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Antifungal Trait and Plant Growth Promotion Potential of *Bacillus* Spp. from Rhizosphere Soils of Black Aromatic Rice, '*Chakhao*'

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Abstract

Manipuri black aromatic rice, '*Chakhao*', is a nutrient rich unique local rice cultivar of Manipur, India. Rhizosphere bacteria may have potential as antifungal agent and seedling growth promoter. Hence, the current investigation was aimed at screening, for the above traits, using bacteria, *Bacillus* spp., isolated from the soil of Rhizosphere of six *Chakhao* rice cultivars. Altogether, 323 bacterial isolates were obtained from the rhizospheric soils of 6 different cultivars of *Chakhao*, namely *Chakhao Amubi* (CA), *Chakhao Poireiton* (CP), *Chakhao Sempak* (CS), *Chakhao Angoubi* (CAng), *Chakhao Angangbi* (CR) and *Chakhao Wairi* (CW). All the rhizobacterial isolates were screened for antifungal activity against 5 rice fungal pathogens viz. *Rhizoctonia solani* (RS), *Fusarium oxysporum* (FO), *Curvularia oryzae* (CO), *Pyricularia oryzae* (PO) and *Aspergillus niger* (AN). The isolates were also subjected to PGP (plant growth promotion) assays such as ammonia, indole acetic acid (IAA), siderophore and 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase production, and phosphate solubilization. Of 323 isolates obtained, 64 were found to exhibit antifungal activity while 69 showed PGP traits. Twenty-five (25) isolates were found to be positive for both antifungal and PGP traits. Of 25 bioactive isolates, 4 (CR12, CW11, CA2 and CP2) potent isolates were shortlisted for further studies. The shortlisted potent isolates were subjected to quantitative estimation of PGP activities like phosphate solubilization, IAA and siderophore production. The strains could produce significant amount of IAA and siderophore and solubilize phosphate. Molecular characterization by 16S rDNA sequence analyses revealed the identity of the isolates: CR12:

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Bacillus subtilis (Accn. No.OM866257), CW11: *Bacillus paralicheniformis* (Accn. No.OM868047), CA2: *Bacillus sp.* CCMB1014 (Accn. No.OM868070) and CP2: *Bacillus licheniformis* (Accn. No.OM892495). Seedling vigor assays of the shortlisted potent strains were carried out on *Chakhao Amubi* rice. The isolates exhibited higher seedling vigor indices (CR12: 668.00, CA2: 618.66, CP2: 510.92 and CW11: 478.91) over the control (164.48). These rhizospheric strains have the potential to be developed as bioinoculants or biostimulants for enhancing *Chakhao* rice growth.

Introduction

The scented rice of Manipur, *Chakhao*, meaning delicious rice in Manipuri language, possess high socio-cultural values and is in close relationship with spiritual cultural practices of Meiteis. *Chakhao* has a unique flavour and aroma. It is served as a special item in cultural and religious ceremonies such as Usob (death ceremonies), Kang-pali (religious festival), Chak-umba (first rice-eating ceremony).[1] *Chakhao*, an indigenous rice variety of Manipur, is nutrient-rich and contains essential amino acids, minerals, and carotenoids. It may help prevent cancer, diabetes, heart disease, and Alzheimer's disease.[2-3] *Chakhao* is grown at certain parts of Imphal valley in Manipur. Due to its poor yield, *Chakhao* is grown in very limited acreage by farmers in Manipur for ceremonial and cultural purposes. There is a pressing need to increase its output because it is becoming more and more popular due to its nutraceutical characteristics and export demand.[4]

In modern agriculture, synthetic agrochemicals are used for enhancing crop production. The dependence on agrochemicals for improving crop production pose serious risks on ecological and human health.[5] Rhizobacteria are associated with the soils near the roots and intimately interact with the exudates of the roots.[6] Plant growth promoting rhizobacteria (PGPR) are among the beneficial soil microorganisms found in the rhizospheric region of the plant.[7] Intensive research on plant growth promoting bacteria (PGPB) is underway worldwide for developing biofertilizers and biocontrol agents (BCAs) as better alternatives to agrochemicals, as the latter harm the environment and human health besides possess the burden of high costs to poor farmers.[8] Biological control is slow but can be long lasting, inexpensive, and harmless to living organisms and the ecosystem; it neither eliminates the pathogen nor the disease, but brings them into natural balance.[9] As most PGPBs show inconsistent performance in the field conditions, there is urgent need for survey of indigenous strains suited to local conditions. The use of bacteria having biocontrol and PGP activities is urgently warranted to increase *Chakhao* growth.[10]

