International Journal of Microbiology Research
ISSN: 0975-5276 & E-ISSN: 0975-9174, Volume 11, Issue 5, 2019, pp.-1580-1584.
Available online at https://www.bioinfopublication.org/jouarchive.php?opt=&jouid=BPJ0000234

Research Article

ASSESSMENT OF ENDOPHYTIC GUARD CELL BACTERIAL STRAINS *KLEBSEILLA OXYTOCA* AND *ACINETOBACTER* SP. OF RICE FOR ABIOTIC STRESS TOLERANCE

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Received: May 02, 2019; Revised: May 24, 2019; Accepted: May 26, 2019; Published: May 30, 2019

Abstract- The endophytic bacterial communities have intense importance in agriculture due to their widespread benefits to plants and the ability to ameliorate biotic and abiotic stresses. In this present study, two of the endophytic bacterial strains *Klebseilla oxytoca* and *Acinetobacter* sp. isolated from the guard cell protoplast of rice genotype in the previous study were tested for the ability to grow under moisture stress of about -10 bars (-1.0 Mpa) induced with 30% polyethylene glycol 6000. Similarly, these strains were tested for the intrinsic antibiotic resistance with the antibiotics *viz*., streptomycin, ampicillin, kanamycin, tetracycline and chloramphenicol at varied concentrations. Also, these strains were analyzed for growth under different cultural parameters such as pH (4.5-8.5), temperature (20°, 30° and 40°C) and NaCl (2%, 5% and 10%) concentrations and the strains *Klebseilla oxytoca* and *Acinetobacter* sp. showed maximum cell density in the pH regime of 5..5 to 8.5, temperature of 30°and 40°C and the NaCl concentrations of 2% and 5% respectively. Correspondingly, these strains were able to solubilize the plant required minerals such as phosphate and zinc. The bacterial strain *Acinetobacter* sp. showed the maximum solubilization index. Approaching to the plant growth promoting activities, these strains produced maximum amount of Indole Acetic Acid (IAA), ACC deaminase activity and Gibberllic acid(GA3) production under induced moisture stress condition. Hence, this study confirms the plant growth and stress ameliorating activities of the selected endophytic bacterial strains and further gene regulations and stress alleviating metabolomic must be analyzed to understand the complete mechanism of these strains under stress environments. percent.

Keywords- *IAA, ACC deaminase, moisture stress, Antibiotic, Polyethylene glycol, solubilization*

Citation: Vibitha Bala B., *et al*., (2019) Assessment of Endophytic Guard Cell Bacterial Strains *Klebseilla oxytoca* and *Acinetobacter* sp. of Rice for Abiotic Stress Tolerance. International Journal of Microbiology Research, ISSN: 0975-5276 & E-ISSN: 0975-9174, Volume 11, Issue 5, pp.-1580-1584.

Introduction

The term endophyte was defined as the organisms that inhabits within the plant system, may be intercellular or intracellularly and confers many vital effects such as plant growth promotion, elicits defense responses against pathogen attack and helps in ameliorating the abiotic stresses. There are many abiotic stresses prevailing in the agro ecosystem such as drought, high temperature, salinity, heavy metal toxicity and oxidative stresses [1, 2]. These bacterial endophytes cope up with abiotic stresses mainly by the production of plant growth hormones, reactive oxygen species (ROS), inducing and expression of the stress responsive gene and by the production of antistress metabolites [3]. The endophytic bacterial communities mainly enhance the plant growth by number of mechanisms such as mineral solubilization activities [4], indole acetic acid production [5] and production of Siderophore [6]. Remarkably, this endophytic organism produces number of secondary metabolites and antibiotics, anti-inflammatory, antimicrobial compounds and other factors such as biological control agents [7]. Hence, this study aims in investigating the bacterial strains *Klebseilla oxytoca* and Acinetobacter sp. for intrinsic antibiotic resistance, mineral solubilization and growth with different cultural parameters such as induced water stress, extreme temperatures, pH and different strengths of NaCl. Also these bacterial strains were screened for the plant growth promoting activities such as Indole acetic acid production, ACC deaminase activity and phytohormones GA3 production under induced moisture stress condition.

