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RESEARCH ARTICLE

Characterization of bacterial endophytes of King chilli for biocontrol potential and plant growth promotion

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Abstract

Bacterial endophytes associated with host plants provide various beneficial effects. This study assessed the diversity of bacterial endophytes in King chilli, focusing on their biocontrol potential and plant growth-promoting (PGP) activities. The survey was carried out in King chilli-growing regions and identified anthracnose and fruit rot diseases as significant contributors to economic yield loss. A total of 20 bacterial endophytic isolates were obtained using the sterility check method and identified as *Pseudomonas* through 16S rRNA gene sequencing. In *in-vitro* studies, isolates *P. fluorescens* KEB15, *P. putida* KEB5, and *P. putida* KEB7 exhibited notable mycelial growth inhibition rates of 66.67 %, 69.26 %, and 66.30 % against *Pythium*, *Fusarium*, and *Colletotrichum*, respectively. Of the 20 isolates, 5, 16, and 17 isolates demonstrated positive production of hydrogen cyanide (HCN), ammonia (NH_3), and indole-3-acetic acid (IAA), respectively. The efficacy of crude antibiotics from the best-performing antagonistic endophytes was tested against the linear growth of *Fusarium*, with KEB11 showing the largest inhibition area of 35.14 mm. Sequence analysis using the maximum likelihood method revealed close relationships among the potent *Pseudomonas* isolates, identifying KEB5 and KEB7 as *P. putida* and KEB15 as *P. fluorescens*. Field evaluations indicated that KEB7 was most effective in controlling bacterial wilt, anthracnose, and dieback diseases, achieving a maximum plant height of 85.10 cm and a yield of 3683.67 kg/ha. This study demonstrates that bacterial endophytes can effectively exhibit antifungal activity and promote plant growth in King chilli.

Keywords

endophytes; growth promotion; King chilli; phylogenetic analysis

Introduction

King chilli (*Capsicum chinense* Jacq) is the world's hottest chilli and a naturally occurring interspecific hybrid of *Capsicum chinense* and *Capsicum frutescens* (1). This chilli is called by different names such as Naga Jolokia, Bhut jolokia, Ghost chilli pepper, Naga King chilli, Raja Mirchi, Umorok in India, and Nagahari in Bangladesh (2-6). This King chilli is known for its unique taste and aroma, widely cultivated in the North Eastern Region of India. The chilli red color is mainly due to the carotenoid pigment capsanthin, while capsaicin, a chemical compound attributed to its hot and pungent taste. Most of the chilli species cultivated in India contain 1 % capsaicin, while King chilli contains capsaicin ranging from 2.45-5.36 % (4, 7-8).

King chillies are a rich source of ascorbic acid, vitamins A, B, C, B_6, and E (9), and contain a good percentage of iron, calcium, and potassium. Still, the concentration depends on the cultivar (10).

King chili has many medicinal properties, primarily recognized for its anti-cancer potential. It is used in low quantities to provide relief for asthma patients and to treat gastrointestinal issues. Additionally, it has anti-arthritic effects, aids in digestion, promotes blood development, and helps regulate insulin levels in diabetes. The tender leaves of the plant are reported to cure boils, headaches, and night blindness. Studies show that capsaicin, the active component in King chili, may have protective effects against obesity and cholesterol (11). Capsaicin interacts with protein molecules to reduce cholesterol absorption in the body, enhance metabolism, and increase endorphin levels, which can elevate mood. King chili also acts as a detoxifier, helping to remove waste products from the body and increasing nutrient supply to tissues. Furthermore, it can serve as a repellent for animals and insects, particularly ants and cockroaches. In some contexts, King chili has been considered a bio-weapon, being used in sprays for riot control and self-defense.

Endophytes are microorganisms that reside within the interior tissues of plants without causing direct harm to their hosts (12). Plants have likely developed closer biological relationships with these endophytes compared to epiphytes or soil-dwelling organisms, resulting in a diverse range of endophytic habitats. With over 300,000 plant species on Earth, each species harbors one or more endophytes (13). Endophytic bacteria can significantly enhance the metabolism and physiology of their host plants through various mechanisms (14), including atmospheric nitrogen fixation, iron sequestration, phosphate solubilization, synthesis of plant growth hormones, inhibition of ethylene production by 1-aminocyclopropane-1-carboxylate (ACC deaminase), degradation of toxic compounds, and suppression of fungal and bacterial pathogens (15). Given their ecological niche similar to that of phytopathogens, these bacterial endophytes are promising candidates for biocontrol agents (16), as numerous studies have documented their ability to manage nematodes, insects, and plant diseases (15, 17–21). Moreover, endophytes can accelerate seedling emergence and support plant establishment in adverse conditions (22-23). Their potential for fungal antagonism further aids in the development of biofungicides as alternatives to chemical fungicides (24). Despite these advantages, research on the endophytic bacteria associated with King chili plants is limited. This study aims to characterize the endophytes in King chili to explore their roles in plant growth promotion and biocontrol, contributing to existing knowledge and highlighting their agricultural significance.