Certain bacterial taxa e.g., actinomycetes and *Bacillus spp* have been shown to play a significant function in the plant rhizosphere by secreting a variety of antimicrobial substances that inhibit the growth of common root diseases. It is reported that rhizospheric bacteria and other beneficial bacteria have the ability to solubilize phosphate and can produce indole acetic acid (IAA), siderophore, ammonia, 1- aminocyclopropane-1-carboxylic acid (ACC) deaminase and cell wall degrading enzymes such as chitinase, glucanase and protease.[11-16] Rhizospheric isolates associated with *Chakhao* varieties may have potential to produce unique metabolites, which can be exploited for application in agricultural, pharmaceutical and other industries. The present study aims at investigating the antifungal and biofertilizing potential of the rhizospheric isolates from 6 different *Chakhao* cultivars found in Manipur.[17]

Materials and Methods

Soil Sampling and Bacterial Isolation

Rhizospheric bacteria were isolated from all 6 rhizospheric soil samples collected from Wairi, Imphal East (93.88E, 24.78N) and Lilong Leihaokhong, Thoubal (93.93E, 21.70N), using 3 different media i.e., Starch Casein Nitrate Agar (SCNA), Nutrient Agar (NA) and Gauze Medium No. 1 (GM1).

1 g of the soil sample was added to 99 ml of sterile distilled water (SDW). The sample was thoroughly vortexed (150 rpm, 30°C, 10 min). Serial dilutions of the soil suspensions were then performed

(10^{-2} to 10^{-6}). The aliquots of various dilutions from 10^{-2} to 10^{-6} were spread plated on agar plates. The isolation plates were incubated for 3-4 days at 30°C. Morphologically distinct bacterial colonies were sub-cultured till pure cultures were obtained.[18]

Antifungal Assay

Antifungal assay of the rhizospheric bacteria were carried out by dual culture method.[19-20] The inhibition zones are due to diffusible compounds by the bacterial isolate in the antifungal assay. After the full growth of fungal mycelia on the control plates, the inhibition in the remaining plates was measured. The percentage of mycelial growth inhibitions was calculated using the formula:

{(R-r)/} ×100

Where,

R represents the radial growth of the test pathogen in the control plates (measured in mm), and r is the radial growth of the test pathogen in the test plates.

Screening of Plant Growth Promoting (PGP) Traits

Indole Acetic Acid (IAA) Production Test

IAA production was determined by inoculating bacterial isolates in NB medium containing 2 mg/ml of L-tryptophan, (150 rpm, 30ºC, 5 d).[21] The fully grown culture broth was centrifuged (10,000 rpm, 10 min) and 1 ml of the supernatant was mixed with 2 ml of Salkowski's reagent. A pink colour indicates a positive test for IAA production.

For the quantitative assay, the bacterial isolates were inoculated in NB containing 2 mg/mL of Tryptophan under shaking conditions (150 rpm, 30ºC). 5 mL aliquot was withdrawn periodically from each culture flask at 24 hours intervals and centrifuged at 10,000 rpm for 10 min.[21] 1 mL of the supernatant was mixed with 2 mL of Salkowski's reagent and incubated at 30ºC for 20 min. The absorbance was measured at 530 nm and the amount of IAA produced was calculated by comparing it with the standard IAA curve.

Phosphate Solubilization Test

Spot inoculation on NBRIP-BP (National Botanical Research Institute's Phosphate Growth Medium) was carried out to determine phosphate solubilization by bacterial isolates. A halo zone around the bacterial colony after 4 days of incubation at 30°C indicated a positive test.[22]

Quantitative estimation of phosphate solubilization was done by inoculating in 100 mL of NBRIP-BP medium (pH 7). It was then incubated in a shaker incubator (150 rpm, 30°C). 5 mL aliquot withdrawn periodically at 24 hours intervals was centrifuged (10,000 rpm, 10 min) and the supernatant was analysed for pH and Phosphate concentration.[23] KH_2PO_4 was used as the standard.[24]

Siderophore Production Test

SCNA (without iron) amended with CAS-substrate (Chrome Azurol S) were inoculated with 6 mm agar plugs of bacterial isolate and incubated at 30°C for 7 days. The formation of an orange colour halo surrounding the colony was considered a positive result for siderophore production.[25]

