RESEARCH ARTICLE Legume Research- An International Journal, Volume 46 Issue 2: 196-203 (February 2023)

Azotobacter vinellandii SINAz1 Increases Growth and Productivity in Rice under Salinity Stress

Ranjan Kumar Sahoo[1], Madhusmita Pradhan[2]

10.18805/LR-4335

ABSTRACT

Background: *Azotobacter vinellandii* is a soil bacterium which fixes atmospheric nitrogen and provides growth hormones to plant. Locally isolated species can be utilized and efficient bio fertilizers can be prepared.

Methods: A nitrogen fixing bacteria was isolated and it was biochemically identified as *Azotobacter vinellandii* and named as *Azotobacter vinellandii SINAz1*. The 16s rRNA was isolated and the sequence was submitted to NCBI data base and got the Accession number as MN135308.1. The presence of nif D, nif K and nif H gene was studied by PCR amplification. The nitrogen fixing efficiency of these bacteria was studied in laboratory by acetylene reduction assay (ARA) and also by pot culture experiments. The plant growth promoting (PGP) activities also studied by isolating and estimating the secretion of hormones like IAA, GA3, ABA and zeatin. These bacteria also provide salinity stress (200 mM NaCl) tolerance to rice plant for 15 days as compared with the control.

Result: The phenotypic growth and yield of rice crop by the application of these bacteria was studied which found to be significantly better than the control. This novel bacterium can be used as a substitute for the chemically synthesized nitrogen fertilizer for better productivity under normal as well as salinity stress condition.

Key words: Azotobacter, Biofertilizer, Nitrogen, Nitrogen fixation, Rice.

INTRODUCTION

The world population is increasing very fast and it will reach around 9 billion by 2050 (Godfrey *et al*., 2010). To feed this huge population, food supplies has to increased upto 70-100 % (Tillman *et al*., 2011). Rice is a very important crop, which is cultivated and consumed by maximum number of world population than other crop (Huang *et al*., 2018). Besides that, rice cultivation provides much of the raw materials needed by today's manufacturing industry (Kenmore, 2003). Thus, rice production directly affects food security as well as the economy of the people. Therefore, the production of rice must be increased to fulfil the requirements of growing world population.

Rice production depends mainly on nitrogen (N) fertilizer. The use of chemical fertilizers for supply of nitrogen intrinsically degrade soil environment and environmental health through emission of nitrogen oxides, (especially N_2O) from the anaerobic (flooded) rice ecologies (Bhattacharjee *et al*., 2008). Therefore, biological (especially associative) nitrogen fixation (BNF) should be exploited to supplement N for rice production which would supplement 20-25% of the total N *i.e.* about 80000 tones atmospheric N/ha (Subba Rao, 2007). *Azotobacter* is generally regarded as a free-living aerobic nitrogen-fixer (Saharan and Nehra, 2011). Besides, nitrogen fixation, *Azotobacter* also promote the plant growth by producing thiamine, riboflavin, indole acetic acid (IAA) and Gibberellins (GA) (Sahoo *et al*., 2012).

In this study, we report the discovery of nitrogen fixing free living bacteria *Azotobacter vinellandii* strain *SINAz1* among 20 rhizospheric bacteria from different locations of Odisha, India. Along with nitrogen fixation, it secretes plant growth promoting hormones like IAA, GA3, ABA and zeatin significantly compared to other. It also protects rice plants from the toxic effect of salinity stress (200 mM NaCl) up to 15 days in pot experiments. This strain notably increased the growth and yield of rice plant without application of any chemical fertilizer. So our bacterium is novel and bears unique characteristics collectively nitrogen fixation, hormone secretion and salinity stress tolerance.

[1]Department of Biotechnology, Centurion University of Technology and Management, Odisha, India.
[2]Department of Botany, Utkal University, Bhubaneswar-751 003, Odisha, India.

Corresponding Author: Ranjan Kumar Sahoo, Department of Biotechnology, Centurion University of Technology and Management, Odisha, India. Email: ranjan.sahoo@cutm.ac.in

How to cite this article: Sahoo, R.K. and Pradhan, M. (2023). *Azotobacter vinellandii SINAz1* Increases Growth and Productivity in Rice under Salinity Stress. Legume Research. 46(2): 196-203. doi: 10.18805/LR-4335.

