https://doi.org/10.33451/florafauna.v28i2pp177-190
FLORA AND FAUNA ISSN 2456 - 9364 (Online)
2022 Vol. 28 No. 2 PP 177-190 ISSN 0971 - 6920 (Print)

Isolation and bioactivity screening of *Streptomyces* spp. associated with *Curcuma caesia (Yaimu)* from Manipur, India

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Received **:** 20.09.2022; ***Accepted*** : 20.10.2022

ABSTRACT

Thirty four (34) endophytic bacteria were isolated from ***Curcuma caesia***, locally known as *Yaimu*. The putative endophytic isolates were subjected to antibacterial screening against four (4) bacterial test organisms and antifungal assays against six (6) indicator rice fungal pathogens. The isolates were further screened for plant growth promoting (PGP) traits such as IAA, siderophore, ammonia production and phosphate solubilisation. Four (4) isolates (MBRL 750, MBRL 752, MBRL 755 and MBRL 757) were positive in all the PGP traits. MBRL 755, the most potent PGP strain and another promising strain MBRL 750 produced significant amounts of IAA (**138 ìg/mL, 76 ìg/mL** respectively) and ammonia (**27.4 µg/ml, 23.2 ìg/mL** respectively) as well as efficiently solubilised phosphate (**50 µg/ml, 22 ìg/mL** respectively). These bioactive isolates were subjected to rice seedling vigor assays (Variety: *Jatra*). The best isolate, MBRL 750, showed higher vigor index (199.8) over the control (181.5). The shortlisted bioactive strains (MBRL 755, MBRL 750) were characterised by 16S rDNA sequencing and designated as *Streptomyces* sp. MBRL 755 and *Streptomyces* sp. MBRL 750. The bioactive strains MBRL 755 and MBRL 750 hold promise for development as bioinoculants for rice cultivation.

Figures : 06 References : 62 Tables : 07
KEY WORDS : Antimicrobial assays, *Curcuma caesia,* Endophytic bacteria, Isolates, Seed vigor index, *streptomyces* spp.

Introduction

Control of plant diseases and practice of environmentally sound and sustainable crop production are the major challenges for agriculture in the 21st century. Crop plants including rice are subjected to many challenges such as fungal diseases, decline of soil fertility as well as climate change, leading to reduce crop yields[40]. Heavy inputs of synthetic agrochemicals such as fertilizers and fungicides are needed to enhance the yields. However, synthetic agrochemicals pose serious cost burdens to poor farmers and cause adverse effects on ecosystem and human health. Therefore, the use of bacteria having plant growth promotion and/or biocontrol activity holds great promise for a sustainable, ecofriendly, productive and organic agriculture. Bacterial bioinoculants may be developed as supplements or alternatives to the use of synthetic agrochemicals. Many bacterial species can promote plant growth by producing hormones such as Indole Acetic Acid (IAA) [11, 39, 44], siderophores[19], ammonia[50] as well as by mobilising phosphate[17].

There is a sharp decline in the discovery of novel bacteria and natural products from terrestrial habitats as they have been over-exploited in the past several decades[52]. Hence, increasing emphasis is now being given to exploration of novel and unique ecosystems such as freshwater and marine ecosystems, pristine habitats, caves and deserts[1, 2, 59]. One promising biotope for discovery of bacteria with potential agricultural and pharmaceutical applications is the endosphere of medicinal plants[36, 46].

Endophytes are microbial entities that live inside the living tissues of higher plants without causing any overt symptoms on the host. They are known to be rich reservoirs of biologically active secondary

ACKNOWLEDGEMENTS : Shaheen K would like to thank the Department of Science & Technology (DST), Govt. of India (GOI) for providing INSPIRE Fellowship (DST/ INSPIRE Fellowship/ 2014/195). DSN and SM would like to thank the Department of Biotechnology (DBT), GOI, for support under the scheme, Establishments of State Biotech Hub (BT/04/NE/2010). The authors are grateful to Eurofins, Bangalore and Agrigenome, Kerala for 16S rDNA sequencing of the endophytic strains.

TABLE-1. Primary screening of endophytic bacterial isolates

S. No	Bacterial isolates	Test organisms			
		MTCC 106	MTCC 121	MTCC 739	DN1
1.	DSN1	+	+	-	-
2.	DSN2	-	-	-	-
3.	DSN3	-	+	-	-
4.	DSN4	-	+	-	-
5.	DSN5	-	-	-	-
6.	DSN6	-	+	-	-
7.	DSN7	-	+	-	-
8.	DSN8	-	+	-	-
9.	DSN9	-	+	-	-
10.	DSN10	-	-	-	-
11.	DSN11	-	-	-	-
12.	DSN12	-	+	-	-
13.	DSN13	-	+	-	-
14.	DSN14	-	-	-	-
15.	DSN15	-	-	-	-
16.	DSN16	-	-	-	-
17.	DSN17	-	+	-	-
18.	DSN18	-	++	-	-
19.	DSN19	-	-	-	-
20.	DSN20	-	-	-	-
21.	DSN21	+	++	+	-
22.	DSN22	-	+	-	-