Quantitative estimation of siderophore production was done on five different iron deficient liquid media: Starch casein nutrient broth (SCNB), Casamino acid medium (CAA), Nutrient broth (NB), Succinic acid medium (SM) and Bharbhiaya and Rao medium (BR) by CAS-shuttle assay.[26-27] 5 mL aliquots were withdrawn periodically at 24 hours intervals and centrifuged (10,000 rpm, 10 min). An equal volume of CAS reagent was added to the supernatant. Absorbance at 630 nm was noted. A reference made of 1 mL uninoculated broth and 1 mL CAS reagent was used. The amount of siderophore produced (percentage siderophore units) was calculated by using the formula:

Percentage siderophore units = (Ar − As)/Ar× 100

Where,

Ar represents the absorbance of reference and As represents the absorbance of the sample at 630 nm.

Ammonia Production Test

The bacterial isolates were inoculated in 10ml of peptone water and incubated in a shaker incubator (150 rpm, 30°C) for 4 days. To each test tube, 0.5 ml of Nessler reagent was then added. A colour change of brown to yellow indicated ammonia production by the bacterial isolates.[28]

1-Aminocyclopropane-1-Carboxylic Acid (ACC) Deaminase Production Test

Nitrogen-free Dworkin and Foster's (1958), DF salts minimal agar medium supplemented with 2 g of $(NH_4)_2SO_4$ as a sole nitrogen source was used for screening ACC deaminase production.[29-30] Isolates were inoculated on the media and then incubated at 30°C for 4 days. Bacterial growth in the plates indicated a positive test for ACC deaminase production.

***In Vitro* Seed Germination (Vigor Index)**

In Vitro rice seed germination by the isolates were carried out according to Tamreihao *et al*. (2016).[31] The bacterial isolates were inoculated in NB and incubated for 3 days to get fully grown cultures. It was then centrifuged (10,000 rpm, 10 min). The pellets were collected and washed thrice with SDW (Sterile Distilled Water) and culture suspensions were prepared using SDW. Black rice (Variety: *Chakhao Amubi*) seeds were surface sterilized with 70% ethanol for 5 min, followed by 0.2% sodium hypochlorite for 5 min, and rinsed four times with SDW. Surface sterilized seeds were soaked in the cell suspensions and kept under shaking conditions (150 rpm, 30°C) for 2 hours. The seeds were then transferred to sterile plates containing wetted filter papers (10 seeds per plate). Untreated sterile seeds soaked in SDW were used as a control. The plates were then incubated at 28°C for 5 days. The number of germinated seeds, roots and shoot lengths were measured and vigor indices were calculated using the following formula[32];

Vigor index= Percentage germination × Seedling length

Where,

Seedling length= shoot length + root length

Statistical Analysis

All the data were subjected to one-way analysis of variance (ANOVA) followed by independent t-test ($p \leq 0.05$) using the SPSS software.

Strain Identification

Genomic DNA extraction and PCR amplification of 16S rDNA sequences were performed.[33] The PCR products obtained were sent to AgriGenome Labs Pvt. Ltd. (Kerala, India) for sequencing. The bacterial sequences were subjected to BLAST alignment analysis using the NCBI GenBank database to obtain the accession number.[34]

Results

Isolation

Altogether, 323 morphologically distinct isolates were obtained from rhizospheric soils of six (6) different cultivars of *Chakhao*. These isolates were preserved in 30% glycerol stock at − 20°C for further studies.

Antifungal Assay

All the 323 isolates were screened for antifungal assays against the test fungal pathogens, of which 64 showed antifungal activities against one or more fungal pathogens. Four (4) isolates (CR12, CW11, CA2 and CP2) exhibited potent antifungal activity (Figs. 1-2 and Table 1).

Plant growth promoting (PGP) Activities

All the isolates were then subjected to screening for PGP traits such as IAA, siderophore, ammonia and ACC deaminase production, and phosphate solubilization. 69 isolates showed positive results for one or more PGP traits (Table 2).

Shortlisting of Promising Isolates

Based on the screening of antifungal and PGP activities, 4 most potent isolates (CR12, CW11, CA2 and CP2) were shortlisted for molecular characterization and seedling vigor studies.

