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ENDOPHYTIC BACTERIAL DIVERSITY FROM VARIED RICE GENOTYPES AND ASSOCIATED PROPENSITY TO PROMOTE PLANT GROWTH

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ABSTRACT

Plant growth promoting endophytic bacteria (PGPEB) are thosebacteria living within plant tissues increasing production by various plant growth promoting activity. Present investigation was carried out to isolate and characterize PGPEB of rice. About 149 endophytic bacteria were isolated from four rice genotypes collectedfrom IIRR, Hyderabad, India. The Shannon -Weaver diversity did not differ between the genotypes as H' was 1.3 for BPT 5204, MTU 1010 and IRGC 1220 and 1.2 for Swarna. The evenness of bacterial communities ranged from 0.8 in BPT 5204 and Swarna to 0.9 in MTU 1010 and IRGC 1220 indicating the similarity in distribution of colonies in the morphotypes of different genotypes. All the isolates were screened for PGP activities (*in vitro*), found81.20% isolates producingIAA,34.89%,67.78% and 40.93% isolates solubilizing phosphorous, zinc and potassium, respectively, 44.29% producing siderophore, 44.29% were antagonistic against *Rhizoctonia solani* and 52.34% against *Pyricularia oryzae*. Owing to the isolated endophytes' improved nutrient acquisition and antagonistic activity against plant infections, these isolates can be mass produced as bioinoculants to aid farming communities and decrease the usage of chemical fertilizers and fungicides.

Key words: Endophytes, Shannon –Weaver index, Acetylene Reduction Assay, Nutrient Solubilization, Antagonistic Activity

Introduction

Increased demand for rice as a result of rising population necessitates increased rice productivity, and one of the most essential reasons for high rice yields is the use of chemical fertilizers and pesticides, which can pollute the environment and harm human health. Rice cultivation must adopt alternative management strategies that can be employed in sustainable agricultural production without harming the environment in order to become more sustainable and less reliant on chemical fertilizers. The use of plant growth promoting bacteria especially endophytic bacteria could be a viable alternative for increasing plant growth and yield in a long-term manner. Endophytic bacteria (EB) are bacteria that reside within plant tissues without causing any obvious symptoms of their presence by promoting growth of the plants through various mechanisms and are also known to confer abiotic and biotic stress tolerance in plants (Eid *et al*., 2021). Plants constitute vast and diverse niches for endophytic organisms. EB have been isolated from a large diversity of plants and from different plant tissues. In general, EB occur at lower population densities than rhizospheric bacteria or bacterial pathogens. Endophyte population density varies greatly, depending on bacterial species and host genotypes, as well as host developmental stage and environmental factors (Ali *et al*., 2021). EB typically enters plants through their roots and aerial parts, such as leaves, flowers, stems and cotyledons. Plant root bacterial endophytes are thought to be primarily recruited from the soil, and subsequently travel to stems and leaves via the apoplast in xylem vessels. Therefore, plant leaf/shoot endosphere microbiomes have significant overlaps with those in roots.

The mechanisms by which EB affect plant growth is similar to that of Plant Growth Promoting Rhizobacteria (PGPR). Few strains from genera such as Pantoea, Serratia, Sphingobacterium, Sphingomonas, Chromobacterium, Azoarcus, Burkholderia, are well known PGPEB in rice (Tambong *et al.*, 2021). The underlying mechanisms of plant growth promotion by PGPR have been comprehensively described in several articles (Bashan *et al.*, 2006). EB can fix atmospheric nitrogen and supply it to plants; they synthesize siderophores that can solubilize and sequester iron from the soil and provide it to the plant. They synthesize several different phytohormones that can act to enhance various stages of plant growth; and they may synthesize some less well-characterized, low-molecular-mass compounds or enzymes that can modulate plant growth and development (Mukherjee *et al.*, 2022). Owing to their plant growth promoting and disease control properties, endophytes can be used in the form of bioinoculants in agriculture as amendments to promote plant growth and health.

In view of the above-mentioned benefits of EB and their role in promoting plant growth by different mechanisms, present study was taken up where EB from root of different rice genotypes were isolated, morphologically characterized and screened for their plant growth promoting abilities under *invitro* conditions. Rice was selected, as it is a widely grown crop in the nation. In the present study, Shannon-Weaver diversity (Hernández-Tasco *et al.*, 2023) was calculated to get a better understanding of number of isolates from individual genotype of rice.

Materials and Methods

Isolation of Endophytic Bacteria

Roots of four different rice genotypes BPT-5204, SWARNA, MTU-1010, IRGC-1220 (non-indigenous genotype) were collected from the fields of ICAR-Indian Institute of Rice Research (IIRR) at flowering stage (60 DAS).

For isolation of endophytic bacteria in the roots, one gram of root sample was added into a tube with 0.1 % Tween 20 (Sevilla and Kennedy, 2000). The tubes were sonicated for 3 - 5 min to remove the bacteria adhering to the surface of roots. Roots were surface sterilized, and plating was done as per the procedure given Liu *et al.*, 2017 to check the success of surface sterilization. Root homogenates were used for serial dilutions and plating on different media (Piromyou *et al.*, 2015).

Serially diluted (10 fold) root homogenates were spread on six different media *i.e.*, Tripticase Soy Agar (TSA), Nutrient Agar (NA), R2A Agar, M9 minimal media, nitrogen limited Rennie's combined carbon medium for nitrogen fixers and defined media for Archaea which were supplemented with filter sterilized cycloheximide and incubated at 28 ± 2ºC for 72 h.

After counting of colonies on each plate, their colony morphology was recorded using the characteristics (Gabani *et al.*, 2014).The colony characteristics recorded were shape, margin, elevation, size, texture, appearance, pigmentation and optical properties (Supplementary Table 1, 2, 3, 4). The isolated colonies are named according to their first letter of genotype and first letter of media used to isolate (Supplementary Table 5).

The isolates on different media from each genotype were grouped together into morphotypes based on similar morphological characteristics. The information obtained was used to study the diversity of the bacterial population in the different genotypes by using diversity indices. The Shannon-Weaver diversity (H') represent the proportion of species abundance (morphotypes) in the population. H' is maximum when all species occur in similar number of individuals and the lowest when the sample contain one species (morphotypes). Shannon diversity indices, H' of colony morphology typing were calculated using the equation:

$$H = \Sigma\ (Pi \times \text{In } Pi)$$

while Pi is the proportional abundance of morphotype for each genotype (Hill *et al.*, 2003)

The richness was determined as the number of distinct colony morphotypes for each genotype. Evenness (EH) expresses how evenly the colonies in the community are distributed over the different species (morphotypes) and it varies between 0 - 1, with zero signifying no evenness and one, a complete evenness. Shannon's diversity index H' is divided by natural logarithm of species richness ln(S) to calculate the species evenness.

Screening for plant growth promoting properties of endophytic bacterial isolates

Nitrogen fixing activity

The nitrogen fixing capacity of the isolates on N - free media were evaluated by using ARA following the standard procedure (Yates. 1981). Rennie media slants were prepared, inoculated with isolates and incubated in an incubator at 28 ± 2ºC for 72 h. After full growth of organism in Rennie media slants, cotton plugs were replaced by suba - seal septa. Ten per cent (v/v) of the inert air was removed and ten per cent pure acetylene gas was injected and incubated. After 24h incubation, 1 ml of gas sample was withdrawn and injected into the gas chromatograph (Trace 1100 Thermo Scientific). At the end of experimental period, the bacterial cells were lysed and the cell protein content of the cultures (Bradford and Williams, 1977).