23.	DSN23	-	+	+	-
24.	DSN24	+	-	-	-
25.	DSN25	-	-	-	-
26.	DSN26	-	-	-	-
27.	DSN27	-	-	-	-
28.	DSN28	-	-	-	-
29.	DSN29	-	-	-	-
30.	DSN30	-	-	-	-
31.	DSN31	-	-	-	-
32.	DSN32	-	-	-	-
33.	DSN33	-	-	-	-
34.	DSN34	-	-	-	-

Note: +++ (> 50%), ++ (< 50%), + (< 30%), - no inhibition zone

metabolites[27, 29]. Endophytes produce a wide spectrum of bioactive natural products such as alkaloids, benzopyranones, chinones, flavonoids, phenolic acids, quinones, steroids, terpenoids, tetralones, xanthones, isocoumarins, lignans and others[30, 58]. These bioactive compounds have a wide range of applications such as agrochemicals, antibiotics, immunosuppressants, antiparasitics, antioxidants, anticancer[21], antitubercular[24] and antidiabetic agents[26].

Manipur lies in one of the 4 biodiversity Hotspots-the Indo-Burma hotspot-in India[25]. There is great promise for discovery of bioactive bacterial strains in underexplored habitats such as Manipur for potential applications in medicine, agriculture and industry. Meagre studies have been done on isolation and characterisation of endophytic bacteria from medicinal plants in Manipur. Plants growing in biodiversity rich zones are likely to house endophytes with equal or greater biodiversity. Moreover, plants with a long ethnobotanical history are expected to harbour potent bioactive endophytes relative to other plants[53, 56].

Curcuma caesia (Black turmeric), locally known as *Yaimu,* is an endangered species of ethnomedicinal plants of North East India[4] which is a perennial herb distinctive from other species of *Curcuma* by its bluish black rhizomes[35]. Traditionally, the rhizome is used in the treatment of various diseases such as leucoderma, cancer, bronchitis, piles and asthma while the leaf paste finds use in curing rheumatic pains and bruises[51]. There are various reports for its wide spectrum of bioactivities including antifungal[7], antibacterial[48], antioxidant[37], antiasthmatic[5], antianxiety and antiepileptic[32], antihelminthic[18], bronchodilating[43] and anti-ulcer[13] activities. The present study aims to isolate and characterise bioactive endophytic bacteria associated with *Curcuma caesia* (Black turmeric).

Materials and Methods

1. Sampling and Isolation of endophytic bacteria

A healthy growing plant of *Curcuma caesia* locally known as *Yaimu*, was collected from Chingmeirong Mamang Leikai, Imphal East, Manipur ($24^0 49^{/} 36^{//}$ N and $93^0 56^{/} 47^{//}$ E). The plant was subjected to five-step surface sterilisation process to isolate the endophytic bacteria. The steps included 4-10 min washing with 4% sodium hypochlorite (NaOCl), 10 min washing with 2.5% sodium thiosulphate ($Na_2S_2O_3$), 5 min wash with 75% ethanol, followed by washing with SDW and final rinsing with 10%

TABLE-2 : Antibacterial activity profile of bacterial isolates (Kirby Bauer method)

S. No	Bacterial isolates	MTCC 106	MTCC 121	MTCC 739	DN1
		Inhibition zone (mm)			
1.	DSN1	20.0	-	-	-
2.	DSN7	-	15.0	-	-
3.	DSN9	-	16.5	-	-
4.	DSN18	15.2	-	-	-
5.	**DSN21**	**-**	**17.5**	**18.0**	**-**

Note: An inhibition zone of 17mm or above is considered potent.

sodium bicarbonate ($NaHCO_3$)[47]. The surface sterilised sample was cut into small pieces (1 cm) using sterile blade. Four different media were used for isolation: Starch Casein Nitrate Agar (SCNA), 2.5% Water Agar (WA), Tap Water Yeast Extract Agar (TWYE) and Tap Water Peptone Agar (TWPA). The master plates were kept for incubation at 30Ú˜C for 3-4 weeks.

2. Antimicrobial assays

Test organisms

Bacterial test organisms used in our study were *Bacillus subtilis* (MTCC 121), *Micrococcus luteus* (MTCC 106), *Escherichia coli* (MTCC 739) and *Pseudomonas aeruginosa* (DN1). Fungal test organisms used were

TABLE-3 : Percentage growth inhibition of the fungal pathogens by the bioactive strains

S. No.	Test Isolates	% mycelial growth=[(R-r)/R]x100					
		MTCC 4633	MTCC 287	MTCC 2605	MTCC 1344	LSMU1	MTCC 1477
	Control	0.00	0.00	0.00	0.00	0.00	0.00
1.	DSN3	-	-	64.2	-	-	61.90
2.	DSN4	70.2	61.9	73.0	67.8	60.0	65.67
3.	DSN12	-	-	-	-	-	61.90
4.	DSN13	-	-	-	-	-	64.28
5.	DSN17	-	-	-	-	-	53.57
6.	DSN21	30.2	-	-	-	-	55.95
7.	**DSN22**	**32.6**	**-**	**-**	**-**	**-**	**58.33**

TABLE-4 : Screening for plant growth promoting (PGP) activities

S. No.	Strains	IAA production	Siderophore production	Phosphate solubilisation	Ammonia production
1.	DSN1	+	+	+	+
2.	DSN3	+	+	-	+
3.	DSN4	+	-	+	+
4.	DSN6	+	+	+	+
5.	DSN7	+	-	+	+
6.	DSN9	+	+	+	+
7.	DSN12	+	-	+	+
8.	DSN13	+	-	-	+
9.	DSN17	+	-	+	+
10.	DSN18	-	+	-	+
11.	DSN21	+	+	+	+
12.	DSN22	+	-	+	-
13.	DSN23	+	-	+	-
14.	DSN24	+	-	+	+

Curvularia oryzae (MTCC 2605), *Rhizoctonia solani* (MTCC 4633), *Aspergillus niger (MTCC 1344), Bipolaris oryzae* (LSMU1), *Fusarium oxysporum* (MTCC 287) and *Pyricularia oryzae* (MTCC 1477)[41].