Quantitative Estimation of PGP Activities

The shortlisted potent strains were subjected to quantitative estimation of PGP activities like phosphate solubilization, IAA and siderophore production. The strain CR12 produced the highest amount of IAA (73 µg/mL) followed by CW11 (71.4 µg/mL), CP2 (70.5 µg/mL) and CA2 (62.91 µg/mL) respectively after 10 d of incubation. The strain CA2 could solubilize the highest amount of inorganic P (up to 200 µg/mL) after 3 d of incubation with concomitant decrease in medium pH (from 5.5 to 2.69) followed by CR12, CW11 and CP2 (up to 156.006, 113.5 and 105.7 µg/mL respectively). CA2 showed maximum siderophore production (74.37% siderophore units in SCNB) followed by CR12, CP2 and CW11 (73.15% in NB, 67.80% in SCNB and 65.50% in NB respectively) after 6 d of incubation.

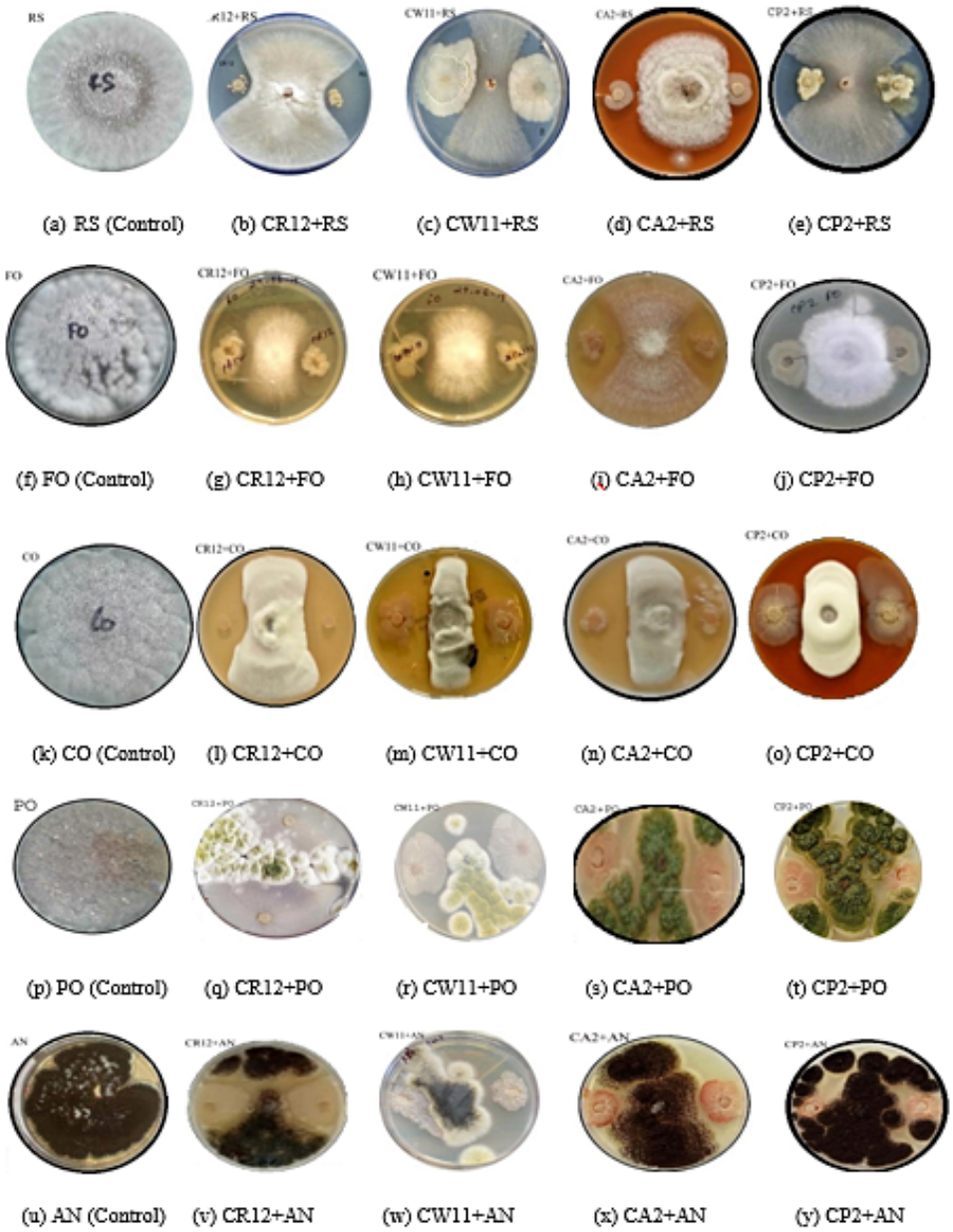

Fig. 1: Antifungal activities of the 4 best strains against the fungal pathogens (a-y)

Characterization of Promising Strains

Strain CR12 showed the highest 16S sequence similarity with *Bacillus subtilis*, CW11 with *Bacillus paralicheniformis*, CA2 with *Bacillus sp. CCMB1014* and CP2 with *Bacillus licheniformis*. They were deposited in NCBI to obtain the accession numbers. Hence, they are designated as *Bacillus subtilis* strain CR12 (Accn. No.OM866257), *Bacillus paralicheniformis* strain CW11 (Accn. No.OM868047), *Bacillus sp. CCMB1014* strain CA2 (Accn. No.OM868070) and *Bacillus licheniformis* strain CP2 (Accn. No. OM892495).