The ARA of the strains were calculated by using the formula:

$$\text{Nano moles of } C_2H_4 \text{ mg protein}^{-1}\text{hour}^{-1} = \frac{C \times Ps \times V}{P_{STD} \times T \times P}$$

Where

C = Concentration of ethylene in standard in nanomoles

Ps= Peak area of the sample

V= Volume of air space in the test tube in ml

P_{STD}= Peak area of standard ethylene

T= Incubation time in hours

P= Protein content of bacterial growth on slant in mg

Qualitative assay of phosphate, zinc and potassium solubilizing activity

Pure cultures of endophytic bacterial isolates were spot inoculated on Sperber's media where the insoluble phosphorous source is in the hydroxyl apatite form (Sperber, 1957), on tris minimal medium containing 0.1 % insoluble zinc compounds (ZnO) (Saravanan *et al*., 2004) and on aleksandrov agar medium containing 0.5 % insoluble potassium aluminium silicate. The plates were incubated at 30 ± 1ºC for 7 - 10 days. The zone of solubilization (mm) formed around colonies was recorded after 10 days (Pikovskaya, 1948).

The solubilizing index and efficiency of the microorganisms was calculated by using following formula:

Solubilizing efficiency (% S.E) = Z /C × 100

Solubilizing index (S.I) = C + Z / C

where

Z = Solubilization zone (mm) C = Colony diameter (mm)

Indole acetic acid production

Bacterial cultures were grown in a nutrient broth amended with tryptophan (5 mM) for 4 - 6 days. Cultures were centrifuged at 10,000 rpm for 20 min. Two ml of supernatant was mixed with two drops of orthophosphoric acid and 4 ml of Salkowski reagent. Tubes were incubated at room temperature for 25 min (Gordon & Weber, 1951). Based on the intensity of color, the endophytic isolates were classified into 4 groups viz., -, +, ++, +++

Screening of endophytic bacterial isolates for biocontrol activity (Siderophore & Hydrogen cyanide production, Antagonistic activity)

Production of siderophore was estimated qualitatively by the method where, CAS media was used (Schwyn & Neilands, 1987). The diameters of the color formation around the colonies were measured; efficiency and index were calculated as described earlier for phosphorus solubilization.

The HCN production was tested by inoculating the test isolates on nutrient agar plates containing 4.4 g per liter of glycine. A disc of whattman filter paper No.1 of the diameter equal to the petri plate size, impregnated with alkaline picric acid solution (0.5 % picric acid (w/v) in 1 % sodium carbonate) was placed in the upper lid of the inoculated Petri plates under aseptic condition. The control plate had no inoculum. The plates were incubated at 28 ± 2ºC for 48 - 72 h. Change in colour of the filter paper from yellow to light brown, moderate or strong reddish brown was taken as indication of HCN production (Castric & Castric, 1983). *Pseudomanas fluorescens* isolate obtained from plant pathology (ICAR-IRRI) division was used as positive check.

Antagonistic activity was verified by following dual culture technique (Skidmore & Dickinson, 1976). Pure isolates of common phytopathogens of rice *viz*. *Rhizoctonia solani* and *Pyricularia oryzae* was obtained from Plant Pathology Section, IIRR, Rajendranagar. Loopful of each endophytic bacterial culture was streaked on the Potato Dextrose Agar (PDA) plate at one end, which was pre-inoculated with 5 days old, 5 mm mycelial disc of test pathogen at the other end. Control plate was maintained by placing only pathogen mycelial disc on the plate without bacteria. Combination media with NA and PDA was prepared to check the antagonistic activity of Archaebacterial isolates as they do not grow on PDA media. The assay plates were incubated at 28 ± 1ºC for 5 days and observations were made on inhibition of mycelial growth of the test pathogens. Based on the intensity of inhibition the isolates were characterizes into four groups. The isolate with no inhibition of pathogen was given -, the isolate with slight inhibition of pathogen was given +, the isolate with moderate inhibition of pathogen was given ++ and the isolate with strong inhibition of pathogen was given +++.

Results and Discussion

The bacterial population in the root endosphere of different rice genotypes was enumerated after plating on different media (Table 1). The highest endophytic bacterial (5.34 log CFU/g of root fresh wt) was found in

Table 1: Endophytic bacterial population in the roots of rice genotypes (log CFU/g root fresh weight).

Media/ Varieties	BPT 5204	Swarna	MTU 1010	IRGC 1220
TSA	5.94	5.78	5.66	5.59
NA	5.82	5.62	5.64	5.60
R2A	5.74	5.75	5.71	5.56
REN	5.50	5.54	5.36	4.70
M9	5.68	5.66	5.43	5.55
Archaea	3.36	3.25	3.04	2.69
Mean	5.34	5.27	5.14	4.95
CD (0.05)	0.0641	0.0768	0.669	0.0744
CV (%)	0.67	0.68	0.70	0.73

the BPT 5204, followed by Swarna, MTU 1010 and IRGC 1220 with population of 5.27, 5.14 and 4.95 log CFU/g root fresh wt, respectively. The endophytic population in different genotypes on different media ranged between 2.69 to 5.94 log CFU/g root tissue. Maximum number of isolates was obtained with TSA media while the lowest number of isolates were present in the media for Archaea (Supplementary Plate 4.1).

Complex and minimal media were used because it is supposed that it might lead to different yields of cultivable endophyte. Complex media contain high amounts of nutrients such as sugars and amino acids, which implies that most (cultivable) fast growing bacterial species can grow easily on these plates. Minimal media, on the other hand, provide a strict amount of nutrients, which leads to a slower and more selective growth, but might also allow slow growing endophytic strains a chance to develop (Eevers *et al.*, 2015). Statistically significant differences were observed in numbers of diazotrophic bacteria retrieved from both genotypes and tissues using the five different media in elephant grass genotypes (Videira *et al.*, 2012).

The rice genotypes used in this study belong to diverse genetic backgrounds. BPT 5204, MTU 1010 and Swarna are indica genotypes while IRGC 1220 is a tropical japonica line. Moreover the lineages of the indica lines are also different. MTU 1010 parentage is MTU 2077 × IR64, and that of BPT 5204 is GEB 24 x TN-1, while the Swarna parentage is Vashista × Mahsuri. Since all the four genotypes belong to different genetically, the physiology of the genotypes also differs, which might result in the differences in supporting endophytic bacterial population density and diversity as observed in this study.

Colony characteristics of endophytic bacteria from different genotypes

Table 2: Nitrogenase activity of isolates grown on N- free media.

S. No	Isolate ID	Nitrogen fixation (nmol C_2H_4/mgprotein/h)
1	BRen1	3.37± 0.02
2	BRen2	0.89± 0.08
3	BRen3	ND
4	BRen4	ND
5	BRen5	0.41± 0.03
6	SRen1	0.08± 0.04
7	SRen2	2.54± 0.04
8	SRen3	13.92± 0.03
9	SRen4	0.85± 0.05
10	SRen5	0.28± 0.06
11	MRen1	0.18± 0.05
12	MRen2	19.28± 0.08
13	MRen3	0.08± 0.05
14	IRen1	0.08± 0.04
15	IRen2	0.09± 0.04
16	IRen3	ND

Values are the means of three replicates ± standard deviation; ND – Not Detected

After 72 h incubation to establish the full growth, the colony morphology of EB isolates was studied on respective media in which the isolate has grown like NA, TSA, R2A, Rennie, M9 and defined media for Archaea. About 43 different colony morphologies were observed in BPT 5204, 41 in Swarna, 34 in MTU 1010 and 31 in IRGC 1220 (Supplementary Table 1, 2, 3, 4).

After classifying the colonies into morphotypes the data was used to calculate the diversity of endophytic bacterial population in each genotype. The number of different colony morphotypes and the number of colonies belonging to each morphotype were used as parameters for further calculation of Shannon diversity indices. The Shannon -Weaver diversity did not differ between the genotypes as H' was 1.3 for BPT 5204, MTU 1010 and IRGC 1220 and 1.2 for Swarna. The evenness of bacterial communities ranged from 0.8 in BPT 5208 and Swarna to 0.9 in MTU 1010 and IRGC 1220 indicating the similarity in distribution of colonies in the morphotypes of different genotypes (Fig. a). Colony morphology is commonly used to distinguish bacterial genotypes on plates, and it is also a good indicator of ecological diversity (Saxer *et al.*, 2010; Pirhadi, 2018).

