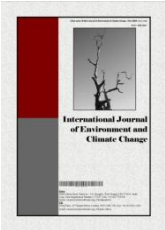

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Identification and *in vitro* Evaluation of Environmental Stress Resilient Plant Growth Promoting Rhizobacterial Consortia for Rice (*Oryza sativa* L.)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The present study screened bacteria isolated from rice rhizosphere for various beneficial traits related to three categories of plant growth promotion, abiotic stress tolerance, and compatibility with multiple agrichemicals to identify a microbial consortium that performs better than individual isolates in improving rice growth under normal and stressful environmental conditions. Among thirty-two isolates screened, 29, 9, 20 isolates were able to solublilize insoluble minerals of nutrients like phosphorous, potassium and zinc respectively. About 29, 31, 23, 17 isolates were found to possess the traits of IAA, ammonia, siderophore and HCN production respectively. Abiotic stress related

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traits of salinity, drought and high temperature tolerance were displayed by 8, 2 and 10 isolates respectively when examined for their growth in NaCl (4%, 6% 8%), in PEG (64 g/l equivalent to -0.73Mpa) and at 45℃ temperature. Agrichemical tolerant isolates were identified by screening for compatibility with fertilizers and commonly used rice pesticides. Based on the scores assigned during screening tests, 9 best isolates, three from each category, were selected for development of consortia. Our aim in this initial study was to identify three consortia (C-1, 2 and 3) consisting of three compatible bacteria, and to evaluate their effect on rice seed germination and seedling growth under normal *in vitro* conditions. C1 enhanced the growth of rice when compared with individual strains, C2 and C3 with highest vigour index I, II values of 1616 ± 18 and 5.47 ± 0.68 respectively. The 16S rRNA sequence analysis identified the bacterial isolates in C1 as *Achromobacter sp*, *Stenotrophomonas sp and Pseudomonas stutzeri*. Therefore, this study has identified a unique consortium that can be used for rice growth promotion while simultaneously demonstrating the advantage of bacterial consortia over single isolate inoculants under non stressed conditions.

Keywords: Rhizobacteria; plant growth promotion; abiotic stress tolerance; agrichemical compatibility; consortia; rice.

1. INTRODUCTION

Plant Growth Promoting Rhizobacteria (PGPR) are a constituent group of beneficial bacteria which reside in the rhizosphere and benefits plants by producing a range of secondary metabolites, growth regulators, siderophores, and organic acids. They are also involved in nitrogen fixation and solubilization of insoluble sources of plant nutrients like phosphorus, potassium, zinc and silicates thereby improving the availability of these nutrients and assisting plants in absorbing nutrients from the soil [1]. These rhizospheric bacteria with multiple plant growth promoting traits are natural choice as bioinoculants to improve soil quality, crop health and productivity in an eco-friendly approach to crop production.

PGPR based technologies cannot totally substitute for fertilizers and other chemical inputs [2,3] and are hence applied in conjunction with agrichemicals like fertilizers and pesticides, consequently forming a vital component of integrated nutrient and pest management strategies [4,5]. During this scenario wherein PGPR are exposed to agrichemicals applied to soil or seed, it is important to study their effect on rhizobacteria and to ensure that there are no adverse effects that impair the beneficial traits of selected bacteria [6,7]. Therefore, compatibility studies between rhizobacterial inoculants and agrichemicals are of foremost importance while selecting PGPR that can act as bioinoculants. In addition, soil edaphic and environmental factors also affect the in-field efficacy of a bioinoculant [8]. Varying abiotic stresses like soil acidity, salinity and sodicity including high temperatures and water stress affect the establishment of PGPR, making the technology inefficient, unpredictable and unreliable under field conditions [9-13]. Therefore, to overcome these challenges, there is a need to study the survival traits of PGPR under drought, heat stress and salinity conditions encountered during interactions with soil [14].

PGPR bioinoculant technology has traditionally relied on the use of single strains of beneficial soil bacteria to improve soil fertility, soil quality and crop yield. However, when a single PGPR strain is introduced into the fields, inability to adapt to varying local conditions can result in the PGPR being incapacitated and unable to provide the desired benefits to the crop [15,16]. Under such conditions, inoculating plants with microbial consortia containing two or more beneficial microorganisms have been known to produce positive results [17,18] probably by providing better plant growth promotion, as the consortia may possess more beneficial traits than an individual microbial inoculum. Microbial consortia development for plant growth promotion is the new frontier in PGPR inoculant technology and unique indigenous consortia that are adapted to local soil and climatic conditions needs to be developed.

Rice is known to benefit from the inoculation with PGPR [6,19,20]. However, studies on the use of microbial consortia that work synergistically towards increasing crop productivity of non-legume crops are rare [21]. The purpose of this study was to identify a bacterial consortium possessing multiple plant growth promoting and abiotic stress tolerance traits including compatibility with frequently used rice agrichemicals that can benefit the rice crop not

only under normal conditions but also under various stresses of edaphic and climatic origin and to test the hypothesis that this microbial consortium would be superior than the individual isolates in improving germination and vigour index of rice.

2. MATERIALS AND METHODS

Thirty-two bacteria (IIRRSS22-1 to IIRRSS22 - 27, P1, P2, R1, O1, M1), isolated from rice rhizosphere and maintained at ICAR -Indian Institute of Rice Research, Hyderabad, Telangana were used for the study. Rhizobacterial isolates were assessed under three categories of i) plant growth promotion, ii) tolerance to abiotic stresses and iii) compatibility to agrichemicals and a trait profile of each isolate was created to enable selection of most competent isolates in each category.