Submitted: 25-01-2020 **Accepted:** 04-04-2022 **Online:** 02-05-2022

MATERIALS AND METHODS

Isolation of nitrogen fixing (*Azotobacter* spp.) organisms

For isolation *Azotobacter* species, soil samples were collected from four different rice fields situated in different locations of Odisha, India, *viz.* OUAT experimental field 1 and 2, Experimental field of Mahanga and Sindhupur, Cuttack, Odisha, India, where rice is cultivated at least for last 20 years. The rice plants (*Oryza sativa* L. var. IR64)

were up rooted and the soil was scrapped off from the root and used for isolation. The isolates were phenotyped (Kennedy *et al.*, 2005; Sahoo *et al.*, 2014) grouped on the basis of phenotypic characters and one representative of each group of isolates were used for the remainder experiments.

Morphological and staining characteristics of the bacteria

Morphological characteristics (shape, size, motility) of the bacteria were checked under a phase contrast light microscope (100X objective). Gram's stain and spore stain (malachite green) of the isolates were done following standard microbial methods (Collee and Miles, 1989).

Physiological and biochemical characterization of isolates

Physiological and biochemical tests (oxidase, catalase, urease, indole production, methyl red, acetoin production, nitrate reduction , citrate utilization, hydrogen sulphide (H_2S) production, carbohydrate fermentation, arginine dihydrolase, starch hydrolysis, lipase, tributyrin and vegetable oil hydrolysis, cholesterol hydrolysis, protein hydrolysis, gelatine and casein hydrolysis, pectin and chitin hydrolysis, lecithin hydrolysis) were done for identification of all the bacterial isolates. The detailed procedure of each test was performed according to the method described by Sahoo *et al.*, (2014).

Acetylene reduction assay (ARA), quantification of IAA, GA3, ABA and zeatin produced by bacterial isolates

Nitrogen fixation efficiency in culture by the four *Azotobacter* isolates were assessed by ARA in the laboratory (Hardy *et al.*, 1968) cultivated on N-free Jensen agar (Jensen, 1954). The extraction and quantification of IAA, GA3, ABA and zeatin were done according to the method described earlier (Sahoo *et al.*, 2014).

Amplification of *nif*K, *nif*D, *nif*H gene

The genomic DNA was amplified using full length *nif K* primers (forward 5′- ATGAGCCAGCAAGTCGATAA-3′ and reverse 5′- TGGTGCTGGACCATGCGATT-3′), *nif D* primers (forward 5′- ATGACCGGTATGTCGCGCCA-3′ and reverse 5′- CGGCGGTCGCGGACT-3') and *nifH* primers (forward 5′- ATGGCTATGCGTCAATGCGC-3′ and reverse 5′- TCAGACTTCTTCGGCGGTTT-3′) designed by using primer-3 software (http://bioinfo.ut.ee/primer3-0.4.0/). These primers were synthesized and supplied by Eurofins Amar Immunodiagnostics, Hyderabad, A.P., India.

Sites for pot experiments

The pot experiments was conducted in the green house of Department of Soil Science and Agricultural Chemistry, Orissa University of Agriculture and Technology, Odisha, India, in the year 2018-2019, to unveil the native efficient *Azotobacter* strain for formulation and production of potent indigenous biofertilizer for commercial exploitation in salinity soil.

Formulation of biofertilizers

Biofertilizers were formulated aseptically under a laminar air flow using the following composition. Sterile (autoclaved) charcoal powder 700 g/kg, $CaCO_3$ 100 g/kg, gum acacia 20 g/kg and liquid culture 180 g/kg (180 ml containing 10^9 cfu/ml) *i.e.* final population 2 $\times 10^8$ cfu/g formulation (according to Bureau of Indian Standards (BIS).

Treatment of seedlings and design of pot experiments

Healthy, 21d old rice (*Oryza sativa* L. var. IR 64, a salt sensitive variety) seedling were dipped separately in biofertilizer suspensions (10% w/v *i.e.* 2 $\times$ 10^8cfu/ml) for 2 h as recommended for commercial formulations by Bureau of Indian Standards (BIS) and transplanted in different pots with three replications each *viz.* Control (C) without any fertilizer; Treatment 1 (T1) with *Azotobacter vinelandii.* isolated from OUAT experimental field 1; Treatment 2 (T2) with *Azotobacter vinelandii.* isolated from OUAT experimental field 2; Treatment 3 (T3) with *Azotobacter vinelandii.* isolated from experimental field Sindhupur, Cuttack.

Growth parameters

Growth parameters like plant height (cm), tiller/hill (no), effective tiller/hill (no), panicle length (cm), leaf area (sq. cm) and panicle length (cm) were measure prior to harvest. The crop was harvested after 90 d and the post harvest observations like root length (cm), root dr. wt. (g), root volume (ml), panicle weight (g), grain yield/plant (g), filled grain/ panicle (no.) and 1000 grain wt. (g) were recorded.