Screening of endophytic bacterial isolates for plant growth promoting activities

The selected morphotypes were tested for their purity and preserved in the microbiology lab, IIRR, Rajendranagar, Hyderabad and were further screened for nine different PGP activities *in vitro*.

Nitrogen fixation ability of endophytic bacterial isolates

Table 3: Solubilization of insoluble P, Zn and K by endophytic bacterial isolates (BPT5204).

Isolate ID	Phosphate solubilization		Zinc solubilization		Potassium solubilization	
	SI	SE%	SI	SE%	SI	SE%
BT1	-	-	3.3 ± 0.16	233 ± 6.26	-	-
BT2	2.2 ± 0.07	120 ± 3.88	2.8 ± 0.11	175 ± 5.37	-	-
BT3	3.0 ± 0.07	200 ± 4.38	3.3 ± 0.08	233 ± 3.95	2.5 ± 0.11	150 ± 18.90
BT4	-	-	3.4 ± 0.14	240 ± 9.91	-	-
BT5	-	-	-	-	2.6 ± 0.23	160 ± 20.52
BT6	-	-	3.3 ± 0.07	227 ± 5.91	3.0 ± 0.21	200 ± 17.24
BT7	-	-	2.3 ± 0.09	133 ± 5.16	-	-
BN1	-	-	3.4 ± 0.10	240 ± 8.56	-	-
BN2	-	-	3.0 ± 0.06	200 ± 5.46	-	-
BN3	-	-	3.8 ± 0.07	280 ± 10.14	-	-
BN4	-	-	-	-	3.0 ± 0.28	200 ± 13.28
BN5	2.6 ± 0.11	166 ± 4.46	4.2 ± 0.24	316 ± 3.50	-	-
BN6	-	-	5.3 ± 0.19	425 ± 17.92	-	-
BN7	3.5 ± 0.10	250 ± 12.71	3.5 ± 0.06	250 ± 2.44	-	-
BN8	-	-	3.4 ± 0.10	240 ± 6.68	-	-
BR1	-	-	2.5 ± 0.08	150 ± 7.93	-	-
BR2	-	-	4.3 ± 0.09	325 ± 9.07	2.3 ± 0.32	125 ± 8.40
BR3	-	-	-	-	-	-
BR4	-	-	-	-	-	-
BR5	-	-	3.4 ± 0.11	240 ± 8.43	2.3 ± 0.28	125 ± 16.06
BR6	-	-	3.0 ± 0.06	200 ± 2.34	2.3 ± 0.23	125 ± 11.64
BR7	-	-	2.6 ± 0.06	166 ± 6.18	-	-
BR8	-	-	3.3 ± 0.16	233 ± 2.70	-	-
BR9	2.3 ± 0.08	125 ± 2.72	3.0 ± 0.06	200 ± 6.02	2.6 ± 0.37	160 ± 6.12
BR10	-	-	2.3 ± 0.05	125 ± 3.27	-	-
BR11	2.2 ± 0.06	116 ± 2.86	2.4 ± 0.06	137 ± 1.64	-	-
BR12	-	-	3.3 ± 0.09	225 ± 6.04	-	-
BR13	-	-	5.8 ± 0.09	475 ± 14.67	-	-
BRen1	4.3 ± 0.09	333 ± 8.95	3.0 ± 0.12	200 ± 5.20	3.8 ± 0.12	275 ± 38.11
BRen2	-	-	2.4 ± 0.06	137 ± 2.14	-	-
BRen3	2.8 ± 0.06	175 ± 5.37	4.0 ± 0.15	300 ± 9.61	4.4 ± 0.14	333 ± 46.40
BRen4	4.0 ± 0.13	300 ± 5.07	4.6 ± 0.16	360 ± 7.41	5.0 ± 0.25	400 ± 14.05
BRen5	-	-	2.4 ± 0.06	142 ± 2.11	-	-
BM1	-	-	-	-	-	-
BM2	-	-	2.4 ± 0.08	140 ± 3.77	2.8 ± 0.15	183 ± 13.77
BM3	2.6 ± 0.13	140 ± 5.78	3.8 ± 0.04	280 ± 15.01	3.0 ± 0.09	200 ± 8.29
BM4	3.0 ± 0.12	200 ± 5.20	3.0 ± 0.12	209 ± 6.72	3.2 ± 0.26	220 ± 3.49
BM5	2.8 ± 0.06	180 ± 6.97	4.0 ± 0.03	300 ± 6.90	3.0 ± 0.19	200 ± 13.27
BM6	3.0 ± 0.12	200 ± 7.13	3.6 ± 0.10	266 ± 5.72	3.5 ± 0.14	250 ± 16.82
BM7	3.0 ± 0.07	200 ± 5.46	2.5 ± 0.13	150 ± 9.86	3.0 ± 0.25	200 ± 11.33
BA1	-	-	-	-	-	-
BA2	-	-	3.5 ± 0.09	250 ± 2.97	-	-
BA3	-	-	3.0 ± 0.10	200 ± 5.94	-	-

Values are the means of three replicates ± standard deviation; SI – Solubilization Index; SE (%) – Solubilization Efficiency

Acetylene Reduction Assay (ARA) was carried out to examine the nitrogen-fixing ability of the 16 endophytic bacterial isolates grown on N free Rennie media under laboratory conditions. The data revealed considerable variability in the nitrogen fixing ability among the studied strains that ranged from to 0.08 to 19.28 η moles C_2H_4 mg protein h^{-1} (Table 2). Nitrogen fixation was maximum with the isolate MRen2 (19.28 η moles C_2H_4 mg protein h^{-1}). As Nitrogenase is a versatile enzyme capable of catalyzing the reduction of several substrates other than

Table 4: Solubilization of insoluble P, Zn and K by endophytic bacterial isolates (Swarna).