2.1. Primary Screening

Initial antibacterial assay was carried out by Cross Streak method against the bacterial indicator organisms. The plates were incubated for 24-48 hours and inhibition zones were measured[42, 60]. Endophytic isolates showing inhibition zones of >50 % against the indicator bacteria were considered to be potent.

2.2. Secondary Screening

The endophytic isolates were then subjected to Kirby Bauer method. Briefly, the isolates were inoculated on Starch Casein Nitrate Broth (SCNB) and incubated in an orbital shaker (150 rpm, 7 d). The fully grown cultures were centrifuged (10,000 rpm, 15 min). The supernatants were transferred into sterile falcon tubes. 100 µl of the freshly grown test organisms were spread plated on Nutrient Agar, pH 7 and kept for drying. Agar wells (6 mm diameter) were punched and filled with 0.7% soft agar up to ½ depths of the wells. The culture supernatants were then put into the wells in duplicates and kept at 4Ú˜C for 3 hours for proper diffusion. The plates were further incubated at 30Ú˜C for 4-5 days. The plates were then observed for presence or absence of inhibition zones around the wells[9].

2.3. Antagonistic activity against rice fungal pathogens

Antagonistic activity of the endophytic isolates was carried out by *in vitro* plate technique on Sabouraud Dextrose Agar (SDA) against the rice fungal pathogens

TABLE-5 : *In vitro* rice seed germination (Vigor index) by the bioactive strains

S. No.	Treatment	Germination percent (%)	Shoot length (cm)	Root length (cm)	Vigor index
1.	Control	82.5	1.09±0.272[a]	1.11 ± 0.107[a]	181.5
2.	DSN1	90	1.14 ± 0.353[a]	1.18 ± 0.212[a]	199.8
3.	DSN6	80	1.21 ± 0.144[b]	1.01 ± 0.59[c]	177.6
4.	DSN9	75	0.86 ± 0.011[b]	1.06 ± 0.034[b]	144
5.	DSN21	77.5	1.21 ± 0.069[c]	1.57 ± 0.017[b]	117.7

*Values with the same letter within a column are not significant at Pd" 0.05.

mentioned earlier. Briefly, agar wells (6 mm diameter) were punched on SDA plates using sterilised cork borer, followed by placing of the agar plugs of the endophytic cultures on the SDA wells in duplicates with each test fungus at the centre. The plates were incubated at 30ÚC for 48 h. A plate containing a fungal agar plug without the actinomycete culture was used as the control and it was incubated at 30ÚC until the control plate showed full growth[22, 33].

The percentage growth inhibition was calculated using the formula:

Inhibition = {(R-r)/R} X 100

Where, R = radius of the fungal growth in control plate

r = radius of the fungal growth in test plate.

All the experiments were performed in triplicates.

3. PGP screening

The endophytic isolates were further subjected to *in vitro* plant growth promoting (PGP) activities such as IAA, siderophore and ammonia production and phosphate solubilisation.

3.1. IAA production

The isolates were inoculated in SCNB containing 2 mg/ml L-tryptophan (trp) and incubated in an orbital shaker (150 rpm, 30°C and 5 d). The culture broths were centrifuged (10,000 rpm, 10 min) and 1 ml of the supernatant was mixed with 2 ml of Salkowski reagent[8]. Appearance of pink colour indicated production of IAA.

3.2. Siderophore production

Agar plugs (6 mm diameter) of the isolates were inoculated on SCNA plates (without iron) amended with CAS-substrate (Chromazurol S) and incubated at 30°C for 7 d[61]. Orange- coloured halo zones surrounding the colonies indicated production of siderophore.

3.3. Phosphate solubilisation

The isolates fully grown on SCNA was spot

TABLE-6 :Quantitative estimation of PGP traits

S. No.	Strains	IAA production (ig\mL)	Siderophore production (%)	Phosphate solubilisation (ig\mL)	Ammonia production (ig\mL)
1.	**DSN1**	76 ± 0.7	18.04	22 ± 1.41	23.2 ± 0.1
2.	**DSN6**	138 ± 0.1	13.84	50 ± 0.2	27.4 ± 0.3
3.	**DSN9**	12 ± 0.4	7.09	69 ± 0.7	19 ± 0.7
4.	**DSN21**	18 ± 0.5	17.56	43 ± 0.1	14 ± 0.1