Materials and Methods

Bacterial strains

The strains used in this study were the guard cell bacterial cultures isolated from the rice genotypes CO51 and IR64 (DRT). They were identified as *Klebseilla oxytoca* and Acinetobacter sp.

Antibiotic assay

The intrinsic antibiotic resistance of the two bacterial strains was done by inoculating the strains in Luria Bertani (LB) broth for 24 h at 34°C. After the incubation period, 1.5 ml of culture was taken in microfuge tubes and then it was centrifuged at 10,000x g for 10 min. Then the cell pellet was washed thrice with one percent saline solution and also suspended using 1ml of that same solution. Appropriate media was prepared and the antibiotic resistance was measured using disc diffusion method proposed by Gaudreau [8]. The antibiotics used in this assay were streptomycin, ampicillin, kanamycin, tetracycline and chloramphenicol at different concentrations *viz*., 10, 50, 100 and 200 ppm.

Growth curve of bacterial strains under induced water stress

The tryptic soy broth (TSB) was prepared with the addition of 30% polyethylene glycol 6000[9]. To this broth, 1% of exponentially grown bacterial cultures were added and incubated at 28°C. After the incubation period, the cell growth was measured at different time intervals by measuring the optical density at 600 nm using a spectrophotometer (M/s, Cary 50 Bio, varian).

Effect of different temperatures, pH and NaCl concentrations on growth of bacterial strains

The growth of the bacterial strains under different temperatures, pH and NaCl concentrations were studied by inoculating the bacterial strains in Tryptic soy broth (TSB).

Table-1 *Intrinsic antibiotic resistance of bacterial strains at different concentrations of antibiotics*

Bacterial strains	Streptomycin (ppm)				Ampicillin (ppm)				Kanamycin (ppm)				Tetracycline (ppm)				Chloramphenicol (ppm)			
	10	50	100	200	10	50	100	200	10	50	100	200	10	50	100	200	10	50	100	200
Klebseilla oxytoca	+	+	+	-	+	+	-	-	+	+	-	-	+	+	+	-	+	-	-	-
Acinetobacter sp.	+	+	+	+	+	-	-	-	+	+	-	-	+	-	-	-	+	+	-	-

++ Highly resistant; + Moderately resistant;- - Low resistance

Table-2 *Plant growth promoting activities of bacterial isolates under induced moisture stress condition*

Bacterial strains	Indole Acetic acid production µg ml^{-1} (without Tryptophan)	ACC deaminase activity Nano moles of α- ketobutyrate produced mg^{-1} hr^{-1}	Gibberllic acid production µg of GA3 produced ml^{-1}
Klebseilla oxytoca	27.7±0.06	279.44±5.09	3981.4±35.2
Acinetobacter sp.	39.5±0.14	291.8±3.34	4720.3±88.4
CD	1.0775**	8.1823**	141.4533**

*Means within each columns followed by the same letters are not significantly different at (p=0.05), and the numbers are mean of three replications. *Significant ** Highly Significant*

Table-3 *Zinc solubilization assay of bacterial strains Klebseilla oxytoca and Acinetobacter sp.*

Bacterial strains	Average of D (cm)	Average of d (cm)	Solubilization index	Solubilization efficiency (%)
Klebseilla oxytoca	1.20	1.70	0.70	70.580±1.29
Acinetobacter sp.	1.55	0.90	1.72	172.22±1.43
CD(0.05) = 13.02**				

*D- Diameter of the Halo-zone (cm) d- Diameter of the growth of the colony (cm)Means within each column followed by the same letters are not significantly different at (p=0.05). And the numbers are mean of three replications. *Significant ** Highly Significant*

Table-4 *Phosphate solubilization assay of bacterial strains Klebseilla oxytoca and Acinetobacter sp.*

Bacterial strains	Colony growth (cm)	Clearing Zone (cm)	Solubilizing efficiency %
Klebseilla oxytoca	1.34	2.11	111.00 ± 0.74
Acinetobacter sp.	1.69	2.25	125.00 ± 2.67
CD(0.05) = 9.11**			