Isolate ID	**Phosphate solubilization**		**Zinc solubilization**		**Potassium solubilization**	
	SI	**SE%**	**SI**	**SE%**	**SI**	**SE%**
ST1	-	-	-	-	-	-
ST2	-	-	3.5± 0.67	250 ± 13.56	-	-
ST3	-	-	2.6± 0.05	166 ± 19.03	3.3 ± 0.28	225 ± 22.26
ST4	-	-	3.3± 0.12	225 ± 33.38	-	-
ST5	-	-	3.7± 0.62	266 ± 24.94	2.6 ± 0.37	160 ± 26.45
ST6	2.6 ± 0.25	160 ± 7.95	2.5± 0.14	154 ± 6.90	2.3 ± 0.20	133 ± 14.67
ST7	-	-	3.0± 0.30	200 ± 11.65	-	-
ST8	-	-	4.0± 0.35	300 ± 27.65	3.2 ± 0.50	216 ± 19.47
ST9	-	-	3.3± 0.30	233 ± 28.23	2.5 ± 0.16	150 ± 9.93
ST10	2.2 ± 0.36	120 ± 12.83	2.6± 0.12	160 ± 16.42	-	-
ST11	3.5 ± 0.38	250 ± 23.56	3.6± 0.38	262 ± 29.06	3.2 ± 0.10	216 ± 17.30
SN1	-	-	4.2± 0.39	320 ± 18.85	-	-
SN2	-	-	-	-	4.0 ± 0.37	300 ± 28.12
SN3	-	-	2.5± 0.07	150 ± 13.01	-	-
SN4	3.4 ± 0.30	240 ± 7.47	2.8± 0.15	180 ± 11.43	3.8 ± 0.71	275 ± 32.99
SN5	-	-	-	-	-	-
SN6	2.3 ± 0.14	125 ± 6.77	3.5± 0.40	250 ± 17.51	-	-
SN7	3.0 ± 0.23	200 ± 22.92	3.2± 0.47	220 ± 16.51	-	-
SN8	-	-	-	-	-	-
SN9	-	-	-	-	-	-
SN10	2.6 ± 0.24	160 ± 23.74	2.5± 0.22	145 ± 17.32	3.3 ± 0.07	233 ± 10.14
SN11	2.7 ± 0.31	166 ± 15.62	3.4± 0.15	240 ± 25.27	-	-
SN12	2.5 ± 0.10	150 ± 6.70	4.5± 0.26	350 ± 12.60	2.5 ± 0.09	150 ± 15.00
SR1	2.5 ± 0.25	150 ± 8.74	3.6± 0.33	260 ± 26.94	-	-
SR2	3.2 ± 0.22	216 ± 19.96	-	-	3.2 ± 0.54	220 ± 15.61
SR3	-	-	-	-	2.8 ± 0.16	175 ± 8.05
SR4	2.3 ± 0.10	133 ± 16.15	2.8± 0.33	175 ± 11.89	3.3 ± 0.32	225 ± 45.69
SR5	-	-	-	-	3.0 ± 0.26	200 ± 18.85
SRen1	4.0 ± 0.81	300 ± 30.79	4.6± 0.47	316 ± 14.80	4.5 ± 0.40	350 ± 31.05
SRen2	4.3 ± 0.40	325 ± 35.98	4.5± 0.49	350 ± 9.02	6.0 ± 0.29	500 ± 72.17
SRen3	2.8 ± 0.24	175 ± 10.31	6.0± 0.35	500 ± 83.35	2.8 ± 0.29	175 ± 15.67
SRen4	5.0 ± 0.72	400 ± 34.70	4.6± 0.39	360 ± 19.62	4.3 ± 0.40	325 ± 52.37
SRen5	-	-	-	-	-	-
SM1	2.2 ± 0.19	116 ± 7.36	2.8± 0.17	185.7 ± 5.47	2.8 ± 0.08	225 ± 15.25
SM2	2.2 ± 0.35	120 ± 8.40	-	-	3.0 ± 0.16	200 ± 6.52
SM3	2.2 ± 0.14	116 ± 8.70	3.0 ± 0.21	200 ±19.75	2.8 ± 0.32	180 ± 17.05
SM4	-	-	4.0± 0.30	300 ±22.65	2.8 ± 0.40	225 ± 43.08
SM5	2.2 ± 0.07	116 ± 13.85	6.3± 0.75	533 ± 98.66	4.5 ± 0.42	350 ± 7.92
SA1	4.0 ± 0.37	300 ± 31.59	9.0± 0.94	800 ± 98.30	7.0 ± 0.31	600 ± 22.75
SA2	-	-	8.0± 0.28	700 ± 46.47	-	-
SA3	2.1 ± 0.41	114 ± 4.11	3.0± 0.31	200 ± 14.18	2.8 ± 0.16	225± 38.61

Values are the means of three replicates ± standard deviation; SI – Solubilization Index; SE (%) – Solubilization Efficiency

nitrogen, including acetylene (C_2H_2), nitrous oxide, azide, nitriles and isonitriles, the observations that acetylene is an inhibitor of dintrogen (N_2) fixation and is converted to ethylene by the nitrogen fixing enzyme nitrogenase provided the basis for the development of the first simple method for estimation of N_2 fixation. Comparable results were obtained by isolation and characterization of diazotrophic bacteria as species of the genera *Azospirillum*, *Herbaspirillum*, *Beijerinckia* and *Pseudomonas* and nitrogen fixing ability of isolates was detected by ARA (Koomnok *et al.*, 2007) and isolation of six closely related N_2-fixing bacterial strains from surface-sterilized roots and stems of four different rice varieties (Gyaneshwar *et al.*, 2001). Inoculation of the

Table 5: Solubilization of insoluble P, Zn and K by endophytic bacterial isolates (MTU- 1010).

Isolate ID	Phosphate solubilization		Zinc solubilization		Potassium solubilization	
	SI	SE%	SI	SE%	SI	SE%
MT1	-	-	2.4 ± 0.48	140 ± 18.29	3.0 ± 0.52	200 ± 31.98
MT2	2.3 ± 0.10	125 ± 8.92	2.5± 0.45	150 ± 6.19	-	-
MT3	2.5 ± 0.09	150 ± 15.64	3.5± 0.60	250 ± 12.86	2.2 ± 0.11	120 ± 13.45
MT4	2.3 ± 0.06	133 ± 13.84	2.5± 0.31	150 ± 12.92	-	-
MT5	2.7 ± 0.56	166 ± 12.51	-	-	-	-
MT6	2.3 ± 0.24	133 ± 17.42	2.6± 0.23	254 ± 39.91	-	-
MT7	-	-	-	-	2.3 ± 0.07	133 ± 13.32
MT8	-	-	2.9± 0.28	185± 10.59	2.7 ± 0.39	166 ± 18.92
MN1	2.2 ± 0.16	120 ± 4.95	2.6 ± 0.30	160 ± 9.16	-	-
MN2	-	-	4.2 ± 0.36	320 ± 41.33	-	-
MN3	2.3 ± 0.22	125 ± 6.43	3.8± 0.65	283 ± 28.19	-	-
MN4	2.3 ± 0.37	133 ± 11.48	3.2± 0.30	220 ± 7.46	2.5 ± 0.10	150 ± 24.40
MN5	-	-	2.6 ± 0.57	160 ± 11.73	-	-
MN6	-	-	-	-	-	-
MN7	-	-	3.0± 0.20	200 ± 32.04	-	-
MR1	2.4 ± 0.25	125 ± 19.64	-	-	-	-
MR2	-	-	-	-	-	-
MR3	-	-	-	-	-	-
MR4	2.3 ± 0.23	133 ± 7.63	3.8 ± 0.29	280 ± 35.28	-	-
MR5	-	-	3.6 ± 0.66	260 ± 33.45	-	-
MR6	2.3 ± 0.25	125 ± 7.15	2.5± 0.20	400 ± 37.22	3.0 ± 0.20	233 ± 14.58
MR7	-	-	3.4± 0.66	240 ± 26.87	3.8 ± 0.34	280 ± 26.70
MR8	-	-	4.5± 0.50	350 ± 42.19	-	-
MR9	-	-	3.6± 0.25	260 ± 34.68	3.0 ± 0.52	200 ± 40.37
MRen1	-	-	2.6 ± 0.34	160 ± 24.05	-	-
MRen2	-	-	-	-	2.3 ± 0.14	125 ± 22.90
MRen3	2.3 ± 0.37	133 ± 17.22	3.0± 0.10	200 ± 18.23	-	-
MM1	-	-	-	-	2.3 ± 0.25	133 ± 23.04
MM2	-	-	-	-	2.8 ± 0.19	180 ± 22.93
MM3	3.0 ± 0.18	200 ± 19.92	-	-	2.8 ± 0.19	180 ± 16.09
MM4	4.0 ± 0.38	300 ± 10.17	4.0 ± 0.42	300 ± 52.03	-	-
MM5	-	-	2.2 ± 0.15	120 ± 6.07	2.8 ± 0.27	175 ± 7.69
MA1	-	-	-	-	-	-
MA2	-	-	-	-	-	-

Values are the means of three replicates ± standard deviation; SI – Solubilization Index; SE (%) – Solubilization Efficiency

two Oryza species with the endophyte strains *Klebsiella pasteurii* BDA134-6 and *Phytobacter diazotrophicus* BDA59-3 led to the highest nitrogenase activity (Bianco *et al*., 2021).