TABLE-7 : Similarity 16S rRNA sequences in NCBI with accession numbers assigned

S. No.	Bioactive isolates	New name assigned	Accession Numbers	Best closest match in NCBI	Similarity (%)
1.	DSN1	*Streptomyces* sp. MBRL 750	OM407399	*Streptomyces cheonanensis* VC-A46 (AY822606)	99.48
2.	DSN9	*Priestia* sp. MBRL 752	OM149848	*Priestia megaterium* NBRC15308 (T) (JJMH01000057)	99.93
3.	DSN6	*Streptomyces* sp. MBRL 755	OM407464	*Streptomyces carpaticus* NBRC 15390 (AB184641)	99.62
4.	DSN21	*Streptomyces* sp. MBRL 757	OM407396	*Streptomyces carpaticus* NBRC 15390 (AB184641)	99.38

inoculated in the National Botanical Research Institute (NBRI, India)'s phosphate growth medium containing Bromophenol Blue (NBRIP-BP) medium and incubated at 30°C[38]. Formation of a halo zone surrounding the colony after 4 d indicated phosphate solubilization.

3.4. Ammonia production

The isolates were inoculated in 10 ml peptone water (broth) and incubated in an orbital shaker (150 rpm, 30^0C, 4 d). 0.5 ml of Nessler's reagent was then added in each tube. Development of brown to yellow colour indicated ammonia production[10].

4. *In vitro* seed germination test (Seed Vigour index test)

The bioactive endophytic isolates which showed positive results in all the PGP traits were shortlisted for studies of seed vigour indices on rice seeds (Variety: *Jatra*). Bioactive endophytic isolates grown in SCNB (7 d) were centrifuged (10,000 rpm, 10 min) and the pellets were dissolved in sterile distilled water (SDW). Rice (Variety: *Jatra*) seeds were subjected to surface sterilisation procedure that involved 5 min 70% ethanol wash, 5 min 0.2% sodium hypochloride wash and final rinse with SDW (4 times). Surface sterilised rice seeds

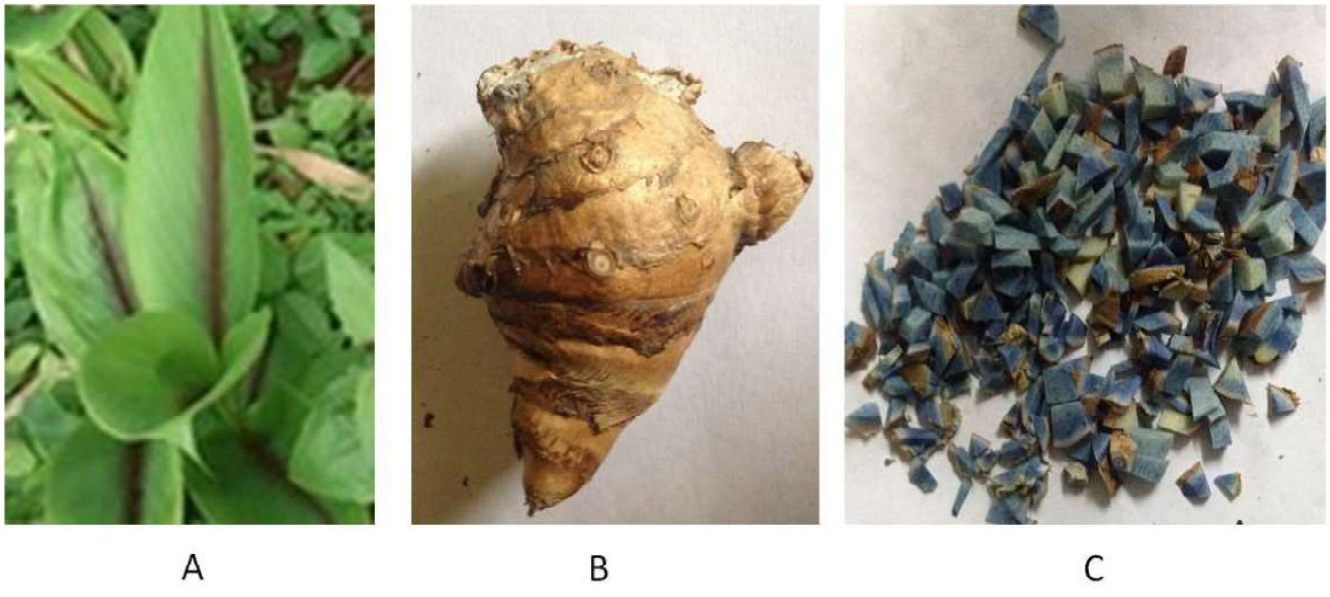

Fig. 1: Plant sample, *Curcuma caesia* for endophytic bacteria isolation: (A) The plant *Curcuma caesia* (Yaimu) (B) The rhizome part (C) The cut pieces of rhizome part showing the peculiar bluish black colour