*Means within each column followed by the same letters are not significantly different at (p=0.05). And the numbers are mean of three replications. *Significant ** Highly Significant*

The intrinsic salinity resistance was studied by observing the growth in TSB (2%, 5% and 10% w/v) of NaCl and incubating at 28°C for 24 hr. Similarly, to study the effect of pH on growth of bacterial strains, they were incubated in TSB with different pH (4.5, 5.5, 6.5, 7.5 and 8.5) and the growth curve was observed by measuring the optical density at 660 nm. And to study the temperature tolerance of the bacterial strains, they were inoculated in the TSB and incubated at different temperature regime (20°C, 30°C and 40°C) and the optical density was measured at 600 nm [10].

Plant Growth promoting activities of bacterial strains under induced water stress condition

Screening for IAA production

The indole acetic acid production by the bacterial isolates was estimated by inoculating the cultures in 50 ml of Tryptic Soy Broth (TSB) induced with moisture stress by the addition of 30% Polyethylene glycol (PEG 6000) and incubated in dark at 28±2°C for 4 days at 180 rpm. After the incubation, the IAA produced by the culture supernatant was estimated by adding 4 ml of Salkowski's reagent in 1 ml of supernatant, incubated in dark for 30 mins and the absorbance was read at 535 nm using UV/ visible spectrophotometer. IAA production was calculated using the standard curve and the result was expressed as µg ml^{-1}[11].

Screening for ACC deaminase activity

The quantitative estimation of ACC deaminase activity was done by inoculating the cultures in 5 ml of Luria Bertani (LB) along with 30% of PEG 6000 to induce moisture stress condition. The ACC deaminase was quantified according to the protocol of Dworkin and Foster, [12]. The absorbance was measured at 540 nm and the quantity of ACC deaminase activity was expressed as nano moles of α-ketobutyrate produced mg^{-1} hr^{-1}.

Screening for production of phytohormones Gibberllic acid (GA3)

The phytohormones Gibberllic acid produced by the bacterial cultures was quantified using by growing the cultures in Luria Bertani (LB) broth) along with 30% of PEG 6000 to induce moisture stress condition. After then, the cultures in broth were centrifuged and the resultant supernatant containing GA3 was extracted using ethyl acetate and the amount of GA3 produced was quantified following the method of Mahadevan and Sridhar, [13]. The absorbance was measured at 254 nm and expresses in µg ml^{-1}.

Mineral solubilization assay

The mineral phosphate solubilization assay was carried out by spot inoculating 10µl of the bacterial strains in Pikovskaya (PVK) medium that contains tricalcium phosphate as the sole source of phosphate and the cultures were incubated at 28°C for 5-6 days. After the incubation period, the formation of halo zones around the colonies shows the positive solubilization result [14]. Zinc solubilization assay was carried out by spot inoculating 10µl of the bacterial cultures in Bunt and Rovira medium which contains 0.1% of insoluble zinc source (Zinc oxide, Zinc sulphate or Zinc carbonate) and the plates were incubated at 28°C for 5-6 days. After the incubation period, the plates were observed for the formation of halo zone around the colonies [15].

Statistical analysis

All the results presented in the current study were the means of the three replicate values. For all the data's, the mean values were analyzed using the analysis of variance and Duncan's Multiple Range Test (DMRT) at the level of significance of 5% using SPSS software

Results

Intrinsic antibiotic resistance of bacterial strains

The endophytic bacterial strains *Klebseilla oxytoca* and *Acinetobacter* sp. were tested for the intrinsic antibiotic resistance against the antibiotics *viz*., streptomycin, ampicillin, kanamycin, tetracycline and chloramphenicol. In this study, both of the bacterial strains showed maximum resistance upto 50 ppm of all the antibiotics tested and the results were depicted in [Table-1]. Among the two strains, the *Acinetobacter* sp. showed resistance to the antibiotic streptomycin upto 200 ppm of concentration.