Salinity stress tolerance study

The above pot experiments were repeated for salinity stress tolerance assay. The 3 selected *Azotobacter vinellandii* (Based on the ARA assay) strains along with control were used for these studies. The treatments (C, T1, T2 and T3) were used for this salinity tolerance assay. Rice plants after 6 weeks in soil were subjected to salinity (200 mM NaCl) stress. All the pots (C, T1, T2 and T3) were kept in one big tank filled with 200 mM NaCl solution. The plants were grown in the green house and the white light was provided (16 h photo period) by white fluorescent tubes (36 W Philips TLD) with a photon flux density of 52 µ /m2s (PAR).

16S rRNA gene sequencing

The 16S rRNA gene was PCR amplified by using the forward (5′- AGAGTTTGATCMTGGCTCAG-3′) and reverse primer 5′- GTTACCTTGTTACGACTTAAGTCGTAACAAGGTAACC-3′ using the genomic DNA isolated from the most efficient *Azotobacter vinellandii* The amplified products were sequenced. The sequencing was done on ABI 3130xl analyser based on Sangers dideoxy termination method and submitted to NCBI gene bank (ncbi.nlm.nih.gov).

Statistical analysis

All statistical analysis were performed using the graph and prism software. The experimental data values were mean values from three independent series, each done with three replicates and the results presented as means ± standard error (SE), based on three replications. The statistical significance at $P<0.05$ has been calculated.

RESULTS AND DISCUSSION

Morphological, colony and biochemical characteristics of tentative Azotobacter on Jensen's media

Five types of soil bacteria isolated from each experimental rice fields using Jensen's medium. The organisms were studied by their colony characters (Table 1). The colonies of the bacteria of Jensen's medium produced convex, circular, fluorescent, brown, off white or white, low convex, flat, plicate, size ranges from 0.60-1.00 mm, gummy, not gummy, mucoid (Table 1). The characteristics *viz.*, shape, size (length and breadth) motility, Gram's stain of the bacteria were checked under a phase contrast microscope (100X objective). The morphological characteristics of all 20 colonies were presented in Table 2.

Biochemical characterization

The biochemical tests such as oxidase test, phosphatase test, nitrate reduction test catalase, carbohydrate utilization, carbohydrate fermentation, nitrate reduction, citrate utilization etc. were carried out for identification of isolates. The isolates were examined for catalase, oxidase and for urease test. In citrate utilization test, the bacteria of Jensen's medium showed positive some of them showed negative. Biochemical characterization of all isolates were given in the Table 3.

Identification of *Azotobacter* spp.

After biochemical tests some of them are identified as *Azotobacter vinellandii*, *Azotobacter chroococcum*, *Klebsiella* spp. *Beijerinckia* spp. *Pseudomonas* spp. from the 20 number of isolates (Table 4). The identified *Azotobacter vinelandii* were used for further assays.

Acetylene reduction assay (ARA) of isolated Azotobacter spp.

In vitro nitrogen fixing efficiency of identified Azotobacter spp. were studied and among the 11 isolates, the *Azotobacter vinelandii* isolated and identified from the different fields showed higher nitrogen fixation efficiency (Table 5). All the 3 *Azotobacter vinelandii* isolates (Az1a, Az2b and Az4a) identified from different fields were used in 3 treatments (T1, T2 and T3) respectively along with control (C) for further studies. Because these three *Azotobacter vinelandii* isolates have higher nitrogenise activity than others.

PGP functions of the *Azotobacter* isolates

Plant growth promotion (PGP) functions of the *Azotobacter vinelandii* isolates (Az1a, Az2b and Az4a) were presented in Fig 1. The activities were highly variable. The isolate Az4a possess higher IAA, ABA, GA3 and zeatin content than other two (Az1a and Az2b).

Amplification of *nif* gene clusters

The gene expected size (1.4kb) was obtained in gel picture of *nif D* gene, size of (1.5 kb) band was obtained in case of *nif K* gene and size of 0.87 kb was obtained in case of *nif H* gene for all the *Azotobacter* isolates (Fig 2).

16S rRNA sequencing of *Azotobacter vinelandii* isolate

The amplified fragment of 16s rRNA of *Azotobacter vinellandii* (Az4a) was sequenced and the sequence was submitted to NCBI gene bank and catalogued the accession number as MN135308.1.

Growth observations in pot experiments under salinity stress

Effects of the the 3 *Azotobacter vinelandii* isolates (Az1a, Az2b and Az4a) on the growth and productivity of the rice plant along with control (C) experiment were studied. The control plants (C) were died and the plants of other treatments (T1, T2 and T3) were grew well and showed better phenotypic growth characteristics. Among them more tiller number, more plant height were observed in case of T3 (Table 6).