***In Vitro* Seed Germination (Vigor Index)**

Seedling vigor assays for the 4 selected promising strains (CR12, CW11, CA2, and CP2) were done on

Chakhao Amubi. The isolates exhibited higher vigor indices (CR12: 668.00, CW11: 478.91, CA2: 618.66 and CP2: 510.92) relative to the control (164.48) as shown in Fig. 3 and Table 3.

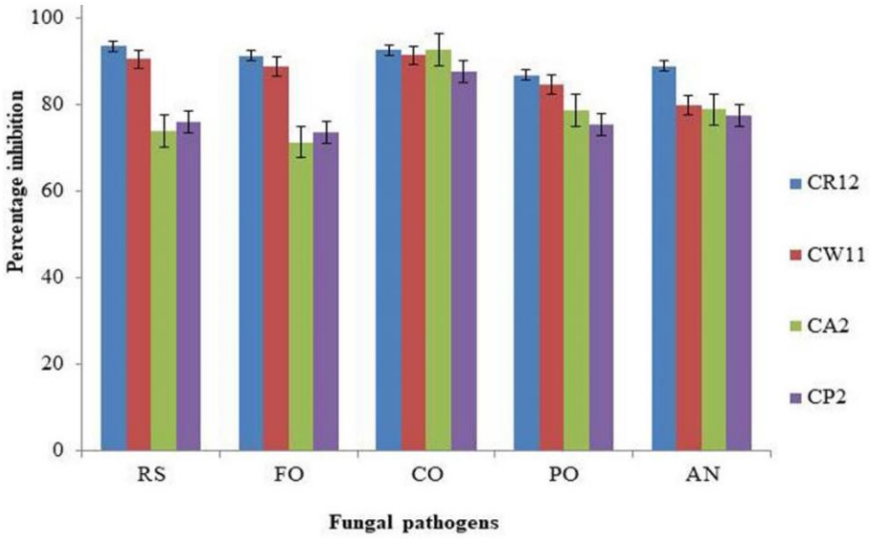

Fig. 2: Graphical representation of antifungal activities of the 4 best strains

Table 1: Antifungal assay of the rhizospheric bacterial isolates

Sl. No.	Bacterial Isolates	Percentage Inhibition Zone				
		MTCC4633 (RS)	MTCC287 (FO)	MTCC2605 (CO)	MTCC1477 (PO)	MTCC1344 (AN)
1.	CA1	67.4	-	69.3	-	-
2.	**CA2**	**73.75**	**71.25**	**92.5**	**78.5**	**78.75**
3.	CA3	-	65.3	57.2	-	-
4.	CA6	45.4	-	51.6	-	-
5.	CA8	-	60.4	71.6	-	-
6.	CA11	52	-	-	-	-
7.	CA12	-	65	-	-	-
8.	CA14	-	43.7	61.4	-	-
9.	CA21	47.3	-	-	-	-
10.	CA23	-	56.8	-	-	-
11.	CA28	-	-	71	-	67.3
12.	CA33	64.3	-	-	-	-
13.	CA37	-	-	53.2	-	-
14.	CA45	47.2	-	-	34.3	-
15.	CA46	-	61	-	-	-
16.	CA51	-	-	56.4	-	-
17.	CA54	53.2	-	55.6	-	-
18.	CR1	43	-	51.5	-	-
19.	CR3	-	54	61.4	-	-
20.	CR8	37	48.5	-	-	-
21.	CR10	45	53.3	-	-	-