Growth curve of bacterial strains under induced moisture stress condition

The bacterial strains *Klebseilla oxytoca* and *Acinetobacter* sp. were screened for the drought tolerance using polyethylene glycol (PEG 6,000). The results showed that the growth of *Klebseilla oxytoca* showed minimum cell density upon the

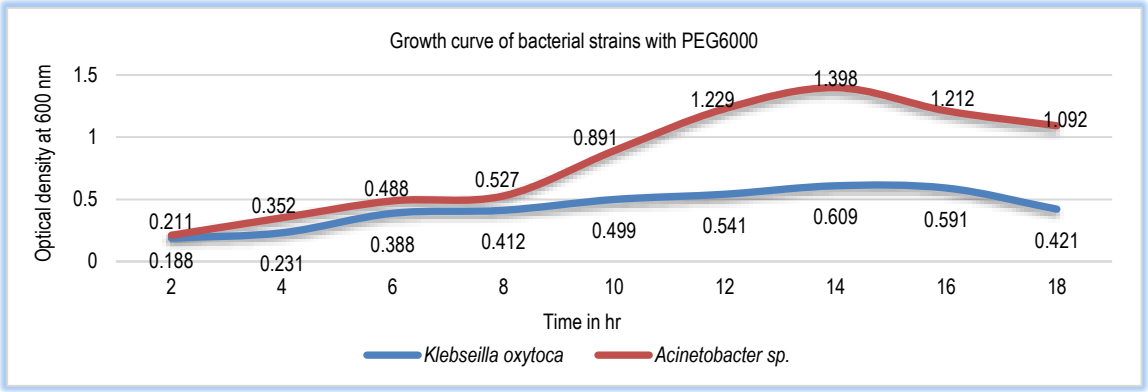

Fig-1 Growth curve of *Klebseilla oxytoca* and *Acinetobacter* sp. under induced moisture stress condition

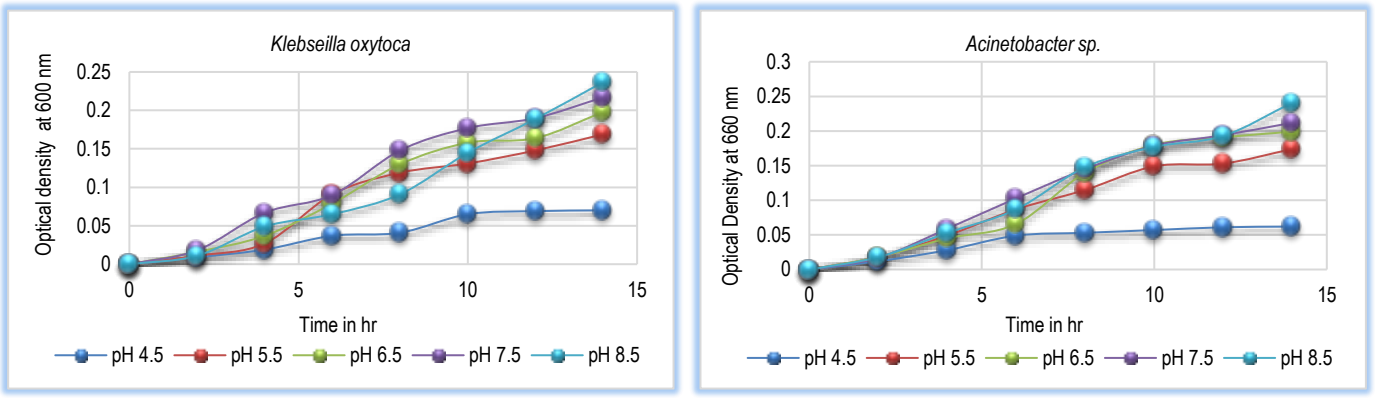

Fig-2a and 2b Growth of *Klebseilla oxytoca* and *Acinetobacter* sp. under different pH regimes