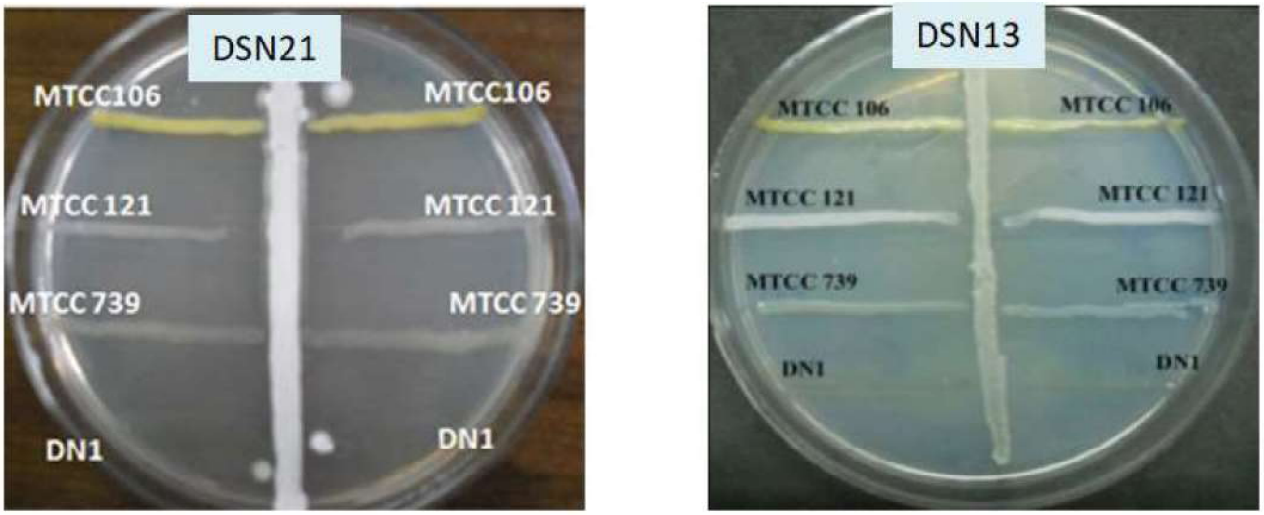

Fig. 2: Primary Screening by Cross Streak method: The figure represents the representative plates for primary screening of the isolates DSN21 and DSN13 showing antibacterial activity against MTCC-121 (*Bacillus subtilis*)

were soaked in the cell suspensions of bioactive isolates and kept in a shaker (150 rpm, 30Ú˜C, and 2 h). The rice seeds were then transferred to sterile petriplates containing wetted filter papers (10 seeds per plate). Sterilised rice seeds soaked with SDW were used as the control. The plates were incubated for 7 days at 30Ú˜C. The number of germinated seeds, their root lengths and shoot lengths were noted and compared with the controls. Vigour index was calculated using the formula[6]:

Vigor index = percent germination X seedling length

Where, seedling length **=** shoot length + root length

All the data were subjected to one-way analysis of variance (ANOVA) followed by independent t-test at *P*d" 0.05 by the use of SPSS 16 software (SPSS Inc).

5. Quantitative estimation of PGP Traits

The PGP activities (IAA, siderophore and ammonia production and phosphate solublisation) of the shortlisted endophytic isolates, namely DSN1, DSN6, DSN9 and DSN21, were estimated quantitatively.

5.1. IAA production

The endophytic isolates were grown in SCNB with 2 mg/ml of tryptophan and kept in shaker incubater (150 rpm, 30°C, 5d). 1 ml of the culture supernatant (after centrifugation at 10000 rpm for 10 min) was taken and 2 mL of Salkowski reagent was added. The amount of IAA was estimated spectrophotometrically at 530 nm by comparing with the standard IAA curve[23].

5.2. Siderophore production

CAS-shuttle assay[45] was employed to estimate the siderophore production using SCNB. 1 mL of the 5 d old endophytic culture was grown in SCNB and kept in shaker incubater (150 rpm, 30°C, 5d). 1 ml of the culture supernatant (after centrifugation at 10000 rpm for 10 min) was taken and 1 mL of CAS reagent was added. The amount of siderophore was estimated spectrophotometrically at 630 nm against a reference (1 mL of uninoculated broth culture and 1 mL of CAS reagent) by the formula given below:

Percentage siderophore units = {(AR-As)/Ar} x 100

Where, Ar = reference absorbance at 630 nm

As = sample absorbance at 630 nm

5.3. Ammonia Production

The endophytic isolates were grown in SCNB and kept in shaker incubater (150 rpm, 30°C, 5d). 5 ml of the culture supernatant (after centrifugation at 10000 rpm for 10 min) was taken and 2.5 mL of Nessler's reagent was added. The amount of ammonia produced was measured spectrophotometrically at 530 nm and estimated by comparing with the standard curve given by ammonium sulphate[20].

5.4. Phosphate Solubilisation

The endophytic isolates were grown in NBRIP medium (pH 7) and kept in shaker incubator (150 rpm, 30°C, 5d)[30]. 5 ml of the culture supernatant (after centrifugation at 10000 rpm for 10 min) was taken and then phosphate concentration was estimated[15]. The amount of phosphate was estimated spectrophotometrically at 530 nm by comparing with the standard curve. Potassium Dihydrogen Phosphate was used as the standard.