2.1 Assessment of Plant Growth Promoting Characteristics of Rhizobacteria

The plant growth promoting traits of solubilization of insoluble phosphates, potassium and zinc were assessed using Pikovskaya agar [22], Aleksandrov agar [23] and tris minimal agar media [24]. The Solubilization Index (SI) [25] was calculated by using formula (halo zone + colony diameter/colony diameter) and scores were ascribed to the isolates based on the indices. The isolates with SI values between 0-1 were assigned a score of 0, while SI values of 1-2, 2-3 and above 3 were given scores of 1, 2 and 3 respectively for each mineral solubilization trait.

The isolates were also examined for other PGPR traits like production of Indole Acetic Acid (IAA), siderophore, ammonia and hydrogen cyanide (HCN). Culture supernatant of bacterial isolates grown at 28℃ for 7 days in nutrient broth amended with tryptophan was used to assay for the production of IAA using Salkowski reagent [26]. Based on intensity of pink colour which is directly correlated to IAA production, the isolates were grouped into 4 categories namely bacteria that do not produce IAA, and bacteria that show low, moderate and high IAA production and given scores as 0 - no production of IAA, 1- low production, 2- moderate production and 3-high production. Ammonia production by bacterial isolates was determined using the culture supernatant obtained after growth of the cultures in peptone water [27] for 4 days at 28℃. Nessler's reagent was used to assay for the presence of ammonia in the supernatant and the isolates where the supernatant color changed to dark brown color indicating high ammonia production were allotted a score of 3, while the isolates with supernatant exhibiting brown, yellow or no change in color were given scores of 2, 1 and 0 respectively. Siderophore production was assessed by inoculating the cultures on CAS agar media [28] and observing for formation of yellow to orange zone around the colonies. Siderophore production index (SPI) [29] was calculated and the isolates with SPI above 4 were ascribed a score of 3, while isolates with SPI of 0-2, 2-3 and 3-4 were assigned scores of 0, 1 and 2 respectively. HCN production was assayed by spreading the culture on nutrient agar media amended with 4.4g/l glycine [30] and incubating at 28 ℃ for 4 days. Picrate paper (filter paper saturated in reagent containing 0.5% picric acid in 1% sodium carbonate solution and dried) placed in the upper-lid of petriplate was used to monitor HCN production. The cultures were rated 0, 1, 2 and 3 based on the change in colour of filter paper [31] after incubation from yellow (no HCN production) to light brown, brown and reddish brown respectively.

2.2 Evaluation of Rhizobacterial Tolerance to Abiotic Stress

The isolates were also screened for tolerance to abiotic stresses like high temperature, salinity and drought. The bacterial isolates were streaked the on tryptone soy agar media [32] and incubated at 45℃ for 3 days and examined for temperature tolerance. The bacterial isolates that showed no growth at 45℃ were given a score of zero indicating susceptibility to heat stress while isolates with low, moderate and good growth were attributed with scores of 1, 2 and 3 respectively. Similarly, screening for salt tolerance was performed by observing the growth of isolates on tryptone soy agar media amended with 4, 6, 8% sodium chloride [33] and scores were assigned to the isolates based on growth characteristics. Tolerance to drought was assayed by growing the isolates at 28 ℃ for 24 hrs in tryptone soy broth amended with poly ethylene glycol to create a water potential of -0.73 MPa [34]. The growth of bacterial isolates was measured at 600nm by spectrophotometer and based on OD values, the isolates were classified as highly sensitive-OD >0.3, sensitive-OD 0.3-0.4, tolerant- OD 0.4-0.5, highly tolerant OD>0.5 with corresponding scores of 0, 1, 2 and 3 [35].

2.3 Screening Rhizobacteria for Compatibility with Agrichemicals

The compatibility of bacterial isolates against agrichemicals like fertilizers (urea, single super phosphate, muriate of potash), insecticides (Cartap, Ferterra, Thiamethaxom), herbicides (Pretilachlor, Bispyribac sodium) and fungicides (Carbendazim, Mancozeb) were evaluated with filter paper disc technique [36]. Filter paper discs wetted with the solutions of 1X, 2X, 3X of the recommended doses of agrichemicals were placed on a lawn of bacterial culture seeded on nutrient agar plates and formation of inhibition zone after incubation at 28 ℃ for 4-5 days is taken as evidence of incompatibility with the agrichemical. Isolates were adjudged to possess high compatibility with the agrichemical when no inhibition zones were observed around discs containing all the three doses (1X, 2X, 3X) of the agrichemical and were rated with a score of 3. Growth in the presence of 1X and 2X dose of agrichemicals resulted in a rating of 2 while the compatibility rating for growth in only 1X was 1. Isolates fell into the zero compatibility rating when susceptibility was exhibited at 1X dose of agrichemicals.

2.4 Selection Framework for Identification of Efficient Rhizobacteria

The rhizobacterial isolates were assessed for seven traits pertaining to plant growth promotion category, three traits related to tolerance to abiotic stresses and ten traits associated with the compatibility to agrichemicals and for every isolate, a bonitur scale [37] was generated based on the scores assigned for each trait. The highest composite score that can be obtained by an isolate was 21, 9 and 30 corresponding to categories related to plant growth promotion, abiotic stress tolerance and agrichemical resistance. Three isolates having the highest score in each category was selected for consortia formation.