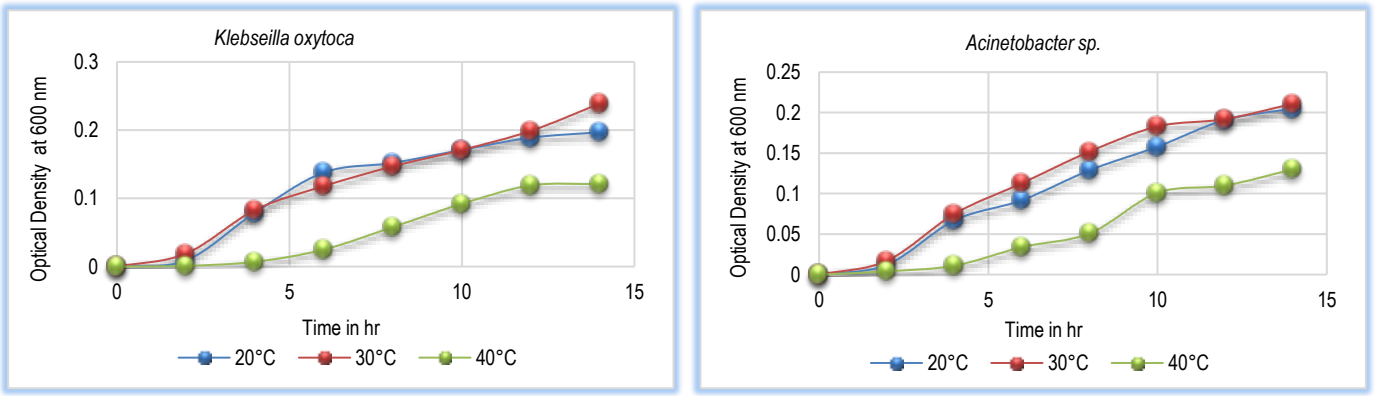

Fig-3a and 3b Growth of *Klebseilla oxytoca* and *Acinetobacter* sp. under different temperature regimes

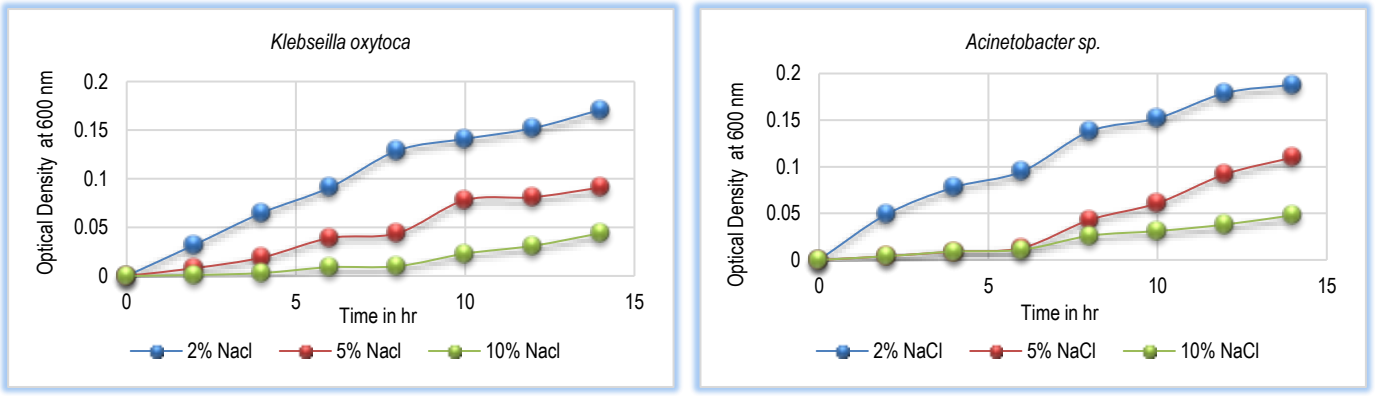

Fig-4a and 4b Growth of *Klebseilla oxytoca* and *Acinetobacter* sp. with different concentrations of NaCl

induction of matric stress [Fig-1]. But *Acinetobacter* sp. showed maximum cell density even at the higher osmotic potential of about (-10 bars).