6. Molecular characterisation

Extraction of genomic DNA and PCR amplication of the 16S rDNA sequences of the bioactive isolates (DSN1, DSN6, DSN9 and DSN21) was performed[55]. The

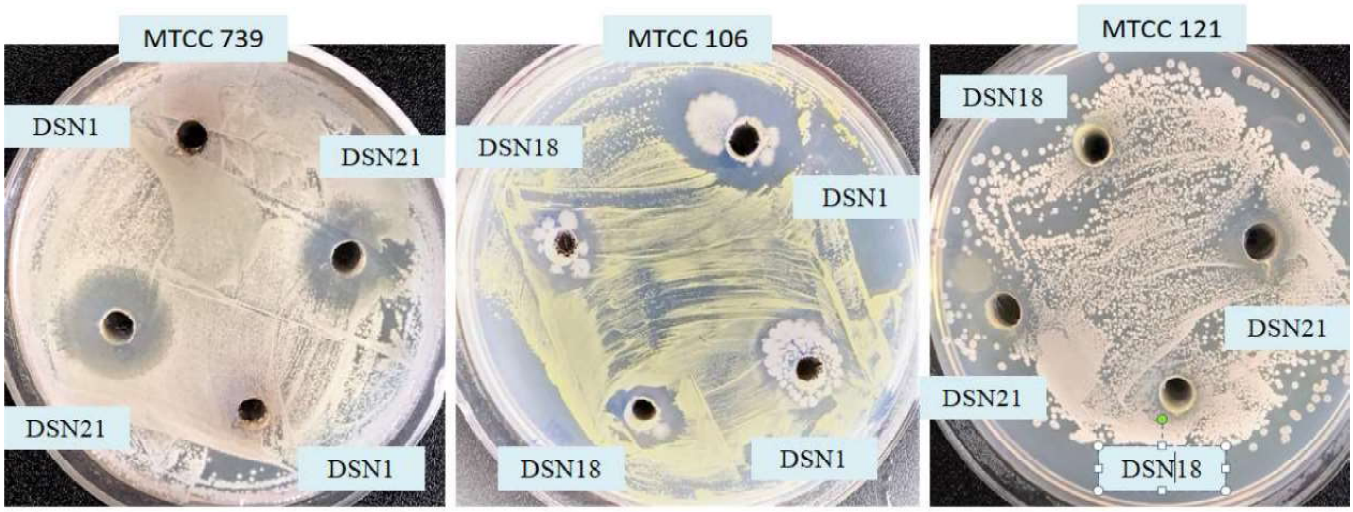

Fig. 3: Antimicrobial assay by Kirby Bauer method: The figure represents the plates of antimicrobial assay of the isolates DSN18 and DSN1 showing antibacterial activity against the test organism MTCC 106 (*Micrococcus luteus*), DSN18 and DSN21 against MTCC 121 (*Bacillus subtilis*) and DSN1 and DSN21 against MTCC 739 *(Escherichia coli)*

almost complete 16S rRNA gene sequences of the strains were identified through Ez-Taxon e-server and NCBI GenBank databases[28].

Results

1. Sampling and Isolation of endophytic bacteria

The plant sample *Curcuma caesia* (*yaimu*) is shown in (Fig. 1). Putative bacterial colonies were sub-cultured till pure cultures were obtained. A total of 34 endophytic bacterial isolates were obtained, 24 from rhizome and 10 from leaf samples. They were designated as DSN1 to DSN24 (endophytic bacterial isolates from rhizome) and DSN25 to DSN34 (from leaf).

2. Antimicrobial assays

2.1. Primary Screening

34 endophytic isolates were subjected to Cross Streak method against the bacterial test organisms, namely *Micrococcus luteus* (MTCC-106), *Bacillus subtilis* (MTCC-121), *Escherichia coli* (MTCC-739) and *Pseudomonas aeruginosa* (DN1), of which 15 showed antibacterial activity against one or more of the test organisms (Table-1, Fig. 2).

2.2. Secondary Screening

All 34 endophytic bacterial isolates were subjected to secondary screening for antibacterial activity, of which 5 exhibited significant activities. Two (2) isolates, DSN1 and DSN21, were found to be most potent among them. None of the isolates were found to have any activity against *Pseudomonas aeruginosa*. (Table-2, Fig. 3).

2.3. Antagonistic activity against rice fungal pathogens

All the endophytic isolates were screened for antifungal activities against indicator rice fungal pathogens using **Dual Culture** technique, of which, 7 were found to have antifungal activity against one or more of the test organisms. Isolate **DSN4** was the most potent among them (Table-3, Fig. 4).

3. PGP screening

The endophytic isolates were further subjected to *in vitro* plant growth promoting (PGP) activities such as IAA, siderophore and ammonia production and phosphate solubilisation.

Four (4) isolates (DSN1, DSN6, DSN9 and DSN21) were positive in all the PGP traits assayed (Table-4, Fig. 5).

4. *In vitro* seed germination test (seed vigour test)

The bioactive endophytic isolates positive for all the PGP traits were subjected to *in vitro* seed germination tests (Table-5, Fig 6). The isolate DSN1 showed higher vigor index of 199.8 as compared to the control (181.5). DSN1 treated seeds showed higher germination percentage (90 %) over the control (82.5 %).

5. Quantitative estimation of PGP traits

The endophytic isolates were subjected to quantitative estimation of PGP traits such as IAA, siderophore and ammonia production and phosphate solubilisation (Table-6).