Materials and Methods

Field survey, sample collection and isolation

Field Survey and Sample Collection

To determine the prevalence of diseases in Nagaland's several King chilli-growing regions, a thorough survey was carried out in 2015–16 (Fig. 1). Healthy fruits and plant materials with the disease were gathered from each site throughout the survey, and samples were sealed in plastic bags and transported to the lab. All the samples were processed immediately to isolate pathogens from infected samples and endophytes from healthy samples. The samples were stored in the refrigerator for future experimental purposes.

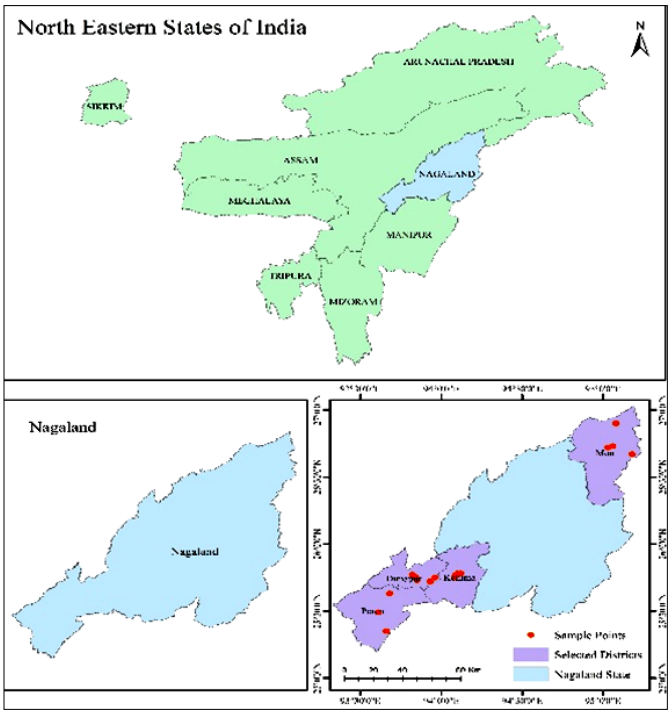

Fig. 1. Collection of samples from different locations.

Isolation of fungal pathogens of King chilli

The disease-affected plant samples were collected during the survey and subjected to isolation. The plant parts showing the different symptoms of damping off, wilting, anthracnose, and fruit rot were collected on tissue paper, separately from infected plant parts. Using the tissue segment method, the pathogen associated with affected tissues was isolated on a potato dextrose agar (PDA) medium (25). Additionally, the isolates underwent purification using the single hyphal tip technique in a plain agar medium (26). The purified isolates were identified based on the morphological and cultural characteristics of the pathogen and proved Koch's postulates.

Isolation of bacterial endophytes

The bacterial endophytic biocontrol agents were isolated from King chilli fruits on nutrient agar media by following the sterility check method (27). The fruit samples were washed under running tap water to remove the soil debris then washed with sterile distilled water. The chosen fruit samples were first surface sterilized with 0.1 % mercuric chloride and then disinfected with 70 % ethanol. Lastly, use double-sterilized distilled water to rinse the fruits three times after surface sterilization. We performed sterility checks in accordance with Shi *et al*. (28), to validate the efficacy of surface sterilization and ensure that there was no biological contamination on the surface. After being macerated in sodium phosphate buffer (pH 7), the material was serially diluted up to 10^{-5} and plated on a nutrient

agar medium (29). For two days, Petri plates were incubated at 28 °C. Standard procedures were used to count the colonies and single colonies were purified by streaking on an agar slant separately (30).

Molecular characterization of bacterial endophytes

Isolation of bacterial genomic DNA: The genomic DNA was extracted by using a CTAB method (31) with slight modifications (32). A 25 mL culture was centrifuged at 6,000 rpm for 5 min at 4 °C. The pellet was resuspended in 1 mL TE buffer and mixed with 0.5 mL butanol, then centrifuged at 5,000 rpm for 5 min. The pellet was washed twice with TE buffer. Next, it was treated with 100 µl of freshly prepared lysozyme (10 mg/mL) for 5 min at room temperature, followed by the addition of 100 µl SDS (10 %) and 25 µl proteinase K (100 mg/mL), incubated at 37 °C for 1 h. Then, 200 µl of 5 M NaCl and 150 µl CTAB were added and incubated at 65 °C for 10 min. The mixture was extracted with 1 mL phenol, centrifuged at 6,000 rpm for 15 min, and the aqueous layer was transferred to a microfuge tube. DNA was precipitated with 0.6 volumes of ice-cold isopropanol and incubated overnight at -20 °C. After pelleting at 12,000 rpm for 15 min, the pellet was rinsed with 70 % ethanol, dried under vacuum for 10 min, and resuspended in 50 µl TE buffer. Finally, 1 µl of DNase and RNase-free solution (10 mg/mL) was added, and the DNA was incubated at 37 °C for 30 min before storage at -20 °C.