22.	**CR12**	**93.4**	**91.2**	**92.5**	**86.7**	**88.8**
23.	CR13	56.3	-	62.3	-	-
24.	CR19	-	57.4	-	-	-
25.	CR25	-	-	80.6	-	-
26.	CR26	-	-	-	45.3	54.2
27.	CR31	58.3	-	60.3	-	-
28.	CR35	34	-	42	-	-
29.	CR41	-	-	56.8	-	-
30.	CR42	-	57.3	-	-	-
31.	CR53	46.7	-	-	-	-
32.	CR58	-	35	46.3	-	-
33.	CR60	-	-	-	-	65.4
34.	CR62	-	-	-	43.6	-
35.	CR65	-	-	79.5	-	-
36.	CR68	-	45.6	57.5	-	-
37.	CR71	76.3	-	-	-	-
38.	CR74	-	-	65.4	-	-
39.	**CP2**	**75.8**	**73.4**	**87.6**	**75.3**	**77.3**
40.	CP4	-	54.8	67.4	-	-
41.	CP12	46	-	-	-	-
42.	CP16	-	-	-	56.4	67
43.	CP22	43	54.5	-	-	-
44.	CP29	41.4	46.7	-	-	-
45.	CP31	-	-	73.7	-	-
46.	CW7	-	-	-	65.4	43.6
47.	CW8	-	-	64.8	-	-
48.	**CW11**	**90.4**	**88.7**	**91.3**	**84.5**	**79.7**
49.	CW13	84.3	78.6	-	-	-
50.	CW24	-	49	58.4	-	-
51.	CW25	72	-	-	-	-
52.	CW34	-	-	69.6	-	-
53.	CW41	-	-	-	-	77
54.	CS5	-	-	37.6	-	-
55.	CS9	53.2	-	47.4	-	-
56.	CS13	81.4	75.8	80.6	-	-
57.	CS23	72	-	-	-	-
58.	CS32	-	43.6	35	-	-
59.	CS40	-	-	56.9	-	-
60.	CAng4	74	-	57.8	-	-
61.	CAng11	-	-	-	43	37.8
62.	CAng16	29	-	-	-	-
63.	CAng24	-	-	43.4	-	-
64.	CAng31	-	-	46.7	-	-

Discussion

According to a survey of *Chakhao* rice cultivation in some selected villages of Manipur by Borah *et al*. (2018), it was found that the yield of *Chakhao* is generally lower (~1.3-1.8 tons/hectare) as compared to hybrid and traditional rice varieties (~2.0-5.5 tons/hectare).[35] Farmers generally do not apply chemical fertilizers and farmyard manure in the cultivation of *Chakhao* rice as application of excess inorganic and organic fertilizer increases lodging and sterility.[1] So, there is an urgent need to search for organic fertilizers that can enhance *Chakhao* rice growth.

Table 2: PGP assays of rhizospheric bacterial isolates

Sl. No.	Bacterial Isolates	Plant growth promoting traits				
		IAA production	Phosphate solubilization	Siderophore production	Ammonia production	ACC production
1.	CA1	+	-	-	+	+
2.	**CA2**	**+**	**+**	**+**	**+**	**+**
3.	CA3	-	-	-	+	-
4.	CA7	+	-	-	-	+
5.	CA8	-	-	-	+	-
6.	CA10	+	-	-	+	-
7.	CA12	-	-	-	+	-
8.	CA14	+	-	-	-	-
9.	CA21	-	+	-	-	+
10.	CA22	-	-	-	+	-
11.	CA25	+	-	-	-	-
12.	CA28	-	+	-	-	+
13.	CA31	+	-	-	+	-
14.	CA37	-	-	-	-	+
15.	CA39	+	-	-	-	-
16.	CA41	-	-	-	+	-
17.	CA44	-	-	-	-	+
18.	CA53	-	-	-	-	+
19.	CA54	-	-	-	-	+
20.	CA57	-	-	-	+	-
21.	CA60	-	-	+	-	-
22.	CA62	-	-	-	+	+
23.	CA69	+	-	-	+	-
24.	CR1	+	-	-	+	-
25.	CR2	+	-	-	+	-
26.	CR4	-	+	-	-	-
27.	CR7	-	+	+	-	-
28.	CR8	-	-	-	+	-
29.	CR10	-	-	-	+	+
30.	**CR12**	**+**	**+**	**+**	**+**	**+**
31.	CR21	-	+	-	-	-
32.	CR26	-	-	-	+	-
33.	CR32	-	-	-	+	-
34.	CR37	+	-	-	-	+
35.	CR39	+	-	-	-	+
36.	CR45	-	+	-	-	+
37.	CR47	-	-	+	+	-
38.	CR54	-	-	-	+	-
39.	CR63	-	-	-	-	+
40.	CR68	-	-	-	-	+
41.	CR71	-	-	-	+	-
42.	CR75	-	-	-	+	-
43.	CR77	-	-	-	+	+
44.	**CP2**	**+**	**+**	**+**	**+**	**+**