Azotobacter vinellandii provide salinity (200 mM) tolerance to plants

There was a significant difference in survival and agronomic parameters of rice plants of 3 different treatments (T3-T6) when compared with the plants of C. Better agronomic characteristics were observed in all the treatments under 200 mM salinity stress except C (Table 1). The rice plants of C pot died due to toxic stress of chromium. But other treatment (T1, T2 and T3) plants survived up to maturity.

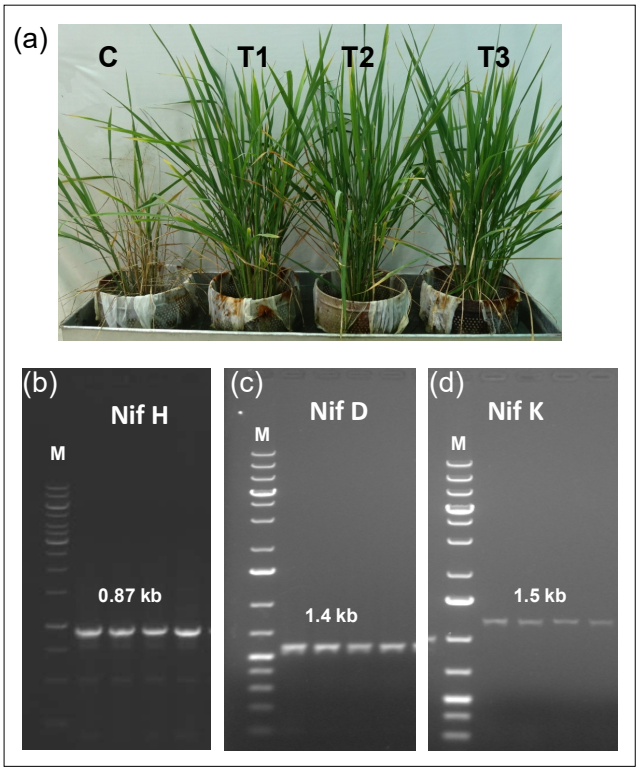

Fig 1: (a) Rice plants of different treatments (C, T1, T2 and T3) inoculated with *Azotobacter vinellandii* exposed to salinity stress (200 mM). (b) PCR conformation of the *nif H* gene showing amplification of 0.87kb. (c) *nif D* gene (1.4 kb) (d) *nif K* gene (1.5 kb).

All together 20 tentative *Azotobacter spp.* were isolated from Jensen's N-free medium and they were phenotyped by morpho-physiological and biochemical characters (Kennedy *et al.*, 2005; Sahoo *et al.*, 2014). The morphological, physiological and biochemical characters identified the isolates *viz.* Az1a, Az2b, Az4a as *Azotobacter vinelandii*, Az1b, Az2c, Az2d, Az3b, Az3d, Az3e, Az4e as *Azotobacter chroococcum* and Az3a as *Azotobacter spp.* (Hill and Sawers, 2009) and the species of other isolates remained unknown. The results proved that the population of *Azotobacter* spp. of rice rhizosphere was diverse. Similarly, diverse species of *Azotobacter i.e. A. vinelandii*,

Table 1: Colony characteristics of the isolated tentative *Azotobacter* spp.

Location	Isolate no.	Form	Colour	Elevation	Margin	Size (mm)	Consistency
OUAT experimental field-1	Az1a	Circular	Brown	Convex	Entire	0.60-0.75	Gummy
	Az1b	Circular	Fluorescent	Flat	Entire	0.85-0.90	Gummy
	Az1c	Circular	Brown	Convex	Entire	0.65-0.75	Mucoid
	Az1d	Circular	Off white	Convex	Entire	0.65-0.75	Not gummy
	Az1e	Circular	Fluorescent	Low convex	Entire	0.85-0.90	Not gummy
OUAT experimental field-2	Az2a	Circular	Brown	Convex	Entire	0.60-0.75	Mucoid
	Az2b	Circular	Brown	Convex	Entire	0.60-0.75	Gummy
	Az2c	Circular	Fluorescent	Convex	Entire	0.85-0.90	Gummy
	Az2d	Circular	Fluorescent	Flat	Entire	0.60-0.75	Gummy
	Az2e	Circular	Fluorescent	Flat	Entire	0.65-0.75	Not gummy
Experimental field of Mahanga, Cuttack	Az3a	Circular	White	Flat	Entire	0.95-1.00	Gummy
	Az3b	Circular	White	Convex	Entire	0.85-0.90	Gummy
	Az3c	Circular	Off white	Convex	Entire	0.65-0.75	Mucoid
	Az3d	Circular	Brown	Low convex	Entire	0.85-0.90	Gummy
	Az3e	Circular	White	Low convex	Entire	0.85-0.90	Not gummy
Experimental field of Sindhupur, Cuttack	Az4a	Circular	Brown	Convex	Entire	0.65-0.75	Gummy
	Az4b	Circular	Brown	Convex	Entire	0.85-0.90	Not gummy
	Az4c	Circular	Off white	Flat	Entire	0.60-0.75	Mucoid
	Az4d	Circular	White	Convex	Entire	0.60-0.75	Mucoid
	Az4e	Circular	Brown	Low convex	Entire	0.65-0.75	Gummy

Table 2: Morphological characteristics of the tentative Azotobacter isolated on Jensen's media.