Solubilization of phosphate, zinc and potassium by endophytic bacteria

Among 43 isolates of BPT-5204, 14 isolates were proven to be solubilizing hydroxy apatite as insoluble P source, 37 and 17 isolates were able to solubilize ZnO and potassium alumino silicate, respectively. Their solubilization efficiency (SE) and solubilization index (SI) were presented in Table 3. Among 41 isolates of Swarna, 22 isolates were proven to be solubilizing hydroxy apatite as insoluble P source, 31 and 25 isolates were able to solubilize ZnO and potassium alumino silicate, respectively (Table 4). Among 34 isolates of MTU-1010, 14 isolates were proven to be solubilizing hydroxy apatite as insoluble P source, 22 and 13 isolates were able to solubilize ZnO and KAl_2SiO_4, respectively (Table 5). Among 31 isolates of IRGC-1220, only two isolates were able to solubilize phosphate and six were able to solubilize potassium insoluble form, Zn solubilization was noticed by 20 isolates (Table 6). Endophytic isolates differ in the ability to produce phosphatase enzyme and production of organic acids (Sanam *et al*., 2021) and hence showed different SE. Similar studies were made using endophytes of soybean cultivars (Kuklinsky Sobral *et al*., 2004) and

Table 6: Solubilization of insoluble P, Zn and K by endophytic bacterial isolates (IRGC 1220).

Isolate ID	Phosphate solubilization		Zinc solubilization		Potassium solubilization	
	SI	SE%	SI	SE%	SI	SE%
IT1	-	-	-	-	-	-
IT2	-	-	3.3± 0.16	233 ± 6.26	-	-
IT3	-	-	3.0± 0.12	200 ± 6.13	-	-
IT4	2.2 ± 0.07	120 ± 4.52	3.4± 0.08	240 ± 4.06	-	-
IT5	-	-	-	-	-	-
IT6	2.3 ± 0.05	125 ± 3.96	3.5± 0.14	250 ± 10.33	-	-
IT7	-	-	3.6± 0.08	266 ± 6.92	-	-
IT8	-	-	3.3± 0.13	225 ± 8.71	-	-
IT9	-	-	6.0± 0.19	500 ± 17.84	2.5 ± 0.06	150 ± 3.45
IN1	-	-	-	-	-	-
IN2	-	-	3.6± 0.08	266 ± 7.26	2.3 ± 0.03	128 ± 2.75
IN3	-	-	2.5± 0.05	150 ± 5.43	-	-
IN4	-	-	3.3± 0.19	233 ± 2.57	-	-
IN5	-	-	3.4± 0.12	240 ± 10.12	-	-
IN6	-	-	3.7± 0.06	266 ± 2.60	2.4 ± 0.07	140 ± 9.20
IN7	-	-	-	-	2.7 ± 0.05	166 ± 1.98
IN8	-	-	-	-	-	-
IR1	-	-	-	-	2.3 ± 0.03	133 ± 3.96
IR2	-	-	3.3± 0.10	225 ± 6.26	-	-
IR3	-	-	-	-	-	-
IR4	-	-	3.0± 0.10	200 ± 10.57	-	-
IR5	-	-	4.0± 0.08	300 ± 8.37	-	-
IRen1	-	-	5.0± 0.16	400 ± 14.05	-	-
IRen2	-	-	-	-	-	-
IRen3	-	-	2.2± 0.04	120 ± 1.39	-	-
IM1	-	-	3.3± 0.08	225 ± 8.35	-	-
IM2	-	-	-	-	-	-
IM3	-	-	4.0 ± 0.20	300 ± 3.47	-	-
IM4	-	-	4.0 ± 0.08	300 ± 9.03	3.3 ± 0.08	225 ± 6.36
IM5	-	-	-	-	-	-
IA1	-	-	-	-	-	-

Values are the means of three replicates ± standard deviation; SI – Solubilization Index; SE (%) – Solubilization Efficiency

endophytic isolate *Gluconacetobacter diazotrophicus* PAl5 (Saravanan *et al.*, 2007). The majority of isolates tend to solubilize and utilize inorganic phosphorus. Since organic Phosphate solubilizing bacteria are scarce, they can be valuable biofertilizer resources functionally supplementing with the more common inorganic PSB that have already been possessed by plants. Along side Most soil potassium exists in mineral forms such as aluminosilicate, which cannot be directly utilized by plants. Endophytic bacteria play a critical role in the generation of soluble potassium for plant utilization.

Indole acetic acid production

Among 43 endophytic bacterial isolates of BPT-5204, IAA production varied with supplementation of L-tryptophan. Out of 43 isolates only eight isolates showed highest (+++) production, six isolates showed moderate (++) production, 21 isolates showed least production of IAA and eight isolates have no IAA production activity. Among 41 isolates of Swarna, 27 isolates have the ability of production and 14 isolates were not having the activity. Among 34 isolates of MTU-1010, 32 isolates produced IAA and the isolate MM1 is having highest (+++) IAA production activity and 23 isolates have least (+) production. The isolates MR9 and MRen1 have no IAA production activity. Among 31 isolates of IRGC-1220, 27 isolates produced IAA and the isolates (IT1, IT8, IM3) were having highest (+++) IAA production activity. The isolates IT4, IN5, IR2, IRen2 do not have IAA producing ability (Table 7) (Supplementary Plate 4.3). Similar results were reported by isolates obtained from *Prosopisstrom buliferain* producing IAA (Sgroy *et al.*, 2009). Literature establishes that the IAA secreted by the microbial resources, in the auxin pool of the plant, enhanced the

Table 7: Screening of EB isolates from different rice genotypes for IAA and HCN Production.

Isolate No.	ID	BPT-5204		ID	Swarna		ID	MTU-1010		ID	IRGC-1220	
		IAA	HCN		IAA	HCN		IAA	HCN		IAA	HCN
1	BT1	+++	-	ST1	+	-	MT1	+	-	IT1	+++	-
2	BT2	+	-	ST2	-	-	MT2	+	-	IT2	+	-
3	BT3	++	-	ST3	+	-	MT3	+	-	IT3	+	-
4	BT4	+++	-	ST4	+	-	MT4	+	-	IT4	-	-
5	BT5	+	-	ST5	+	-	MT5	+	-	IT5	+	-
6	BT6	+	-	ST6	+	-	MT6	++	-	IT6	+	-
7	BT7	+	-	ST7	+	-	MT7	+	-	IT7	+	-
8	BN1	+++	-	ST8	-	-	MT8	+	-	IT8	+++	-
9	BN2	+++	-	ST9	+	-	MN1	+	-	IT9	++	-
10	BN3	+	-	ST10	-	-	MN2	++	-	IN1	+	-
11	BN4	++	-	ST11	+++	-	MN3	++	-	IN2	++	-
12	BN5	+	-	SN1	+	-	MN4	+	-	IN3	++	-
13	BN6	+	-	SN2	++	-	MN5	+	-	IN4	+	-
14	BN7	++	-	SN3	+	-	MN6	+	-	IN5	-	-
15	BN8	+++	-	SN4	+	-	MN7	+	-	IN6	+	-
16	BR1	++	-	SN5	+	-	MR1	+	-	IN7	+	-
17	BR2	+	-	SN6	-	-	MR2	+	-	IN8	+	-
18	BR3	+	-	SN7	-	-	MR3	+	-	IR1	+	-
19	BR4	+	-	SN8	-	-	MR4	+	-	IR2	-	-
20	BR5	+++	-	SN9	++	-	MR5	+	-	IR3	+	-
21	BR6	+	-	SN10	-	-	MR6	+	-	IR4	+	-
22	BR7	-	-	SN11	-	-	MR7	+	-	IR5	++	-
23	BR8	+	-	SN12	+	-	MR8	+	-	IRen1	++	-
24	BR9	+	-	SR1	+	-	MR9	-	-	IRen2	-	-
25	BR10	-	-	SR2	-	-	MRen1	-	-	IRen3	++	-
26	BR11	-	-	SR3	-	-	MRen2	+	-	IM1	+	-
27	BR12	-	-	SR4	-	-	MRen3	++	-	IM2	++	-
28	BR13	-	-	SR5	-	-	MM1	+++	-	IM3	+++	-
29	Bren1	+++	-	SRen1	+	-	MM2	++	-	IM4	++	-
30	Bren2	++	-	SRen2	-	-	MM3	++	-	IM5	++	-
31	Bren3	+	-	SRen3	+	-	MM4	++	-	IA1	++	-
32	Bren4	++	-	SRen4	+	-	MM5	++	-	-	-	-
33	Bren5	-	-	SRen5	-	-	MA1	+	-	-	-	-
34	BM1	+	-	SM1	+++	-	MA2	+	-	-	-	-
35	BM2	+	-	SM2	+++	-	-	-	-	-	-	-
36	BM3	+++	-	SM3	+++	-	-	-	-	-	-	-
37	BM4	+	-	SM4	++	-	-	-	-	-	-	-
38	BM5	+	-	SM5	+++	-	-	-	-	-	-	-
39	BM6	+	-	SA1	+	-	-	-	-	-	-	-
40	BM7	+	-	SA2	+	-	-	-	-	-	-	-
41	BA1	-	-	SA3	+	-	-	-	-	-	-	-
42	BA2	+	-	-	-	-	-	-	-	-	-	-
43	BA3	-	-	-	-	-	-	-	-	-	-	-