2.5 Compatibility Studies Between Bacterial Isolates for Consortium Development

Based on the above screening tests the bacterial isolates were further selected for compatibility studies. For consortia formulation, the bacterial isolates were checked for compatibility with other isolates by cross streak method [38]. The plates were observed for inhibition zone formation. Development of inhibition zone indicates that the isolates are incompatible.

2.6 Molecular Identification of Bacteria Isolates

Genomic DNA was isolated from selected bacteria using Gsure bacterial RNA isolation kit (GCC Biotech, India) according to manufacturer's protocol. Gene amplification of 16S rRNA was carried out in BioRad T100 thermocycler (BioRad, USA) using 2 primers developed in-house for amplification of 16S rRNA. The primers used were i) 8F (5'-AGAGTTTGATCCTGGCTCAG-3') and 907R (5'-CCGTCAATTCCTTTRAGTTT-3') and ii) 785F (5'- GGATTAGATACCCTGGTA-3') and 1492R (CGGTTACCTTGTTACGACTT-3'). The PCR reaction mixture of 20 µl, which comprised of bacterial DNA- 2 µl, dNTPs- 1.6 µl, 10X buffer-2µl, taq polymerase- 0.2µl were subjected to an initial denaturation at 94℃ for 5 min, followed by 30 cycles of 94℃ for 30 sec, 54 ℃ for 30 sec, 72 ℃ for 1 min, and a final extension at 72 ℃ for 7 min for amplification of 16S rRNA genes. The amplified DNA after sequencing using sanger sequencing platform were aligned and the contigs created using Cap3 Bioedit Software were compared against the sequences of 16S rRNA of bacterial isolates available in the National Centre for Biotechnology Information (NCBI) Nucleotide Database (http://www.ncbi.nih.gov/blast) and the isolated bacteria were identified based on maximum percentage of similarity of the sequences.

2.7 Consortia Formulation and *In vitro* Germination Assay with Rice Seeds (RNR-15048)

Based on compatibility studies**,** 3 different consortia (containing 3 isolates, one each from each category related to plant growth promotion, abiotic stress tolerance and agrichemical resistance) were formulated by dissolving the pellet of bacteria in phosphate buffered saline and optical density of culture solution was measured colorimetrically at 600 nm. The surface sterilized seeds were soaked in the culture and its consortia (containing three organisms in equal proportions and having optical density-1 at 600 nm) for 24 hrs at 28 ℃. After incubation the culture was drained off and the seeds are placed on water agar plates for germination. The plates are incubated at 28℃ for 7 days. The parameters like germination percentage, root length, shoot length, fresh and dry weights were taken after 7 days.

3. RESULTS AND DISCUSSION

3.1 Characterizing Bacterial Isolates for Plant Growth Promoting Traits

Phosphorous in insoluble forms is unavailable to plants and many rhizobacteria are capable of solubilizing insoluble phosphates majorly by secreting organic acids [39]. In this study phosphate solubilizing bacteria were screened using Pikovskaya agar plate assay method (Fig. 2). Out of 32 isolates, 29 isolates showed solubilization activity (Fig. 1). The highest activity was shown by IIRRSS22-18 with Solubiliztion Index (SI) of 3.1 ± 0.05.

Potassium is one of the major macronutrients which has enough reserves in soil, but only 1-2 % of it is available for plants and 90-98% of soil K is fixed as silicate minerals [40]. The potassium solubilizing bacteria use mechanisms such as acidolysis, chelation, exchange reactions for solubilization of potassium [41]. In this study potassium solubilization (Fig. 2) potential of bacterial isolates was studied on Aleksandrov agar media. Among all the isolates, 9 isolates have shown potassium solubilizing activity (Fig. 1). Highest SI of 2.1 ± 0.06 was observed with P1.

Zinc is the essential micronutrient required by plants for plant growth. Zn when applied in field as zinc sulphate converts into insoluble ZnS within 7 days and completely becomes unavailable to plants [42]. The zinc solubilizing bacteria solubilize the unavailable form of zinc by secreting organic acids and also by chelating ligands [43]. Among 32 isolates screened for zinc solubilization, 20 isolates (Fig. 1) have shown solubilizing activity which is seen as halo zones around the colonies on tris minimal media (Fig. 2). Highest zinc solubilizing activity was observed in P1 with SI of 2.5 ± 0.07.

IAA is a major plant growth hormone and one of the potent signaling molecule, crucial for interaction between plants and microbes [44]. Plant roots exude tryptophan into rhizosphere which is utilized by bacteria as a precursor for IAA production [45]. Among 32 isolates, 29 isolates have shown IAA production in nutrient broth amended with tryptophan (Fig. 2). Of the 29 isolates, high production of IAA shown by 3 isolates, moderate by 7 isolates, less production by 19 isolates, no production by 3 isolates (Fig. 1).

Ammonia functions as a metabolic inhibitor affecting pathogen growth and fungal activity [46] and also supplies nitrogen directly to plants aiding plant growth promotion [47]. In present study, ammonia production was observed in 31 isolates as the cultures produced a yellow to brown color on the addition of Nessler's reagent (Fig. 2). About 6 isolates have high production of ammonia, 9 isolates produced ammonia in moderate quantity, 16 isolates produced ammonia in low quantity and 1 isolate tested negative for ammonia production (Fig. 1).