The bioactive isolate DSN6 showed highest amount of IAA (138 ìg/mL) followed by DSN1 (76 ìg/mL) after 7

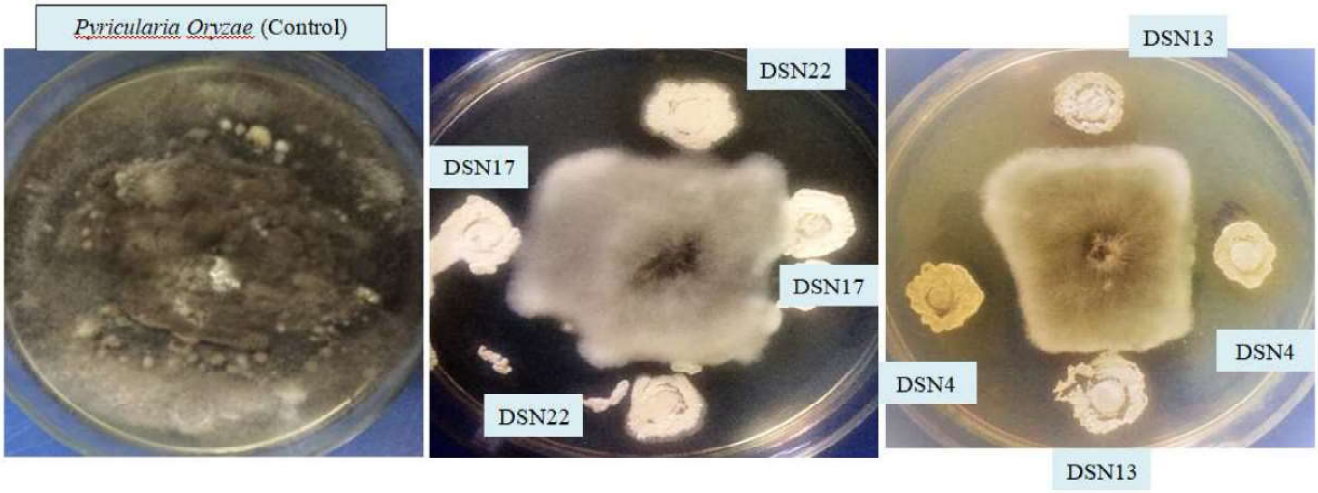

Fig. 4: Biocontrol assay by Dual Culture method: The figure represents the plates of biocontrol assay of the isolates DSN4, DSN13, DSN17 and DSN22 showing antifungal activity against the rice fungal pathogen *Pyricularia oryzae*/ MTCC 1477 (PO).

days of incubation. None of the 4 bioactive isolates produced significant amount of siderophore. The isolate DSN9 could solubilise the highest amount of inorganic phosphate (69 µg/ml) after 7 days of incubation, followed by DSN6 (50 µg/ml), and the isolate DSN6 produced the highest amount of ammonia (27.4 µg/ml), followed by DSN1 (23.2 µg/ml) (Table-6).

6. Molecular characterisation

Extraction of genomic DNA and PCR amplication of the 16S rDNA sequence of the shortlisted bioactive isolates (DSN1, DSN6, DSN9 and DSN21) were carried out. The almost complete 16S rRNA gene sequences of the strains were identified through Ez-Taxon e-server and NCBI GenBank databases (Table-7). DSN1 has the highest 16S rRNA sequence similarity with *Streptomyces cheonanensis* (99.48%), DSN6 with *Streptomyces carpaticus* (99.62%), DSN9 with *Priestia megaterium* (99.93%) and DSN21 with *Streptomyces carpaticus* (99.38%). These bioactive endophytic strains have been, therefore, designated as *Streptomyces* sp. MBRL 750, *Streptomyces* sp. MBRL 755, *Priestia* sp. MBRL 752 and *Streptomyces* sp. MBRL 757 respectively and the 16S rRNA sequences were submitted to GenBank database under accession numbers OM407399, OM407464, OM149848 and OM407396 respectively.

Discussion

Plants growing in biodiversity rich zones are likely to house endophytes with equal or greater biodiversity. Moreover, plants with a long ethnobotanical history are expected to harbour potent bioactive endophytes relative to other plants[53, 56]. During the present study, 34 endophytic bacteria were isolated from *Curcuma caesia* (Black turmeric), locally known as *yaimu*, an ethnomedicinally important plant of Manipur, India. The endophytic isolates were subjected to antimicrobial assays. Primary screening (*cross streak method*) against indicator test organisms indicated that 15 of 34 isolates were inhibitory to one or more of the test organisms. During Secondary Screening (*Kirby Bauer method*), 5 isolates showed antibacterial activity against one or more of the test organisms. Of the 5 isolates, DSN21 was found to be most potent. In a similar study, 5 endophytic bacteria isolated from *Centella asiatica* and *Tinospora cordifelia* showed antimicrobial activities against *Pseudomonas aeruginosa, Streptococcus mutans, Entarobacter aeruginosa, Klebsiella pneumonia, Proteus mirabilis, Staphylococcus aureus*, and *Lactobacillus* spp.[49]. In biocontrol assay (*dual culture method*) against five rice fungal pathogens, it was found that 7 isolates have antifungal activity against one or more of the fungi. Of the 7 antifungal isolates, DSN4 was found to be the most potent and it holds promise for further development as a biocontrol agent for rice cultivation. In a similar study[62], 560 endophytic bacteria isolated from 26 herbal species were reported to exhibit promising antimicrobial properties. In another study, two (2) endophytic strains isolated from rhizomes of *Curcuma longa* L exhibited promising antimicrobial activity: *Paenibacilus alvei* against *C. albicans* and *S. aureus* and *Enterobacter agglomerans* against *Salmonella enterica ser. Typhi* and *S. aureus*[57].