Growth of bacterial strains under different pH, temperatures and NaCl concentrations

The bacterial strain *Klebseilla oxytoca* showed higher growth rate in the various pH regime of 5.5- 8.5. But the same culture showed lesser cell density in acidic pH of 4.5. Similarly *Acinetobacter* sp. showed increased cell density in the pH regimes of 5.5-8.5 and however the growth was reduced in the acidic pH of about 4.5. The results were depicted in the Fig-2 [Fig-2a and Fig-2b]. *Klebseilla oxytoca* and *Acinetobacter* sp. showed higher growth in various temperatures of 20°C and 30°C, but the cell growth was slightly reduced in the higher temperature of 40°C

[Fig-3a and Fig-3b]. Both of the bacterial strains showed higher growth upon the induction of salt stress of 2% NaCl concentration. However, the cell density gets decreased with the supplementation of 5% and 10% NaCl concentration. The results were illustrated in the Fig-4 [Fig-4a and Fig-4b].

Estimation of IAA, ACC deaminase activity and GA3 production under induced water stress condition

The bacterial strains were tested for the production of indole acetic acid without the amendment of tryptophan, *Klebseilla oxytoca* produced 27.7µg ml^{-1} and *Acinetobacter* sp. produced 39.5 µg ml^{-1} and the results were presented in the [Table-2]. And coming to the ACC deaminase activity, *Klebseilla oxytoca* produced 279.44 nano moles of α- ketobutyrate produced mg^{-1} hr^{-1} and *Acinetobacter* sp. recorded the higher amount compared to *Klebseilla oxytoca* of about 291.8nano moles of α- ketobutyrate produced mg^{-1} hr^{-1} respectively. Similarly, both strains produced maximum amount of phytohormones GA3 of about 3981.4 and 4720.3µg ml^{-1} respectively, even upon the induction of moisture stress of -10 bars of osmotic potential [Table-2].

Phosphate and Zinc solubilization assay

Among the two bacterial strains, *Acinetobacter* sp. showed the maximum solubilization efficiency in both zinc and phosphate solubilization assay of about 172.22 and 125% respectively and *Klebseilla oxytoca* showed solubilization efficiency of about 70.58 and 111.00% respectively. The results are presented in the [Table-3 and 4].

Discussion

Nowadays curiosity is growing on studying the endophytic microbial communities in plants due to their beneficial effects of enhancing the plant growth and development by the production of active secondary metabolites, phytohormones such as Gibberllic acid, Abscisic acid etc., production of reactive oxygen species and compatible solutes [16]. This has been exemplified in our study that both of the endophytic bacterial strains showed the production of higher amount of phytohormones GA3 which can be further explored for betterment of the growth of agriculturally important crops. In plants, most of the metabolic activities are regulated by the level of ethylene and the biosynthesis of ethylene in plants was synchronized by the biotic and abiotic stresses. Under the stress conditions, the plant hormone ethylene will endogenously regulates the homeostasis in plants that results in retarded plant root and shoot growth [17]. This plant produced ACC could be easily sequestered and degraded by the ACC deaminase producing endophytic and rhizobacteria for the supply of nitrogen and energy. Besides, this can reduce the detrimental effects of ethylene and helps in ameliorating plant stress and plant growth promotion [18]. In our present study, both of the endophytic bacterial strains produced maximum amount of ACC deaminase activity under the induced moisture stress condition. Hence these strains could be exploited for crop growth under biotic and abiotic stresses. Moreover, the physiologically most active auxin in plant growth and development is Indole Acetic Acid (IAA). This study shows that these bacterial strains are capable of producing maximum amount of active IAA that will helps in surviving in the stressful environments. The endophytic bacterial strains were capable growing under drought stress conditions due to the production of Exopolysaccharides (EPS) that leads the bacteria to grow under low osmotic conditions as the response of the matric stress [19]. Here in this study, the selected bacterial strains were capable of growing under the induced moisture stress of about 30% (-10 bars/ -1.0 Mpa).Similarly, the stress tolerant endophytic bacterial strains will have the ability to grow under varying regimes of cultural parameters such as pH, temperature and NaCl concentrations as their stress adaptive mechanism [20]. Similarly the endophytic bacterial strains used in this study have the ability to grow under the acidic to alkaline pH range, tolerating higher temperature of about 40ºC and salinity tolerance of about 5% NaCl concentration. An essential requirement for the plant growth and development is the availability of minerals that is made available to the plants by endophytic bacterial communities that has the capacity to solubilize the insoluble forms of minerals such as phosphorous, potassium, zinc *etc*., mainly by the mechanism of alteration of pH and production of organic acids and made it accessible to the plants [21]. The above-mentioned key features endorsed to the endophytic bacterial strains *Klebseilla oxytoca* and *Acinetobacter* sp. made them inimitable from other groups of endophytic bacterial communities and gained interest on further research on drought tolerance mechanisms exhibited by these strains and gene regulations needs to be identified in future works.