– No production+ Weak production, ++ Moderate production, +++ Strong production

nutrient uptake, elevated the photosynthesis process, and assisted the host in various growth promoting processes and yield (Arora *et al.*, 2024).

Production of Siderophores

Out of 149 isolates sixty-five isolates produced siderophores. Among 43 isolates of BPT-5204, 25 isolates have the ability to produce siderophore (Table 8a), with a

Table 8a: Screening of four rice genotype isolates for siderophore production *in vitro*.

Isolate No.	Isolate ID	BPT-5204		Isolate ID	Swarna	
		Siderophore production			Siderophore production	
		SI	SE%		SI	SE%
1	BT1	3.3 ± 0.11	227 ± 19.24	ST1	4.6 ± 0.22	360 ± 61.78
2	BT2	2.8 ± 0.13	175 ± 8.32	ST2	2.5 ± 0.09	150 ± 17.89
3	BT3	-	-	ST3	-	-
4	BT4	2.5 ± 0.13	150 ± 15.30	ST4	-	-
5	BT5	-	-	ST5	-	-
6	BT6	2.3 ± 0.07	125 ± 16.73	ST6	2.4 ± 0.26	140 ± 87.44
7	BT7	4.3 ± 0.35	325 ± 30.26	ST7	-	-
8	BN1	3.0 ± 0.19	200 ± 11.48	ST8	-	-
9	BN2	-	-	ST9	-	-
10	BN3	3.4 ± 0.14	240 ± 9.36	ST10	-	-
11	BN4	-	-	ST11	3.0 ± 0.22	200 ± 14.20
12	BN5	-	-	SN1	-	-
13	BN6	-	-	SN2	-	-
14	BN7	-	-	SN3	-	-
15	BN8	2.6 ± 0.22	166 ± 19.42	SN4	2.6 ± 0.14	160 ± 14.52
16	BR1	4.3 ± 0.43	333 ± 48.79	SN5	4.3 ± 0.49	333 ± 16.58
17	BR2	-	-	SN6	-	-
18	BR3	-	-	SN7	-	-
19	BR4	-	-	SN8	-	-
20	BR5	-	-	SN9	2.2 ± 0.32	120 ± 12.83
21	BR6	-	-	SN10	-	-
22	BR7	2.3 ± 0.16	125 ± 10.62	SN11	3.6 ± 0.33	262 ± 24.74
23	BR8	2.3 ± 0.28	125 ± 11.71	SN12	-	-
24	BR9	-	-	SR1	2.8 ± 0.12	180 ± 5.60
25	BR10	2.4 ± 0.30	137 ± 5.74	SR2	-	-
26	BR11	3.3 ± 0.28	233 ± 24.23	SR3	2.3 ± 0.13	133 ± 7.21
27	BR12	3.0 ± 0.19	200 ± 25.70	SR4	-	-
28	BR13	-	-	SR5	2.5 ± 0.22	145 ± 16.62
29	Bren1	2.5 ± 0.16	150 ± 6.86	SRen1	4.0 ± 0.48	300 ± 44.51
30	Bren2	-	-	SRen2	4.0 ± 0.41	300 ± 28.13
31	Bren3	3.0 ± 0.38	200 ± 12.12	SRen3	3.5 ± 0.38	250 ± 11.18
32	Bren4	-	-	SRen4	4.5 ± 0.26	350 ± 20.40
33	Bren5	2.3 ± 0.20	125 ± 6.37	SRen5	4.6 ± 0.39	360 ± 33.18
34	BM1	-	-	SM1	4.0 ± 0.25	300 ± 36.35
35	BM2	3.3 ± 0.12	225 ± 18.35	SM2	4.0 ± 0.28	300 ± 30.79
36	BM3	2.6 ± 0.36	160 ± 17.08	SM3	5.0 ± 0.37	400 ± 44.28
37	BM4	4.3 ± 0.59	325 ± 16.63	SM4	4.6 ± 0.54	360 ± 21.21
38	BM5	4.3 ± 0.15	333 ± 28.53	SM5	4.6 ± 0.48	300 ± 26.02
39	BM6	3.4 ± 0.25	240 ± 22.65	SA1	-	-
40	BM7	4.0 ± 0.16	300 ± 27.99	SA2	-	-
41	BA1	2.8 ± 0.44	183 ± 21.55	SA3	-	-
42	BA2	4.6 ± 0.30	360 ± 38.37	-	-	-
43	BA3	-	-	-	-	-

highest siderophore production index of 4.6 ± 0.30 by BA2 and least of 2.3 ± 0.20 by BT6, BR7, BR8 and BRen5. Among 41 isolates of SWARNA, a total of 21 isolates has siderophore production ability (Table 8a). Highest siderophore production index of 5.0 ± 0.37 was recorded in SM3 and least 2.2 ± 0.32 was shown by SN9. Among 34 isolates of MTU-1010, eight isolates have siderophore production ability (Table 8b) with MM4 having highest siderophore production index of 5.8 ± 0.66 and least 2.2 ± 0.21 by MM3. Among 31 isolates of IRGC-

Table 8b: Screening of four rice genotype isolates for siderophore production *in vitro*

Isolate No.	Isolate ID	MTU-1010		Isolate ID	IRGC1220	
		Siderophore production			Siderophore production	
		SI	SE%		SI	SE%
1	MT1	-	-	IT1	3.0 ± 0.09	200 ± 4.36
2	MT2	3.5 ± 0.70	250 ± 27.99	IT2	2.3 ± 0.04	125 ± 4.04
3	MT3	-	-	IT3	2.6 ± 0.13	166 ± 8.47
4	MT4	-	-	IT4	-	-
5	MT5	-	-	IT5	-	-
6	MT6	-	-	IT6	-	-
7	MT7	-	-	IT7	2.5 ± 0.06	150 ± 3.65
8	MT8	-	-	IT8	-	-
9	MN1	-	-	IT9	2.6 ± 0.08	166 ± 4.03
10	MN2	-	-	IN1	-	-
11	MN3	-	-	IN2	-	-
12	MN4	-	-	IN3	-	-
13	MN5	-	-	IN4	2.3 ± 0.09	125 ± 2.72
14	MN6	-	-	IN5	-	-
15	MN7	-	-	IN6	-	-
16	MR1	-	-	IN7	-	-
17	MR2	-	-	IN8	3.0 ± 0.07	200 ± 4.91
18	MR3	-	-	IR1	-	-
19	MR4	-	-	IR2	3.3 ± 0.14	233 ± 6.26
20	MR5	-	-	IR3	4.3 ± 0.10	333 ± 10.22
21	MR6	-	-	IR4	-	-
22	MR7	-	-	IR5	-	-
23	MR8	-	-	IRen1	-	-
24	MR9	-	-	IRen2	-	-
25	MRen1	-	-	IRen3	-	-
26	MRen2	2.3 ± 0.42	133 ± 16.07	IM1	2.3 ± 0.09	133 ± 2.25
27	MRen3	2.6 ± 0.44	160 ± 21.34	IM2	-	-
28	MM1	2.3 ± 0.28	125 ± 18.79	IM3	2.3 ± 0.07	137 ± 5.68
29	MM2	2.6 ± 0.23	160 ± 14.58	IM4	-	-
30	MM3	2.2 ± 0.21	116 ± 20.22	IM5	4.0 ± 0.09	300 ± 7.81
31	MM4	5.8 ± 0.66	475 ± 24.03	IA1	-	-
32	MM5	2.3 ± 0.19	125 ± 4.17	-	-	-
33	MA1	-	-	-	-	-
34	MA2	-	-	-	-	-

