction
Can3	+++	+++	+++	++	++	+	+	–	–	–	+	Shows alkaline reaction
Can4	+++	+++	+++	++	++	+	+	–	–	–	+	Shows alkaline reaction
Can5	+++	+++	+++	++	++	+	+	–	–	–	+	Shows alkaline reaction
Can6	+++	+++	+++	++	++	+	+	–	–	–	+	Shows alkaline reaction
Can7	+++	+++	+++	++	++	+	+	–	–	–	+	Shows alkaline reaction
Can8	+++	+++	+++	++	++	+	+	–	–	–	+	Shows alkaline reaction

Table 5 Efficiency of nitrogen fixation and salinity tolerance of the isolates

Strain isolated and code	ARA*(nmol of C ₂ H ₄ formed ml ⁻¹ culture media in 72 h)		mg Nitrogen fixed 50 ml ⁻¹ culture media		Culture OD in nitrogen free mineral salt yeast extract broth containing extraneous addition of NaCl % increase (+)/decrease (-) over control			Identification Based on 16S rDNA sequence analysis
	0% NaCl N ₂ free media	1% NaCl N ₂ free media	0% NaCl N ₂ free media	1% NaCl N ₂ free media	1% NaCl N ₂ free media	2% NaCl N ₂ free media	3% NaCl N ₂ free media	
Isolates from canning agricultural field soils								
Can1	31.50	5.317	3.75	2.04	−91	−93	−56	<i>Bacillus sp. isolate 28A</i>
Can2	71.26	21	2.42	2.408	−97	−100	−100	<i>Bacillus sp. MOLA 87</i>
Can3	4.51	3.10	3.45	3.13	−72	−73	−40	<i>Bacillus sp. By113(B)Ydz-dh</i>
Can4	98.54	100.6	4.45	4.08	−86	−93	−87	<i>Bacillus sp. PN13</i>
Can5	27.62	4.20	2.96	2.96	−87	−92	−88	<i>Bacillus licheniformis strain RH101</i>
Can6	164.52	47.22	3.71	4.08	−61	−69	−77	<i>Bacterium Antarctica-14</i>
Can7	75.02	34.18	3.33	2.96	−86	−76	−92	<i>Bacillus sp. PN13</i>
Can8	35.41	1.72	3.33	3.71	−98	−93	−100	<i>Bacillus megaterium</i>
Reference								
<i>Azotobacter vinelandii</i>	128	5.03	5.23	1.64	−95	−100	−100	

* Acetylene reduction assay

NaCl may be due to the inhibitory action of NaCl. However, presence of the organisms in such highly saline soils may be due to the fact that the organisms are more tolerant to salinity stress in soil than in culture media [17].

Identification of the Strains on the Basis of 16S rDNA Sequences

Based on the sequencing of 16S rDNA of the isolates and subsequent comparison with GenBank, the isolates were similar to *Bacillus sp. isolate 28A* (Can1), *Bacillus sp. MOLA 87*(Can2), *Bacillus sp. By113 (B)Ydz-dh*(Can3), *Bacillus sp. PN13*(Can4), *Bacillus licheniformis* strain RH101(Can5), *Bacterium Antarctica 14* (Can6), *Bacillus sp. PN13*(Can7) and *Bacillus megaterium*(Can8). *Bacillus* has been previously reported to exhibit diazotrophic activity [26]. *Bacterium Antarctica 14* as isolated in this study was not previously reported as a diazotroph.

Conclusion

Non-symbiotic diazotrophic bacterial populations of coastal saline soils varied temporarily and they were adapted to their physico-chemical environment. Even at high salinity (10dsm⁻¹), they could substantially fix

atmospheric nitrogen, indicating their potentiality of being used as microbial inoculants for coastal agriculture. Molecular characterization of the isolates revealed the presence of some unusual diazotrophic bacteria in those soils. There is a need to conduct field trials with the efficient strains to test their effectiveness for crop production.

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