45.	CP3	+	-	-	+	-
46.	CP7	+	-	-	-	-
47.	CP8	-	+	-	+	-
48.	CP13	-	-	-	+	-
49.	CP16	-	-	-	-	+
50.	CP24	-	-	-	+	+
51.	CW1	-	-	-	+	-
52.	CW2	-	-	-	+	-
53.	CW4	+	-	-	-	+
54.	CW6	-	-	-	-	+
55.	CW11	+	+	+	+	+
56.	CW13	-	+	-	+	-
57.	CW27	-	-	-	+	+
58.	CW45	+	+	-	-	-
59.	CS4	-	-	-	+	-
60.	CS6	-	-	-	+	-
61.	CS13	+	-	+	-	+
62.	CS28	+	-	-	-	+
63.	CS37	+	-	-	-	+
64.	CS42	-	+	+	-	-
65.	CAng1	+	-	-	-	-
66.	CAng4	+	-	-	-	-
67.	CAng9	-	+	-	-	-
68.	CAng24	-	-	-	+	-
69.	CAng31	-	-	-	+	-

Table 3: Vigor indices of the shortlisted best strains against control

Treatments	Fresh weight (gm)	Dry weight (gm)	Root lengt (cm)	Shoot length (cm)	% seed germination	Vigor index
Control	0.07±0.01b	0.02±0.00b	1.42±0.78b	0.95±0.53b	69.4±2b	164.48
CR12	0.09±0.01a	0.03±6.01a	4.29±0.7a	3.39±0.53a	86.98±1.2a	668.00
CW11	0.06±0.00c	0.02±0.00b	3.7±0.74c	2.70±0.48c	74.83±1.88c	478.91
CA2	0.07±0.00b	0.03±0.00a	4.29±0.77a	3.38±0.53a	80.66±9.90d	618.66
CP2	0.06±0.00c	0.02±0.00b	3.83±0.67c	3.04±0.47a	74.37±2.13c	510.92

*Values with different alphabet within a column are significant at P≤0.05

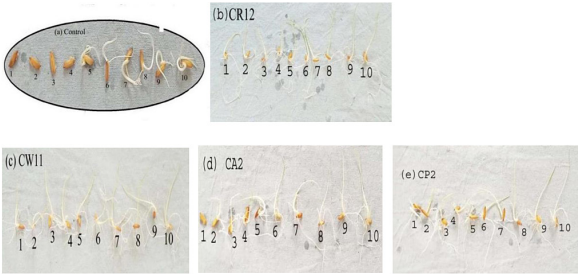

Fig. 3: Seed germination assays of *Chakhao Amubi* rice- (a) Control, (b) CR12, (c) CW11, (d) CA2 and (e) CP2

In our study, plant growth promoting rhizobacteria (PGPR) were isolated from the rhizospheric soils of 6 *Chakhao* rice cultivars cultivated at different locations of Manipur, India. Even though there are many reports on the nutritional value of *Chakhao* rice and its importance to the people of Manipur, the application of PGPRs in *Chakhao* cultivation is still an underexplored area.

In the present study, 4 multi-trait PGP rhizospheric strains viz.CR12 (*Bacillus subtilis* strain CR12), CW11 (*Bacillus paralicheniformis* strain CW11), CA2 (*Bacillus sp. CCMB1014* strain CA2) and CP2 (*Bacillus licheniformis* strain CP2) were chosen as potent biocontrol and biofertilizing agents as they can inhibit all the 5 fungal pathogens tested and were positive for all the PGP traits. The shortlisted potent strains exhibited antifungal activities against the 5 test fungal pathogens and showed significant mycelial growth inhibition against the fungal pathogens. *Bacillus amyloquefaciens* strain Bk7, a PGPR isolated from rice rhizosphere could inhibit the mycelial growth of *Magnaporthe oryzae, Rhizoctonia solani, Botrytis cinerea and Fusarium graminearum*.[36] *Bacillus spp*. strains B-1, B-2, B-3 and B-4 isolated from rice rhizospheric soils of Odisha, India showed strong antifungal activity (upto 90%) against the phytopathogens like *Rhizoctonia solani*, *Sclerotium rolfsii* and *Sclerotium oryzae invitro*.[37] A recent report showed a rice rhizospheric antifungal *Bacillus sp.* KM5 synthesized prominent antibiotics such as Bacillomycin F, Bacillomycin D, Bacillopeptin A, etc.[38] Significant inhibition of the five (5) fungal pathogens by the potent shortlisted strains can also be noted. Therefore they may hold potential to be developed as antifungal agents for rice cultivation.