Siderophores are the biological molecules produced by the bacteria which have the ability to chelate Fe and provide it to plants [48], and slow down the growth of pathogens by limiting the iron available for pathogen [49]. In the present study, 23 isolates have produced siderophores, which is indicated by formation of orange zones around the colonies on CAS agar plates (Fig. 2). Highest siderophore producing activity was shown by IIRRSS22- 7 with SI of 4.3 ± 0.06 and least solubilization index was shown by IIRRSS22-8 with SI of 2.2 ± 0.52. A total of nine isolates have not produced iron chelating molecules (Fig. 1).

Hydrogen cyanide is a volatile secondary metabolite produced by rhizospheric bacteria and it plays an important role in biological control of pathogens [50]. In the present study, 17 isolates have produced HCN (Fig. 1) and were given score 1 because the change in colour of filter paper to reddish brown (Fig. 2) was minimal.

3.2 Screening for Abiotic Stress Tolerance

The bacterial isolates exhibiting various PGPR traits and perform effectively under *in vitro* studies, show differences in performance and inconsistencies when applied to crop under pot or field conditions. It could be due to the abiotic stresses such as high temperature, salinity stress and drought stress [51] endured by the PGPR under such conditions. The plant growth promoting rhizobacteria are directly involved in plant growth promotion and protects the plants from diseases and abiotic stress by production of regulatory chemicals in the vicinity of rhizosphere [52].

The results of salinity tolerance have shown that about 20, 15, 8 isolates have grown at 4%, 6%, 8% NaCl respectively (Fig. 3). A total of 8 isolates have grown at all NaCl concentrations. It

was observed that with the increase in the concentration of NaCl there was a gradual decrease in the growth of bacterial isolates (Fig. 4). And no growth was observed in 12 isolates at all salt concentrations.

A total of 32 bacterial isolates were tested for high temperature tolerance by subjecting them to grow at 45℃ (Fig. 4). It was observed that 12 isolates were able to grow at 45℃ (Fig. 3).

Out of 32 isolates screened for drought tolerance**,** 10 isolates were grouped as highly tolerant, 7 isolates were tolerant, 3 isolates were sensitive and 12 isolates were highly sensitive (Fig. 3). Rhizobacteria can withstand stress conditions by producing exopolysaccharides, formation of biofilm under drought stress [53]. Many PGPR have been reported to alleviate plants from stress conditions, promote plant growth, and mitigate harmful effects of abiotic stress [54]. Therefore, these highly tolerant strains can be used for plant growth promotion under environments where these stresses are encountered.

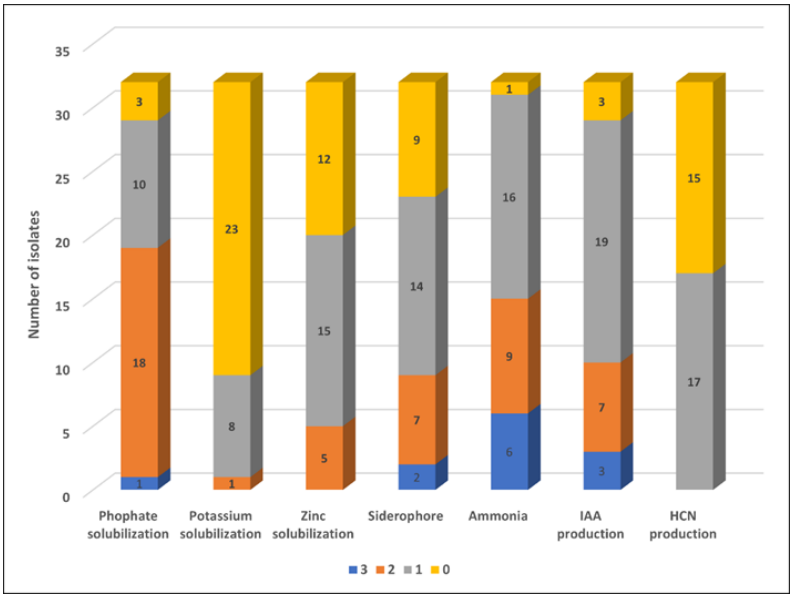

Fig. 1. Number of isolates exhibiting plant growth promoting (PGP) traits
Legend 3, 2 1 and 0 are scores indicating high, moderate, low and no PGP activity of isolates