Application of research: Studies related to assessment of abiotic stress tolerance of endophytic bacterial cultures.

Research Category: Agricultural Microbiology

Acknowledgement / Funding: Authors are thankful to Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore, 641003, India.

***Research Guide or Chairperson of research: Dr N. O. Gopal**
University: Tamil Nadu Agricultural University, Coimbatore, 641003, Tamil Nadu
Research project name or number: PhD Thesis

Author Contributions: All author equally contributed

Author statement: All authors read, reviewed, agreed and approved the final manuscript. Note-All authors agreed that- Written informed consent was obtained from all participants prior to publish / enrolment

Study area / Sample Collection: Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore, 641003, Tamil Nadu

Cultivar / Variety name: Rice cultivars Co51 and IR64 (DRT)

Conflict of Interest: None declared

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.
Ethical Committee Approval Number: Nil

References

[1] De Bary A. (1866) *Handbook of Physiological Botany. Leipzig:Wilhelm Engelmann*.
[2] Khare E.K. and Arora N.K. (2015) *Applied Facets, ed. N. K. Arora (Netherland: Springer)*, 353-381.
[3] Lata R., Chowdhury S., Gond S.K. and White J.F.Jr. (2018) *Lett. Appl. Microbiol*., 66, 268-276.
[4] Wakelin S., Warren R., Harvey P. and Ryder M. (2004) *Bio Fert Soils,* 40, 36-43.
[5] Lee S., Flores-Encarnacion M., Contreras-Zentella M., Garcia- Flores L., Escamilla J.E. and Kennedy C. (2004) *J. Bacteriol*., 186, 5384-5391.
[6] Costa J.M. and Loper J.E. (1994) *Mol Plant Microbe Interact.,* 7, 440-448.
[7] Shweta S., Hima Bindu J., Raghu J. (2013) *Phytomedicine*, 20(3), 913-917.
[8] Gaudreau C., *et al*. (2008) *Antimicrob. Agents Chemother*, 52, 4475-4477.
[9] Michel B.E., Kaufmann M.R. (1973) *Plant Physiol*., 51, 914-916.
[10] Jida M. and Assefa F. (2011) *African Journal of Microbiology Research, 5,* 4133-4142.
[11] Gordon S.A. and Weber R.P. (1951) *Plant Physiol*., 26,192-5.
[12] Dworken M. and Foster J. (1958) *Journal of Bacteriology,* 75, 592-601.
[13] Mahadevan A. and Sridhar R. (1982) *In: Methods in Physiological Plant Pathology, 2nd edition, Sivakami Publishers, Madras,* 24-44.
[14] Gaur A.C. (1990)*Phosphate Solubilizing Micro-Organisms as Biofertilizers, ed. A.C. Gaur, Omega Scientific Publisher*, 16-72.

[15] Saravanan V.S., Kalaiarasan P., Madhaiyan M. and Thangaraju M. (2007) *Letters in Applied Microbiology*, 44, 235-241.
[16] Jasim B., Jimtha C.J., Jyothis M., *et al*. (2013) *Plant Growth Reg*.,71,1-11.
[17] Hardoim P.R., Van Overbeek L.S. and Van Elsas J.D. (2008) *Trends Microbiol.,* 16, 463-471.
[18] Glick B.R. (2005) *FEMS Microbiol. Lett*., 251,1-7.
[19] Roberson E.B. and Firestone M.K. (1992) *Appl. Environ. Microbiol*., 58, 1284-1291.
[20] Jiang L., Guli J., Bao A., Guo H. and Ndayisaba F. (2017) *Sci. Total Environ*., 59, 967-980.
[21] Kim K.Y., Jordan D. and McDonald G.A. (1998) *Soil Biol Biochem*., 30, 995-1003.