Location	Isolate no.	Shape	Length			Breadth			Motility	Gram stain
			Range	Mean	SE	Range	Mean	SE		
OUAT experimental field-1	Az1a	Ovoid	1.0-2.0	1.5	0.024	0.75-1.0	0.87	0.043	+	-ve
	Az1b	Large ovoid	1.5-2.0	1.75	0.021	0.5-1.0	0.75	0.027	+	-ve
	Az1c	Rod	1.5-2.0	1.75	0.025	0.5-0.75	0.62	0.036	+	-ve
	Az1d	Rod	1.5-2.0	1.75	0.031	0.5-0.75	0.62	0.027	-	-ve
	Az1e	Large ovoid	1.0-2.0	1.5	0.038	0.5-0.75	0.62	0.024	+	-ve
OUAT experimental field-2	Az2a	Large ovoid	1.5-2.0	1.75	0.032	0.75-1.0	0.87	0.018	+	-ve
	Az2b	Ovoid	1.0-1.75	1.4	0.035	0.75-1.0	0.87	0.029	-	-ve
	Az2c	Rod	0.5-1.3	0.9	0.021	0.5-0.75	0.62	0.031	-	-ve
	Az2d	Rod	0.5-1.0	0.75	0.026	0.75-1.0	0.87	0.027	+	-ve
	Az2e	Large ovoid	1.0-2.0	1.5	0.031	0.5-0.75	0.62	0.018	+	-ve
Experimental field of Mahanga, Cuttack	Az3a	Ovoid	1.5-2.0	1.75	0.035	0.75-1.0	0.87	0.027	+	-ve
	Az3b	Ovoid	1.0-1.75	1.4	0.027	0.75-1.0	0.87	0.031	+	-ve
	Az3c	Rod	0.5-1.5	1.0	0.023	0.5-1.0	0.75	0.057	-	-ve
	Az3d	Rod	0.5-1.0	0.75	0.053	0.5-1.0	0.75	0.058	-	-ve
	Az3e	Ovoid	0.5-1.3	0.9	0.057	0.75-1.0	0.87	0.053	-	-ve
Experimental field of Sindhupur, Cuttack	Az4a	Rod	0.5-1.0	0.75	0.028	0.5-1.0	0.75	0.041	+	-ve
	Az4b	Ovoid	1.5-2.0	1.75	0.027	0.5-1.0	0.75	0.037	+	-ve
	Az4c	Ovoid	1.0-1.75	1.4	0.025	0.75-1.0	0.87	0.034	-	-ve
	Az4d	Ovoid	1.0-1.75	1.4	0.027	0.5-1.0	0.75	0.037	-	-ve
	Az4e	Ovoid	0.5-1.0	0.75	0.021	0.75-1.0	0.87	0.031	-	-ve

Table 3: Biochemical characteristics of the *Azotobacter spp.* isolated.

Name of test	OUAT experimental field-1					OUAT experimental field-2					Experimental field of Mahanga, Cuttack					Experimental field of Sindhupur, Cuttack				
	Az1a	Az1b	Az1c	Az1d	Az1e	Az2a	Az2b	Az2c	Az2d	Az2e	Az3a	Az3b	Az3c	Az3d	Az3e	Az4a	Az4b	Az4c	Az4d	Az4e
Oxidase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Phosphatase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Utilization of carbon source																				
Rhamnose	+	+	-	-	+	-	+	+	-	+	+	-	-	+	-	+	+	-	+	-
Caprylate	+	+	+	+	+	-	+	-	-	-	-	+	+	+	+	+	+	-	+	-
Meso-inositol	+	+	+	-	-	-	+	-	-	-	-	-	-	+	+	+	+	+	+	-
Mannitol	+	+	-	-	-	+	+	+	+	+	+	+	-	+	+	+	+	+	+	
NO_3 reaction test	+	+	-	-	-	-	+	+	+	-	-	-	-	+	+	+	+	+	-	+
H_2S production	+	+	-	-	+	+	+	-	-	-	+	-	-	+	+	+	+	+	-	+
Utilization of sole carbon source																				
Fructose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Glutarate	+	+	-	-	-	-	+	+	+	+	+	+	+	-	-	+	+	-	-	-
Oxaloacetate	+	+	+	+	-	-	+	+	+	+	+	+	+	-	+	+	-	+	+	-
D-galactose	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Glycerol	+	+	+	-	+	+	+	-	+	-	+	-	+	-	-	+	-	-	+	-

Table 4: Identification of tentative *Azotobacter* spp.