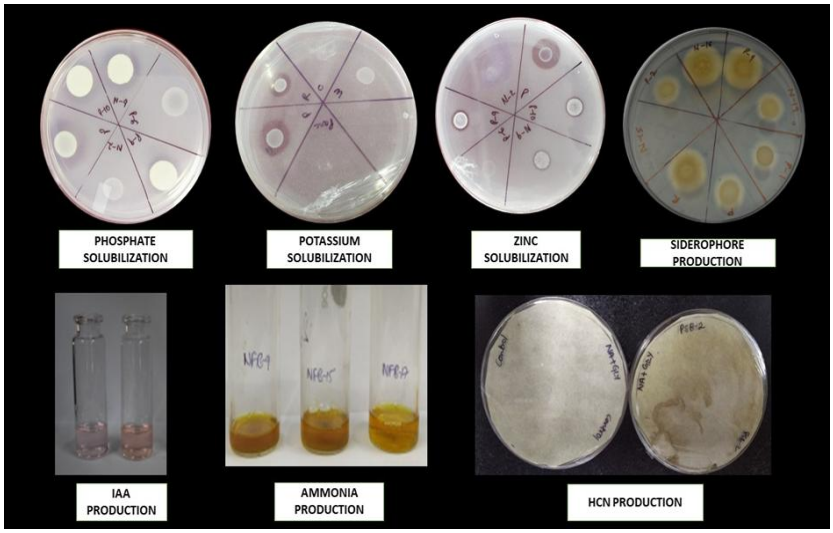

Fig. 2. Assessment of plant growth promoting traits of rhizobacteria

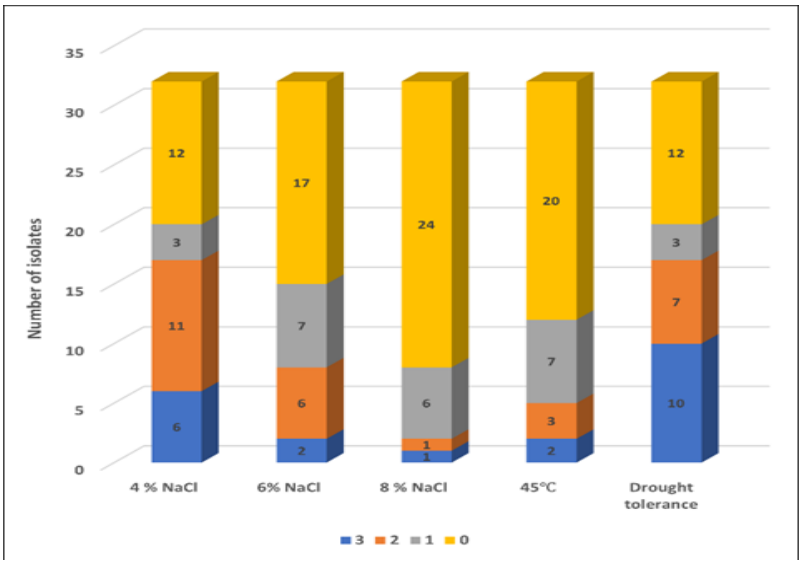

Fig. 3. Number of isolates exhibiting abiotic stress tolerance

Legend 3, 2 1 and 0 are scores indicating high, moderate, low and no tolerance of isolates to abiotic stresses

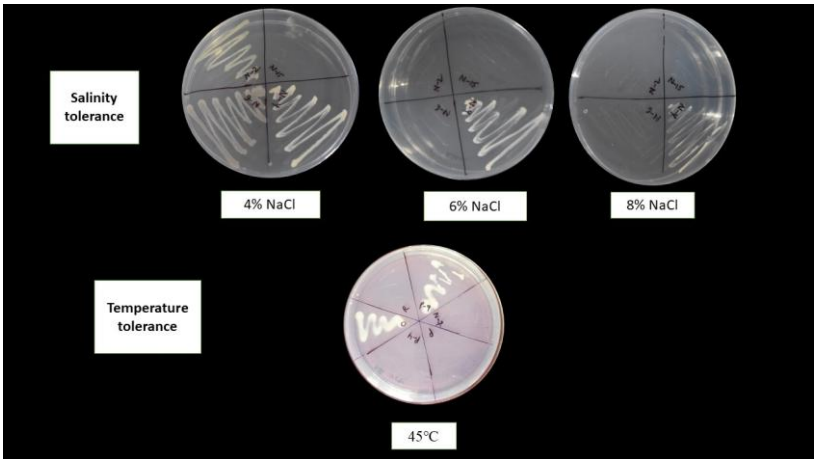

Fig. 4. Assessment of salinity and temperature tolerance of rhizobacteria

3.3 Compatibility of Bacterial Isolates with Agrichemicals

Use of agrichemicals has become an indispensable part of crop production as they are applied to soil, seeds or on plant as fertilizers and to control pests and diseases. Hence there is need to study the compatibility of microbial inoculants with agrichemicals [7]. In the present study the compatibility of bacterial isolates with different agrichemicals was studied using disc diffusion method.

In case of fertilizers like urea, 22 isolates have grown at all the concentrations used and were hence considered as highly tolerant. Eight isolates have exhibited inhibition of growth at all concentrations (Fig. 5). The results obtained with Single Super Phosphate (SSP) has shown that 19 isolates were highly tolerant to the SSP concentrations tested and 11 isolates were not tolerant as there was formation of inhibition around the disc (Fig. 5). Tolerance of bacterial isolates when tested with Muriate of Potash (MOP) has resulted in identifying 18 isolates showing high tolerance to all concentrations of MOP and 12 isolates displaying incompatibility with the agrichemical by developing inhibition zones (Fig. 5).

The isolates were tested for compatibility with common insecticides such as cartap, ferterra and thiamethaxom used in rice cultivation. The results of compatibility tests of isolates with

cartap hydrochloride revealed that 19 isolates were highly tolerant for the chemical at all concentration and about 8 isolates have shown inhibition of growth (Fig. 5). Compatibility of isolates tested with ferterra has shown that 13 isolates were highly tolerant and nine isolates were not tolerant to chemical as the inhibition of growth of bacterial isolate was observed around the disc (Fig. 5). With thiamethaxom, while 6 isolates have shown high tolerance to the chemical, 14 isolates were showed no tolerance indicating the incompatibility of isolates with agrichemicals (Fig. 5).