The endophytic isolates from *Curcuma caesia* were subjected to *in vitro* assays of PGP traits such as IAA, ammonia and siderophore production and phosphate solubilistion. Four (4) bioactive isolates (DSN1, DSN9, DSN6 and DSN21) were found to be positive in all the traits tested. Endophytes are reported to be involved in plant growth promotion and plant disease control[3]. An endophytic isolate, *Pantoea alhagi*, promoted growth and drought tolerance of wheat plants[12]. In our study, the

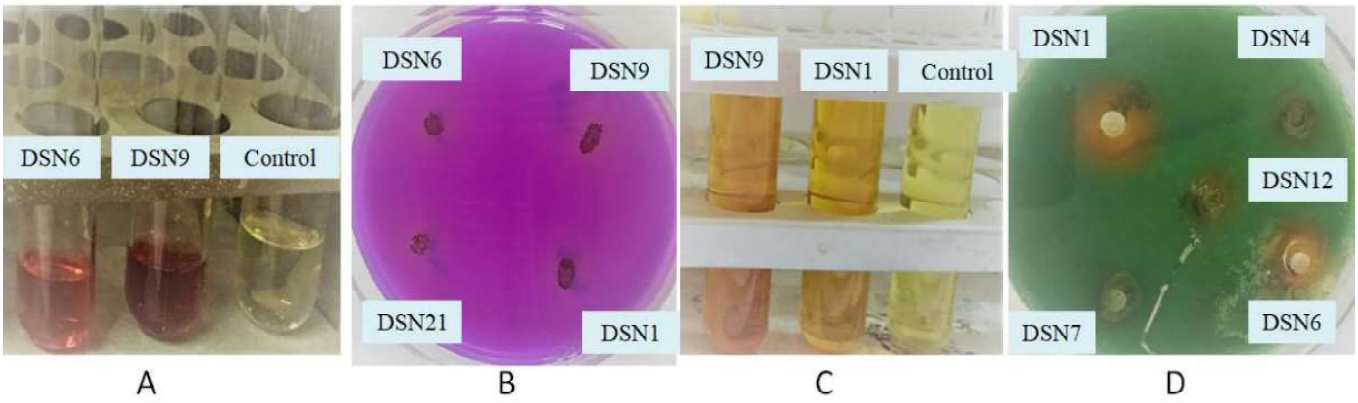

Fig. 5: Representative plates of *in vitro* tests for PGP activities: The figure represents the plates and tubes of *in vitro* tests for (A) IAA showing positive results of two isolates (DSN6 and DSN9) with a control (B) Phosphate solubilisation showing positive results of isolates DSN1, DSN6, DSN9 and DSN21 (C) Ammonia production showing positive results of two isolates (DSN1 and DSN9) with a control and (D) Siderophore production showing positive results of two isolates (DSN1, DSN6) and three isolates (DSN4, DSN7 and DSN12) showing negative results

bioactive endophytic isolates were further subjected to quantitative estimation of PGP traits. The endophytic isolate DSN6 produced highest amount of IAA (138 ì/mL), followed by DSN1 (76 µg/ml). The isolate DSN9 could solubilise the highest amount of inorganic phosphate (69 µg/ml), followed by DSN6 (50 µg/ml). The endophytic strain DSN6 produced the highest amount of ammonia (27.4 µg/ml), followed by DSN1 (23.2 µg/ml). The isolate DSN1 showed higher vigor index of 199.8 as compared to the control (181.5). Bacterial endophytes from a related medicinal plant *Curcuma longa* L., were also reported to be positive in many PGP activities[16, 34]. In the present study, based on the biocontrol and PGP assays, DSN1 and DSN6 showed highest PGP potential relative to other isolates. These isolates are, therefore, selected for studies of their potential for rice growth promotion under pot and limited field trial conditions.

The shortlisted endophytic isolates (DSN1, DSN6,

Fig. 6 : Representative images of seed germination test: The figure represents the representative plates of seed germination test for the four bioactive isolates (DSN1, DSN6, DSN9 and DSN21) with a control

DSN21 and DSN9) were identified as *Streptomyces* sp. MBRL 750, *Streptomyces* sp. MBRL 755, *Streptomyces* sp. MBRL 757 and *Priestia* sp. MBRL 752 respectively. *Streptomyces* spp. are prolific producer of natural products and, of late, they have been shown to have excellent PGP properties. In a similar study, two *Streptomyces* spp. strains isolated from *Centella asiatica* exhibited PGP potential with significant production of IAA[14]. Five strains of *Streptomyces* were reported to have PGP properties in rice and sorghum under greenhouse and field conditions in another study[54].

Conclusions

Of 34 endophytic bacteria, four (4) isolates showed significant PGP potential. Two (2) bioactive strains MBRL 755 and MBRL 750 hold promise for development of bioinoculants for rice cultivation. Further optimization studies of these bioactive strains by changing different cultural conditions could be targets of future studies leading to biotechnological exploitation of these bioactive strains. The study corroborates that Manipur (a part of Indo-Burma hotspot) holds great potential for discovery of novel bacteria and development of novel natural products with commercial biotechnological applications.

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