Location	Isolate no.	Name of bacteria
OUAT experimental field-1	Az1a	*Azotobacter vinelandii*
	Az1b	*Azotobacter chroococcum*
	Az1c	*Pseudomonas*
	Az1d	*Klebsiella* spp.
	Az1e	*Beijerinckia*
OUAT experimental field-2	Az2a	*Klebsiella* spp.
	Az2b	*Azotobacter vinelandii*
	Az2c	*Azotobacter chroococcum*
	Az2d	*Azotobacter chroococcum*
	Az2e	*Klebsiella* spp.
Experimental field of Mahanga, Cuttack	Az3a	*Azotobacter* spp.
	Az3b	*Azotobacter chroococcum*
	Az3c	*Beijerinckia* spp..
	Az3d	*Azotobacter chroococcum*
	Az3e	*Azotobacter chroococcum*
Experimental field of Sindhupur, Cuttack	Az4a	*Azotobacter vinelandii*
	Az4b	*Pseudomonas* spp.
	Az4c	*Klebsiella* spp.
	Az4d	*Beijerinckia* spp.
	Az4e	*Azotobacter chroococcum*

chroococcum etc. were identified from rice rhizosphere elsewhere (Saharan and Nehra, 2011). Nitrogen fixation efficiency (acetylene reduction assay, ARA) of the *Azotobacter* spp. varied between 26.16-128.57 nmole C2H4/mg bact./h. The *Azotobacter vinelandii* SINAz1 (isolate no. Az4a) of experimental field Sindhupur, Cuttack which produced 128.57 nmole C2H4/mg bact./h, was more efficient nitrogen fixing organism in culture than the other isolated bacteria. The results proved that nitrogen fixation efficiency of the *A. vinelandii* SINAz1 was superior to other indigenous BNFs *viz.* acetylene reduction by heterotrophic or endophytic *Azotobacter* spp. which fixed 79.6-329.50 nmol C_2H_4/h/culture or 57-686 nmole C_2H_4/mg protein/h (Barua *et al*., 2012). In rice, ARA of heterotrophic or endophytic *Azotobacter* spp. was 12.10-53.40 nmol C2H4/ mg bact./h (Barua *et al*., 2012) which conformed with the present study. The 16S rDNA of the most potent *A. vinelandii (*Az3*)* (other isolates were not done) produced an amplicon of 1.4 kbp size which conformed to that of the other *Azotobacter* spp. (Sahoo *et al*., 2014). Plant hormones control plant growth and developmental and played a role in adaptation to different stresses (Peleg and Blumwald, 2011).

Table 5: Nitrogen fixation efficiency (Acetylene reduction assay) by *Azotobacter* isolates.

Field	Isolate no.	Organism	N_2-ase activity (nmole C_2H_4/mg bact./h)
OUAT experimental field 1	Az1a	*Azotobacter vinelandii*	87.53±3.09
	Az1b	*Azotobacter chroococcum*	69.19±1.38
OUAT experimental field 2	Az2b	*Azotobacter vinelandii*	87.27± 2.16
	Az2c	*Azotobacter chroococcum*	84.53±2.78
	Az2d	*Azotobacter chroococcum*	63.11±4.23
Experimental field Mahanga, Cuttack	Az3a	*Azotobacter spp.*	63.68±2.11
	Az3b	*Azotobacter chroococcum*	58.12±4.78
	Az3d	*Azotobacter chroococcum*	45.38±4.11
	Az3e	*Azotobacter chroococcum*	76.11±2.58
Experimental field Sindhupur, Cuttack	Az4a	*Azotobacter vinelandii*	128.57±2.13
	Az4e	*Azotobacter chroococcum*	26.16±1.22
CD, P 0.05	-		11.98

Table 6: Phenotypic growth characteristics (plant height, root length, root dry weight, leaf area); photosynthetic characteristics (chlorophyll content, net photosynthetic rate, stomatal conductance and internal CO_2 concentration, total protein); nutrient content (nitrogen, phosphorus, potassium, sodium) of rice plants at different treatments (Az1, Az2, Az3) and control (C) after 15 days salinity (200 mM) stress.