1220, twelve isolates have siderophore production ability with highest siderophore production index of 4.3 ± 0.10 by IR3 and least 2.2 ± 0.04 by IT2, IN4 (Table 8b) (Supplementary Plate 4.3). Similarly, siderophore production by endophytic bacteria has been investigated in few cases as a mechanism of certain bacteria to antagonize pathogenic fungi. Thus, it was observed that all the endophytic bacterial isolates from cotton roots having antagonistic activity with siderophores production (Li *et al*., 2010). Microorganisms release phosphorus (P) from inorganic forms by lowering pH with organic acids or by increasing chelation activities. Calcium-bound phosphorus (Ca–P) solubilizes at a pH of 2.5–4.0, while iron-bound phosphorus (Fe–P) requires an even lower pH (2.0–2.5) but is harder to dissolve due to its lower dissolution rate and higher solubility product. Organic acids are less effective for Fe–P solubilization because of soil-buffering and particle sorption (Cui *et al*., 2022). Siderophores, iron-chelating compounds produced by microorganisms, may play a key role in releasing P from Fe–P complexes, offering potential benefits for improving P and Fe availability to plants.

Production of HCN

The ability of the 149 isolates to produce HCN was determined by the picric acid assay (Table 7). No endophytic bacterial isolates of rice genotypes BPT-5204,

Table 9: Antagonistic activity of endophytic bacterial isolates against *Rhizoctonia solani* (*Rs*) and *Pyricularia oryzae* (*Po*)

Isolate No.	ID	BPT-5204		ID	Swarna		ID	MTU-1010		ID	IRGC-1220	
		Rs	***Po***		***Rs***	***Po***		***Rs***	***Po***		***Rs***	***Po***
1	BT1	+++	-	ST1	+	+	MT1	+	+	IT1	++	+
2	BT2	-	-	ST2	++	++	MT2	-	+	IT2	+++	+
3	BT3	-	++	ST3	-	+++	MT3	+++	++	IT3	++	-
4	BT4	-	-	ST4	-	+++	MT4	+	++	IT4	-	+++
5	BT5	-	+	ST5	-	+	MT5	-	-	IT5	-	-
6	BT6	-	-	ST6	-	-	MT6	-	-	IT6	-	-
7	BT7	-	-	ST7	-	+	MT7	-	+	IT7	-	-
8	BN1	-	-	ST8	+++	+++	MT8	+++	+	IT8	-	+
9	BN2	-	-	ST9	-	+	MN1	+	-	IT9	+++	++
10	BN3	-	+	ST10	-	-	MN2	-	-	IN1	-	++
11	BN4	-	-	ST11	-	+	MN3	+++	+++	IN2	-	+
12	BN5	+++	++	SN1	+++	++	MN4	-	+	IN3	-	+
13	BN6	-	-	SN2	-	+	MN5	-	-	IN4	-	++
14	BN7	-	-	SN3	+++	-	MN6	-	-	IN5	+	-
15	BN8	-	+	SN4	+++	-	MN7	-	-	IN6	+++	+++
16	BR1	+	++	SN5	-	-	MR1	-	-	IN7	+++	-
17	BR2	+++	++	SN6	+++	+++	MR2	-	-	IN8	+++	-
18	BR3	+++	++	SN7	+	+++	MR3	-	-	IR1	-	-
19	BR4	+	-	SN8	++	+	MR4	-	-	IR2	+++	-
20	BR5	+	-	SN9	-	+	MR5	+++	++	IR3	+++	+++
21	BR6	-	++	SN10	-	-	MR6	+	-	IR4	-	-
22	BR7	-	-	SN11	-	-	MR7	++	-	IR5	+	-
23	BR8	-	++	SN12	+++	++	MR8	-	-	IRen1	-	-
24	BR9	-	+	SR1	-	++	MR9	-	++	IRen2	-	++
25	BR10	-	+	SR2	+++	+	MRen1	+++	-	IRen3	-	-
26	BR11	-	-	SR3	-	+	MRen2	+	-	IM1	-	+
27	BR12	-	+	SR4	-	+	MRen3	+++	++	IM2	+++	++
28	BR13	-	+	SR5	-	+	MM1	-	-	IM3	+++	++
29	Bren1	++	+	SRen1	++	+	MM2	+	+	IM4	-	-
30	Bren2	+	+	SRen2	-	+	MM3	-	+	IM5	-	+
31	Bren3	+	-	SRen3	+	-	MM4	+	-	IA1	++	-
32	Bren4	++	++	SRen4	+	++	MM5	+++	+	-	-	-
33	Bren5	-	-	SRen5	-	-	MA1	+	-	-	-	-
34	BM1	+++	++	SM1	-	+	MA2	+	-	-	-	-
35	BM2	++	+	SM2	+	-	-	-	-	-	-	-
36	BM3	+	+	SM3	-	-	-	-	-	-	-	-
37	BM4	++	+	SM4	-	-	-	-	-	-	-	-
38	BM5	+	-	SM5	+	+	-	-	-	-	-	-
39	BM6	+	-	SA1	-	-	-	-	-	-	-	-
40	BM7	+	+	SA2	-	-	-	-	-	-	-	-
41	BA1	+	-	SA3	-	-	-	-	-	-	-	-
42	BA2	-	-	-	-	-	-	-	-	-	-	-
43	BA3	-	-	-	-	-	-	-	-	-	-	-

– No production+ Weak production, ++ Moderate production, +++ Strong production

SWARNA, MTU-1010, IRGC-1220 produce HCN while the positive control plate of *Pseudomons fluorescens* was positive for HCN production. Similar study was made using endophytes from black pepper wherein all positive isolates were proven to be pseudomonas genera (Szilagyi-Zecchin *et al.*, 2014; Aravind *et al.*, 2009). Not only phytoconstituents (Sanam *et al.*, 2024) and fungicides, but also endophytic bacteria producing HCN help in preventing fungal damage to crop. HCN produced by the bacteria increases the efficacy of antifungals against fungal infections aiding in healthy growth of plant and inturn high yields.

Antagonistic Activity

Antagonistic activities of 149 endophytic isolates were evaluated against *Rhizoctonia solani* and *Pyricularia oryzae*under in vitro conditions using dual culture plate technique on PDA medium (Table 9). Among 43 isolates of BPT-5204, 19 isolates have antagonistic activity against *R. solani* and 22 isolates have antagonistic activity against *P. oryzae*. Among 41 isolates of Swarna, 16 have activity against *R. solani*and 26 isolates against *P. oryzae*. Among 34 isolates of MTU-1010, 17 have antagonistic activity against *R. solani* and 14 have antagonistic activity against *P. oryzae*. Among 31 isolates of IRGC-1220, 14 isolates have antagonistic activity against *R. solani*and 16 isolates have antagonistic activity against *P. oryzae*. Another study wherein most of the chilli pepper endophytes had antagonistic activity against *R. solani* (Calvo *et al.*, 2010). This biocontrol activity may be due to production of ammonia (Sanam *et al.*, 2021), which is not studied in the present research, whereas siderophore production studies has been carried out.