The results obtained with pretilachlor, a herbicide, revealed that 19 isolates were able to grow at all concentrations of herbicide tested and 9 isolates have shown inhibition of growth around the disc (Fig. 5). With herbicide bispyribac sodium, 17 isolates have shown high tolerance for herbicide and 9 isolates showed no tolerance at all herbicide concentrations (Fig. 5).

The fungicides such as carbendazim and mancozeb were also used in this study to evaluate the compatibility with bacterial isolates. The results with carbendazim have shown that 20 isolates were able to grow at all concentrations of chemical and 3 isolates have shown no growth near the disc, hence are not tolerant to fungicide (Fig. 5). Compatibility of isolates when tested with mancozeb revealed that 23 isolates were not tolerant and 3 isolates have shown moderate tolerance (Fig. 5).

Similar results of compatibility of bacterial isolates with different agrichemicals were reported by Verma et al. [36].

3.4 Scheme for Selection of Promising Isolates for Consortia Formulation

A bonitur scale was generated similar to that described by Hazarika et al. [55] and Krechel et al. [37] to select the best bacterial isolates for consortia formulation. PGP traits, abiotic stress tolerance and compatibility of isolates with agrichemicals were the three categories employed for preparing the scale and scoring of isolates, following which three isolates with the highest score in each category were selected. The nine selected isolates were P1, IIRRSS22-1, IIRRSS22-6, in the plant growth promotion category with scores of 11, 10 and 9; IIRRSS22-3, R1, IIRRSS22-7 all with a score of 7 in abiotic stress tolerance category and IIRRSS22-5, IIRRSS22-4, IIRRSS22-2, which showed highest compatibility with agrichemicals with scores of 28 and 27 respectively. The isolates were grouped into three consortia with each consortium consisting of one bacterial isolate from each category (Table 1).

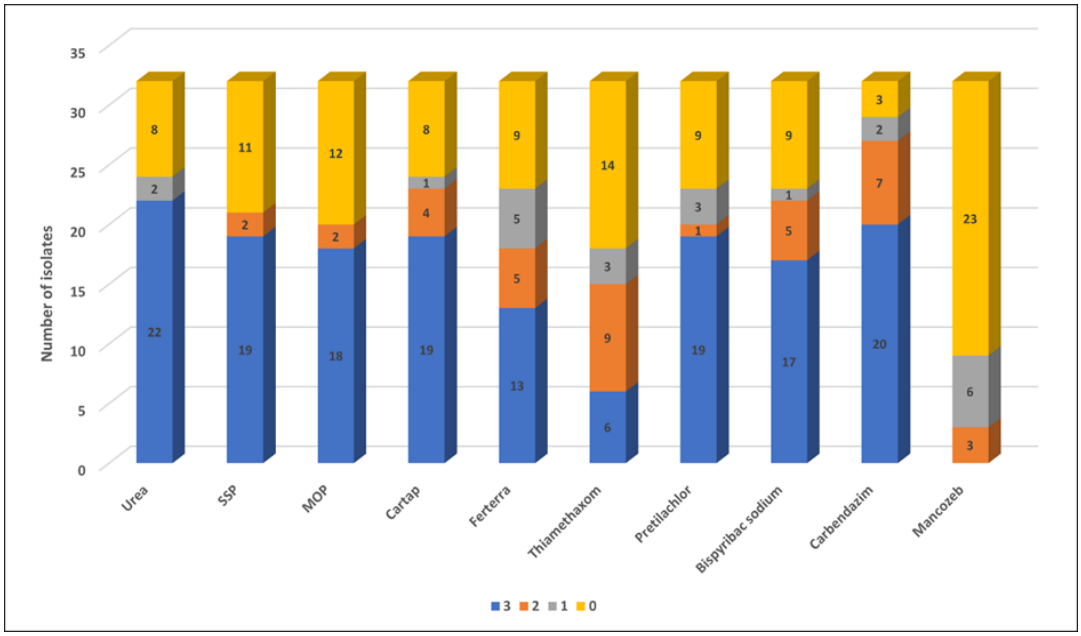

Fig. 5. Number of isolates exhibiting compatibility with agrichemicals

Legend 3, 2 1 and 0 are scores indicating high, moderate, low and no compatibility of isolates to agrichemicals

Table 1. Rhizobacterial partner composition of three consortia

Consortium group	Plant growth promoting traits	Abiotic stress tolerance	Compatibility with agrichemicals
C-1	P1 (11)	IIRRSS22-3 (7)	IIRRSS22-5 (28)
C-2	IIRRSS22-1 (10)	R1 (7)	IIRRSS22-2 (27)
C-3	IIRRSS22-6 (9)	IIRRSS22-7 (7)	IIRRSS22-4 (28)

Numbers in parenthesis indicate the score of the isolates on bonitur scale

3.5 Compatibility Studies Between Bacterial Isolates for Consortia Formulation

Based on the above screening tests, a total of 9 (IIRRSS22-1, IIRRSS22-2, IIRRSS22-3, IIRRSS22-4, IIRRSS22-5, IIRRSS22-6, IIRRSS22-7, R1 and P1) best performing bacterial isolates were selected and compatibility between these isolates were checked by cross streak method. Based on the growth of the isolates, it was observed that all the isolates were compatible with each other. Compatibility of microbes, when applied as consortia is a pre-requisite for the success of plant growth promotion in crops [38]. From the selected isolates, three consortium groups were formed in such a way that each group has 3 isolates, one each from each category. Three consortium groups used for the study were C1(IIRRSS22-5, IIRRSS 22-3, P1), C2(IIRRSS22-1, R1, IIRRSS22-2), C3(IIRRSS22-6, IIRRSS22-7, IIRRSS22-4).