Bacillus spp. strains K3 and N4 isolated from rice rhizospheric soil samples from different villages of Bardoli, Gujarat, India were reported to exhibit PGP activities like phosphate solubilization, IAA and siderophore production39. Phosphate solubilizing rhizobacteria (PSRB) such as Pseudomonas aeruginosa, *Bacillus subtilis* strain 1, *Bacillus subtilis* strain 2 and *Bacillus subtilis* strain 3, isolated from rice rhizosphere have been reported as promising agents for rice cultivation in soils deficient in phosphorus.[40] A rhizobacterial isolate UPMB19, belonging to the genera Lysinibacillus obtained from rice rhizosphere has been reported as a PGPR showing IAA and siderophore production and phosphate solubilization which can promote rice seed growth significantly.[41] ACC deaminase producing *Bacillus sp.* strain SB1-ACC3 isolated from the coastal rice field soils of West Bengal, India promoted rice seed germination.[42] Streptomyces corchorusii strain UCR3-16, obtained from rice rhizospheric soils showed antifungal activities, found positive for plant growth promoting traits such as IAA, ammonia, siderophore, ACC deaminase production and phosphate solubilization and the strain showed significant increase in growth and grain yield of rice plants.[27] The strains (CR12, CW11, CA2 and CP2) can produce IAA, siderophore, ammonia, ACC deaminase and solubilize phosphate. The shortlisted potent strains have the potential of plant growth promoting traits.

Strain CR12 exhibited production of phytohormone IAA in significant amount. It produced 73.83 µg/mL IAA on 10th day of incubation. A rhizospheric *Bacillus sp.* BPR7 isolated from common bean produced 17 µg/mL of IAA in 24 hrs incubation.[43] Goswami *et al*. (2014) has reported IAA production by PGPR from saline desert of Kutch in the range of 25 µg/mL of IAA or more after 72 hrs of incubation.[44] Strain CA2 could solubilize phosphate significantly (200 µg/mL) with a corresponding drop in pH (2.69) on the 3rd day of incubation. *Bacillus sp.* BPR7 isolated from common bean rhizosphere could solubilize upto 23 µg/mL of TCP (tricalcium phosphate) on the 7th day of incubation.[43] Yu *et al*. (2011) has reported several phosphate solubilizing bacterial (PSB) strains, from Walnut could solubilize phosphate in the range of 81.09 mg/L – 233.35 mg/L.[45] The effects of PSB on the growth of several crop plants have been reported by several workers who conducted their research under both greenhouse and limited field trial experiments.[46-47] Strain CA2 could produce siderophore in significant amount (74.37%) in SCNB. A common bean rhizospheric *Bacillus sp.* BPR7 could produce siderophore upto 38 µg/mL in 72 hrs.[43] The application of siderophore producing rhizobacteria as plant growth promoters have been documented by various researchers.[48-49]

Chakhao rice seedling germination by CR12, CW11, CA2 and CP2 were performed and exhibited higher vigor indices over the control. Vigor indices of CR12 (668.00), CW11 (478.91), CA2 (618.66) and CP2 (510.92) were found to be higher than vigor index

of control (164.48). The PGPR strains UPMB10 (*Bacillus sphaericus*), *Rhizobium strains* (SB16, UPMR1006 and UPMR1102) reported by Mia *et al*. (2012) showed significant increase in seedling vigor and overall root growth of lowland rice variety MR21950. *Bacillus subtilis* strain MBI600, a PGPR obtained from Department of Entomology and Plant Pathology, Auburn University, AL, USA enhanced seedling vigor index of rice (13192) relative to the control (5028).[51]

Conclusion

The best approach for sustainable *Chakhao* rice agriculture will be the use of microbes especially rhizospheric bacteria associated with the host plants. In the present study, 4 most promising rhizospheric bacteria from 6 different cultivars of *Chakhao* with PGP potential were found. All 4 shortlisted strains showed good inhibition against major rice fungal pathogens and so they can be used as promising antifungal agents. Moreover, these isolates also showed significant PGP activities. Then shortlisted strains showed notable higher seedling vigor indices over the control under *In Vitro* conditions. Further studies are underway to investigate and confirm their growth promotion potency and biocontrol efficacy under pot trial and limited field conditions.

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Conflict of Interest

The authors declare no conflict of interest.

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