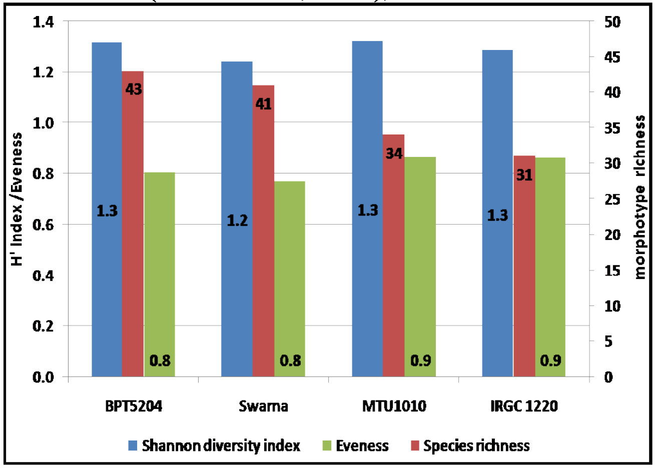

Fig. 1: Diversity indices of bacterial morphotypes from rice genotypes.

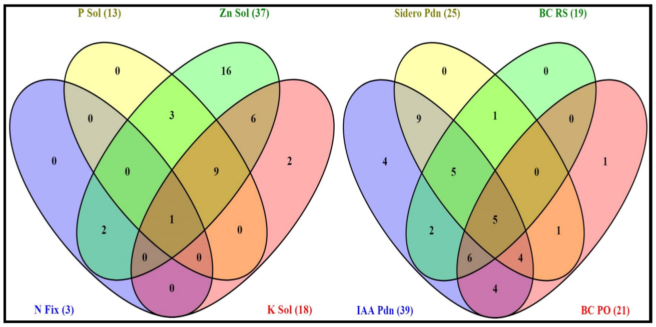

Fig. 2: Distribution of PGP activities in the morphotypes of BPT-5204. [P Sol- Phosphorous solubilization; Zn Sol- Zinc Oxide Solubilization; N Fix- Nitrogen Fixation; K Sol- Potassium Solubilization; Sidero Pdn- Siderophore Production; IAA Pdn- Indole Acetic Acid Production; BC RS- Biocontrol against *Rhizoctonia solani*; BC PO- Biocontrol against *Pyricularia oryzae*.]

The Distribution of PGP activities in the morphotypes of rice genotype

The Distributionof PGP activities in the morphotypes

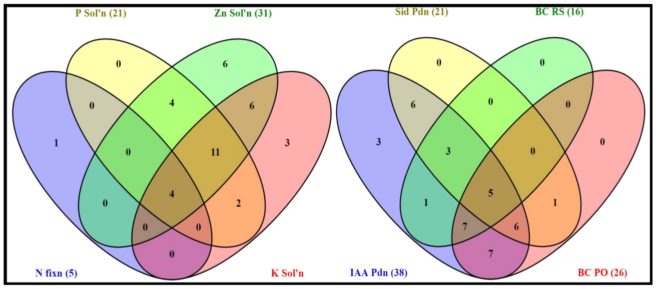

Fig. 3: Distribution of PGPR activities in the morphotypes of SWARNA. [P Sol- Phosphorous solubilization; Zn Sol- Zinc Oxide Solubilization; N Fix- Nitrogen Fixation; K Sol- Potassium Solubilization; Sidero Pdn- Siderophore Production; IAA Pdn- Indole Acetic Acid Production; BC RS- Biocontrol against *Rhizoctonia solani*; BC PO- Biocontrol against *Pyricularia oryzae*.]

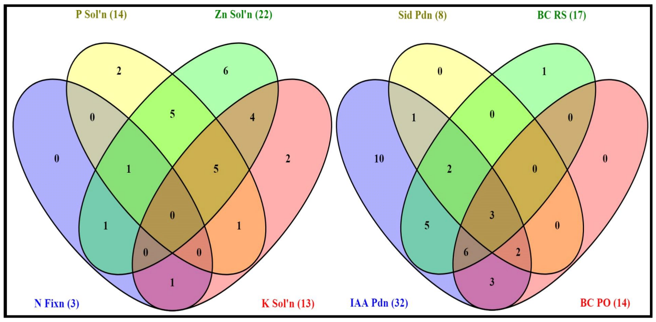

Fig. 4: Distribution of PGPR activities in the morphotypes of MTU-1010. [P Sol- Phosphorous solubilization; Zn Sol- Zinc Oxide Solubilization; N Fix- Nitrogen Fixation; K Sol- Potassium Solubilization; Sidero Pdn- Siderophore Production; IAA Pdn- Indole Acetic Acid Production; BC RS- Biocontrol against *Rhizoctonia solani*; BC PO- Biocontrol against *Pyricularia oryzae*.]

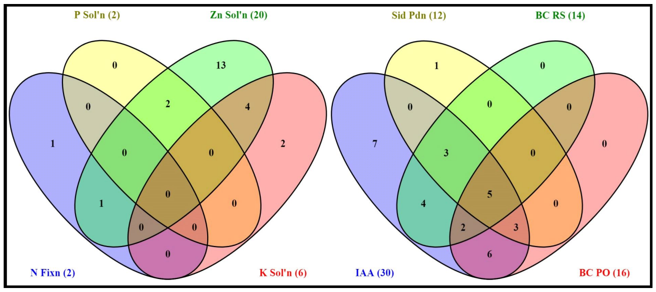

Fig. 5: Distribution of PGPR activities in the morphotypes of IRGC - 1220. [P Sol- Phosphorous solubilization; Zn Sol- Zinc Oxide Solubilization; N Fix- Nitrogen Fixation; K Sol- Potassium Solubilization; Sidero Pdn- Siderophore Production; IAA Pdn- Indole Acetic Acid Production; BC RS- Biocontrol against *Rhizoctonia solani*; BC PO- Biocontrol against *Pyricularia oryzae*.]

of rice genotype using venn diagram is presented in Fig. 2, 3, 4 and 5 for BPT-5204, Swarna, MTU 1010 and IRGC 1220, respectively. From Fig. 2 we can interpret that among the endophytes of BPT-5204 only one isolate was able to soluble all the three insoluble forms of P, K and Zn and nitrogen fixation, only five were able to produce IAA, siderophores and have antagonistic activity against selected plant pathogens. There exists a positive correlation between PGP activities and crop yields (Sanam *et al*., 2022). Among the 41 isolates from SWARNA genotype, only four were able to solubilize nutrients and fix nitrogen, whereas only five were able to have biocontrol activity, produce IAA and siderophore (Fig. 3). The figures d and e represent distribution of PGP activities of endophytes isolated from MTU 1010 and IRGC-1220, respectively, from the diagram it is very clear that none of isolates from both genotypes were able to solubilize all the three insoluble nutrients forms that are used in study and have nitrogen fixation activity. Nevertheless, three endophytes from MTU-1010 and five endophytic bacteria from IRGC-1220 had all the three activities (Siderophore and IAA production, biocontrol activity).

Conclusion

Endophytic bacteria isolated from root using six different media were observed to be more in BPT-5204 genotype with 43 isolates. Due to the presence of more endophytes might be one reason for better performance of BPT 5204 under field condition and gain more popularity among the rice genotypes. Endophytic bacterial isolates from BPT-5204 and SWARNA genotype are much more efficient than MTU 1010 and IRGC-1220 genotype isolates in having more number of endophytes and PGP activities namely nutrient solubilization, production of siderophores, nitrogenase enzyme and indole acetic acid, biocontrol activity against plant pathogen *R. solani* and *P. oryzae*. Further screening has to be carried out to know the isolates performance on inoculation to plants under in-vitro and in-vivo conditions and to identify the strain with best performance for mass multiplication as bioinoculant to benefit the farming community.

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Conflicts of Interest: There are no conflicts among authors

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