3.6 *In vitro* Germination Assays

The nine promising isolates selected based on screening tests were evaluated under *in vitro* conditions on growth of rice (RNR-15048), singly and as consortia. The consortia for treating rice seeds were prepared by mixing together equal volume of each isolate containing 1x 10^6 CFU/ml. Consortia and all 9 isolates individually were used to treat rice seeds by soaking surface sterilized rice seeds (25) for 24 h in microbial inoculum followed by transfer to water agar plates (Fig. 6). After 7 days, the effect of consortia and individual bacterial inoculations on germination, root and shoot growth were studied. The results showed that germination percentage was highest (100 %) when the seeds were inoculated with C1, C2, R1, IIRRSS22-6, IIRRSS22-7 and the least germination was observed with IIRRSS22-4 (93.34 ± 2.31). The root length and shoot length was highest with C1 with values of 9.45 ± 0.06 and 6.07 ± 0.24 respectively. Vigor index - I and II was also found to be highest in C1 with 1616 ± 18 and 5.47 ± 0.68 respectively. All parameters of growth of rice when inoculated with C1 showed significant difference (P=0.05) in seedling length, germination percentage, vigor index I and II compared to control and individual culture inoculation. And among the other consortia groups also C1(IIRRSS22-5, IIRRSS22-3, P1) performed better than C2 and C3 (Table 2). Thus, the results of present study (Table 2) revealed that consortium-1 performs more effectively in terms of plant growth promotion. Therefore, this study suggest that consortium has synergistic effect on plant growth of rice when compared with individual inoculations.

Similar results with consortium were obtained by Akintokun et al. [56] in tomato plants, where there was significant difference in root length, shoot length and vigour index when the seeds were inoculated with consortium containing four organisms when compared with control treatments.

Sherpa et al. [57] has conducted studies on use of consortia for promotion of rice plant growth by using 3 different consortia. Greenhouse and field studies showed that the consortia-3 had the highest plant growth-promoting activity in terms of root length, number of leaflets per plant, grains per panicle, test grain weight, dry root weight per plant, and total dry biomass per plant.

3.7 Molecular Identification of Bacterial Isolates

The assembled 16S rRNA gene sequences of approximately 1500 bp, obtained after amplification and sequencing, were used to identify the selected nine isolates by searching for homology against the National Center for Biotechnology Information (NCBI) non-redundant nucleotide sequence database using Basic Local Alignment Search Tool (BLAST). Based on the homology of the sequences to that in the NCBI, the molecular identity of the bacterial isolates was established. The identity of the isolates is shown (Table 3) along with the accession numbers of 16S rRNA gene sequences that have been deposited in the National Center for Biotechnology Information database.

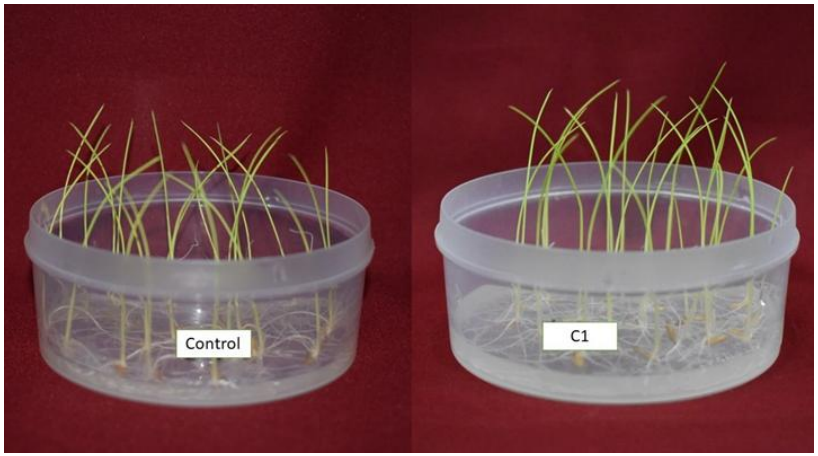

Fig. 6. Evaluation of the effect of rhizobacterial consortia on rice seed germination

Table 2. Effect of bacterial treatment on germination and vigour index of rice seedlings