Attributes	C (Control)	Az1	Az2	Az3
Plant height (cm)	61±2.0^c	75±3.1^a	75±3.2^a	79±3.0^b
Root length (cm)	20±1.1^b	30±1.2^a	30±0.8^a	30±1.0^a
Root dry weight (g)	1.9±0.11^b	2.5±0.1^a	2.4±0.12^a	2.7±0.1^a
Leaf area (cm^2/plant)	74±1.0^c	87±1.5^a	91±2.1^a	91±1.5^b
Total chlorophyll (mg/g f wt)	6.62±0.4^b	9.12±0.1^a	9.02±0.21^a	9.11±0.3^a
Total protein (mg/g f wt)	1.51±0.82^b	1.72±0.81^a	1.71±0.52^a	1.72±0.51^a
Net photosynthetic rate (P_N, μ mol CO_2 $m^{-2}s^{-1}$)	6.01±0.3^b	9.10±0.1^a	9.21±0.2^a	9.05±0.3^a
Stomatal conductance (gs, m mol $m^{-2}s^{-1}$)	211±10.5^c	243±9.3^a	242±10.4^a	245±10.2^b
Intracellular CO_2 (*Ci*, μ mol mol^{-1})	212±9.5^b	221±10.4^a	221±10.2^a	225±10.1^a
Nitrogen (%)	0.253±0.011^c	0.274±0.011^a	0.275±0.010^a	0.279±0.011^b
Phosphorus (%)	0.219±0.010^b	0.241±0.011^a	0.242±0.010^a	0.241±0.011^a
Potassium (%)	0.123±0.001^b	0.163±0.002^a	0.165±0.003^a	0.163±0.001^a
Sodium (%)	0.042±0.001^a	0.042±0.001^a	0.041±0.001^a	0.042±0.001^a

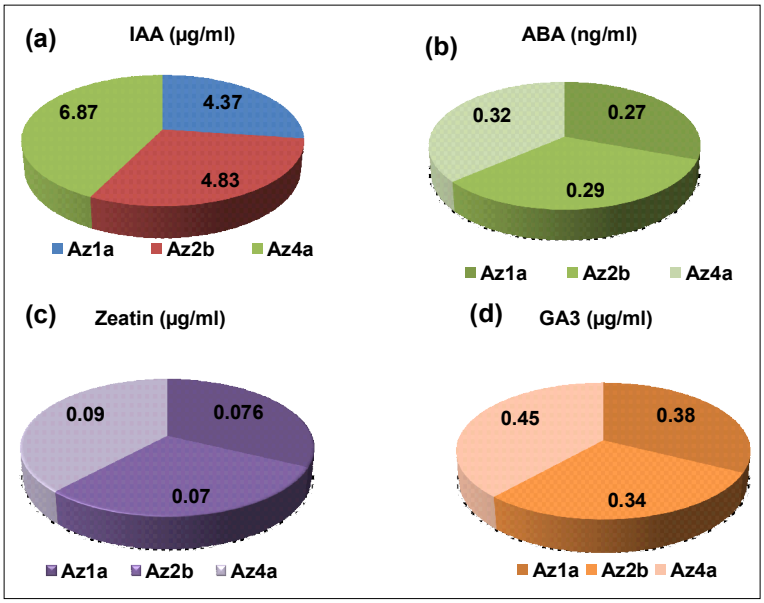

Fig 2: Endogenous hormone content of rice plants (C, T1, T2, T3) inoculated with Azotobacter vinellandii under 200 mM NaCl stress. (a) Endogenous content of IAA; (b) Content of ABA; (c) Content of zeatin; (d) Content of GA3.

The gibberellic acids (GA3) mitigate plant from the negative effects of salinity (Qin *et al.*, 2011). The stress-induced production of cytokinin in plants confers tolerance to transgenic plants to stress (Ha *et al.*, 2012). In the present study, we reported higher GA3, zeatin and IAA in rice plants inoculated with *A. vinellandii SinAz1*. It has been reported that the root and shoot biomass was increased with improved tolerance to salinity in the presence of growth promoting microorganisms (Fan *et al.*, 2011). Our study agree with the similar report on *Azotobacter* inoculation on chickpea (*Cicer arietinum* L.). *Azotobacter* inoculation increases the growth and yield of chickpea under saline (5.8 dS m^{-1}) arid condition (Abdiev *et al.*, 2019).

CONCLUSION

Thus, the conformity of the phenotypic and genetic pattern profiles confirmed the identity of the re-isolates as introduced *Azotobacter vinelandii SINAz1*. The results also proved that the introduced *Azotobacter* SINAz1 established in the experimental pots under salinity conditions and substantially survived up to harvest of the crop with improved yield compared to uninoculated plants.

Conflict of interest: None.

REFERENCES

Abdiev, A., Khaitov, B., Toderich, K., Park, K.W. (2019). Growth, nutrient uptake and yield parameters of chickpea (*Cicer arietinum* L.) enhance by *Rhizobium* and *Azotobacter* inoculations in saline soil. Journal of Plant Nutrition. 42: 2703-2714.

Barua, S., Tripathi, S., Chakraborty, A., Ghosh, S., Chakrabarti, K. (2012). Characterization and crop production efficiency of diazotrophic bacterial isolates from coastal saline soils. Microbiological Research. 167: 95-102.

Bhattacharjee, R.B., Singh, A., Mukhopadhyay, S.N. (2008). Use of nitrogen-fixing bacteria as biofertilizer for non-legumes: Prospects and challenges. Applied Microbiology and Biotechnology. 80: 199-209.

Collee, J.G., Miles, P.S. (1989). Tests for Identification of Bacteria. In: Practical Medical Microbiology, [(Eds). Collee, J.G., Duguid, J.P., Fraser, A.G., Marmion, B.P.]. Churchil Livingstone, NY, USA, pp. 141-160.

Fan, L., Dalpé, Y., Fang, C., Dubé, C., Khanizadeh, S. (2011). Influence of arbuscular mycorrhizae on biomass and root morphology of selected strawberry cultivars under salinity stress. Botany. 89: 397-403.

Godfray, H.C.J., Beddington, J.R., Crute, I.R. (2010). Food security: The challenge of feeding 9 billion people. Science. 327: 812-818.

Ha, S., Vankova, R., Yamaguchi-Shinozaki, K., Shinozaki, K., Tran, L.S. (2012). Cytokinins: metabolism and function in plant adaptation to environmental stresses. Trends in Plant Science. 17: 172-179.

Hardy, R.W.F., Holsten, R.D., Jackson, E.K., Burns, R.C. (1968). The acetylene-ethylene assayf or N2 fixation: Laboratory and field evaluation. Plant Physiology. 43: 1185-1207.

Hill, S., Sawers, G. (2000) Azotobacter. In: Enclyopedia of Microbiology, Vol. 1A-C, ed. Lederberg J, Acad Press, NY, USA. pp. 359- 371.

Huang, M., Fan, L., Chen, J., Jiang, L., Zou, Y. (2018). Continuous applications of biochar to rice: Effects on nitrogen uptake and utilization. Scientific Reports. 8: 11461.

Jensen, H.L. (1954). The Azotobacteriaceae. Bacteriol Rev. 18: 195-214.

Kenmore, P. (2003). Sustainable Rice Production, Food Security and Enhanced Livelihoods. In: Rice Science: Innovations and Impact for Livelihood, Beijing, China, pp. 27-34.

Kennedy, C., Rudnick, P., Mac Donald, M.L., Melton, T. (2005). Genus III. Azotobacter. In: Bergey's Manual of Systematic Bacteriology, Vol 2B, eds Brenner DJ, Krieg NR, Staley JT. Springer, New York, USA. pp. 384-402.

Peleg, Z., Blumwald, E. (2011). Hormone balance and abiotic stress tolerance in crop plants. Current Opinion Plant Biology. 14: 290-295.

Qin, F., Kodaira, K.S., Maruyama, K., Mizoi, J., Tran, L.S., Fujita, Y., Morimoto, K., Shinozaki, K., Yamaguchi-Shinozaki, K. (2011). SPINDLY: A negative regulator of gibberellic acid signaling, is involved in the plant abiotic stress response. Plant Physiology. 157: 1900-1913.

Saharan, B.S., Nehra, V. (2011). Plant growth promoting rhizobacteria: A critical review. Life Sciences and Medical Research. 21: 1-30.

Sahoo, R.K., Ansari, M.W., Dangar, T.K., Mohanty, S., Tuteja, N. (2014). Phenotypic and molecular characterisation of efficient nitrogen-fixing Azotobacter strains from rice fields for crop improvement. Protoplasma. 251: 511-523.

Sahoo, R.K., Bhardwaj, D., Tuteja, N. (2012). Biofertilizers: A Sustainable Eco-Friendly Agricultural Approach to Crop Improvement. In: Plant Acclimation to Environmental Stress. Edited by Tuteja N, Gill SS. LLC 233 Spring Street, New York, 10013, USA: Springer Science plus Business Media; pp. 403-432.

Subba Rao, N.S. (2007). Soil Microorganisms and Plant Growth. Oxford IBH Publication, New Delhi.

Tilman, D., Balzer, C., Hill, J. (2011). Global Food Demand and The Sustainable Intensification of Agriculture. Proceedings of the National Academy of Sciences of the United States of America. 108: 20260-20264.