Isolate ID	Root length (cm)	Shoot length (cm)	Seedling length (cm)	Germination percentage	Vigor index I	Vigor index II
Control	6.84 ± 0.03	5.28 ± 0.23	12.12 ± 0.2	96.67 ± 1.15	1171.46 ± 10.02	4.978 ± 0.03
P1	8.36 ± 0.82	6.42 ± 0.1	14.78 ± 0.72	97.34 ± 2.31	1437.62 ± 44.1	4.99 ± 0.33
IIRRSS22-3	8.13 ± 1.2	6.29 ± 0.41	14.42 ± 0.78	97.34 ± 4.62	1406.12 ± 136.74	4.54 ± 0.14
IIRRSS22-5	9.19 ± 0.04	6.38 ± 0.02	15.58 ± 0.07	97.34 ± 2.31	1516.36 ± 30.19	5.05 ± 0.25
IIRRSS22-1	8.49 ± 0.08	6.2 ± 0.07	14.69 ± 0.15	99.34 ± 1.15	1459.6 ± 8.6	4.89 ± 0.18
R1	7.99 ± 0.70	6.40 ± 0.14	14.4 ± 0.56	100	1440 ± 56	4.52 ± 0.33
IIRRSS22-2	8.8 ± 1.44	6.66 ± 0.05	15.46 ± 1.38	99.34 ± 1.15	1536.19 ± 139.65	4.54 ± 0.28
IIRRSS22-6	7.57 ± 1.48	6.24 ± 0.08	13.81 ± 1.4	100	1381 ± 140	4.05 ± 0.1
IIRRSS22-7	7.04 ± 1.42	6.4 ± 0.3	13.44 ± 1.72	100	1344 ± 172	5.15 ± 0.2
IIRRSS22-4	9.08 ± 0.15	6.12 ± 0.16	15.21 ± 0.01	93.34 ± 2.31	1419.58 ± 34.31	4.69 ± 0.27
C1	**9.45 ± 0.06**	**6.70 ± 0.24**	**16.16 ± 0.18**	**100**	**1616 ± 18**	**5.47 ± 0.68**
C2	9.40 ± 0.36	6.55 ± 0.10	15.96 ± 0.26	100	1596 ± 26	4.8 ± 0.05
C3	8.58 ± 0.19	5.88 ± 0.34	14.46 ± 0.14	98.67 ± 1.15	1427.31 ± 30.03	5.00 ± 0.42
CD (0.05)	1.473	0.332	1.423	3.146	151.408	0.485
CV (%)	10.389	3.103	5.721	1.886	6.187	5.954

Data presented is mean of replicates ± standard deviation

Table 3. Identity of bacterial isolates using16S rRNA gene sequencing

S. No.	Isolate ID	Identified strain	Accession number
1	IIRRSS22-1	*Rhizobium sp.*	OP580066
2	IIRRSS22-2	*Stenotrophomonas maltophilia*	OP580125
3	IIRRSS22-3	*Stenotrophomonas sp.*	OP592247
4	IIRRSS22-4	*Ochrobactrum anthropi*	OP580149
5	IIRRSS22-5	*Achromobacter sp.*	OP592244
6	IIRRSS22-6	*Achromobacter insuavis*	OP580150
7	IIRRSS22-7	*Achromobacter xylosoxidans*	OP580160
8	R1	*Rhizobium sp.*	KY348774
9	P1	*Pseudomonas stutzeri*	MW231894

Based on 16S rRNA sequencing, the isolates in consortia C1 have been identified as *Pseudomonas stutzeri, Stenotrophomonas sp., Achromobacter sp.* The three isolates have been reported by several authors as plant growth promoting rhizobacteria when used individually as bioinoculants. *Pseudomonas sp* are the most extensively studied plant growth promoting rhizobacteria and are known to protect against many soil pathogenic microorganisms [58]. Pham et al. [59] studied the effect of inoculation of *Pseudomonas stutzeri* in rice plants which showed a significant increase in shoot and root dry weight when compared with unioculated rice seedlings. Plant growth promoting potential of S*tenotrophomonas sps* has been reported in wheat plants along with resistance against biotic and abiotic stress by Singh et al. [60]. Inoculation of wheat with this species resulted in significant increase in root/shoot length by 20-39% and fresh/dry weight by 28-42% under salt stress conditions. Studies by Joe et al. [61] on influence of *Achromobacter xyloxidans* on rice growth has shown an increase in seed germination by 31% and seedling vigour by 114%. This study helped to establish that three isolates *Pseudomonas stutzeri, Stenotrophomonas sp., Achromobacter sp* in the consortia functioned synergistically to improve rice seed germination and seedling growth.

4. CONCLUSION AND RECOMMENDA-TIONS

In this study rhizospheric bacterial isolates of rice were screened for plant growth promotion, abiotic stress tolerance and compatibility with agrichemicals. The best performing isolates were selected for formulation of consortia. The effect of these rhizobacterial isolates as consortia and as individual strains was studied on growth promotion in rice. In this study consortia C1 which consisted of a unique combination of isolates viz., *Pseudomonas stutzeri, Stenotrophomonas sp., Achromobacter sp* has shown the high germination percentage (100%), root (9.45cm) and shoot length (6.70 cm), vigor index I and II (1616 and 5.47) when compared with individual strains and uninoculated treatments. The results of this study revealed that the selected combination of bacteria has shown complimentary effect in improving plant growth in rice when compared with individual treatments. Thus, this study not only reiterated the superiority of consortia over single isolate treatments in rice seedling growth promotion but also helped to identify a novel combination of bacteria that can be used as consortia for rice.

The consortium was selected to improve plant performance under both normal and stressful edaphic and climatic environments. Here we present the results of the initial study on the effect of the chosen consortium on rice germination and seedling growth under normal unstressed conditions. In future studies, the consortium will be evaluated under invitro, glasshouse, and field conditions to study its efficacy under various abiotic stress conditions. Further, the consortium can be assessed under different agroclimatic zones experiencing high temperatures/moisture stress and under stressed soil conditions such as salinity, sodicity and soils with agrichemical contamination. before being recommended for use in rice fields as bioinoculant.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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