Detection of Pseudomonas species-specific in endophytes

DNA coding for 16S-23S rRNA was amplified using specific primers in a gradient PCR. Primers ITSIF (5' AAGTCGTAACAAGGTAG 3') and ITS2R (5' GAC-CATATATAACCCCAAG 3') were designed for a 560 bp amplicon (33). The PCR reaction mixture (20 µl) contained 10X buffer (2.5 mM $MgCl_2$, 2 µl), 2 mM dNTPs (2 µl), 2 M primers (5 µl), Taq DNA polymerase (Genei), water (8 µl), and 50 ng of template DNA. Amplification was performed on an Eppendorf Master Cycler with the following conditions: 92 °C for 4 min, followed by 40 cycles of 92 °C for 1 min, 28 °C for 1 min, and 72 °C for 2 min, concluding with a 10 min final extension at 72 °C. PCR products were resolved on a 2 % agarose gel at 50 V, stained with ethidium bromide (0.5 µg/mL), and visualized using a UV transilluminator. The bands were photographed and analyzed with a gel documentation system (Alpha Innotech Corporation). Sizes of the PCR products were determined using a 100 bp ladder (Bangalore Genei Pvt. Ltd.).

In vitro screening of endophytes against the fungal pathogens of King chilli

The antifungal activity of bacterial endophytes was assessed using the dual culture method against *Pythium*, *Fusarium*, and *Colletotrichum* (33). In Petri plates with solidified nutrient agar, a fungal disc and the endophytic bacteria were inoculated side by side, while control plates contained only the fungal disc. The plates were incubated at 28 ± 2 °C until the fungal cultures reached full size. The diameter of the pathogenic colonies was measured, and the percentage of radial growth inhibition compared to the control was calculated using a specific formula (34).

$$I = \frac{C-T}{C} X\ 100$$

Where, I = Per cent inhibition, C = Growth of the pathogen in the control plate, T = Growth of the pathogen in endophyte inoculated plate.

Studies on plant growth promoting properties

Determination of indole acetic acid (IAA)

Isolates of *Pseudomonas* sp. were cultured in King's broth supplemented with 0.5 mM tryptophan for 48 hours. IAA-producing isolates were identified by observing the appearance of a pink color change in the solution after reacting with the Salkowski reagent (35).

Hydrocyanic acid (HCN) production

Bacteria were cultured on tryptic soy agar, and filter paper discs soaked in a picric acid solution (2.5 g picric acid and 12.5 g sodium carbonate in 1 liter of distilled water) were placed in the lids of each Petri plate. The plates were sealed with parafilm and incubated at 28 °C for 48 hours. A color change of the discs from yellow to light brown, brown, or reddish brown indicated weak, moderate, or strong production of HCN, respectively, for each strain (36).

Determination of ammonia production

Using a standard method, bacterial isolates were tested for ammonia production (37). They were grown in tubes of 5 mL of peptone water, incubating at 30 °C for four days. After incubation, 1 mL of Nessler's reagent was added to each tube. The development of a faint yellow color (+) indicated significant ammonia production and was considered positive for ammonia production.

Bioassay of crude antibiotics from potent endophytes

Isolation of crude antibiotics

To extract crude antibiotics from endophytes, selected isolates were grown in Pigment Production Broth (PPB) at 28 ± 2 °C for five days (38). After incubation, the broth culture was centrifuged at 5,000 rpm for 10 minutes, and the supernatant was adjusted to pH 2.0 with concentrated HCl and incubated for 8 hrs. An equal volume of ethyl acetate was added, and the mixture was shaken at 120 rpm at 25 ° C for 3 hours. The ethyl acetate layer was separated and evaporated using a vacuum flask evaporator at 40 °C. The resulting residues were resuspended in 1 mL of methanol for bioassays.

Bioassay of crude antibiotics by agar diffusion well method

The antagonistic strains of endophytic isolates (KEB2, KEB5, KEB6, KEB7, and KEB11) were evaluated for their efficacy against the Fusarium wilt pathogen using the agar diffusion well method (39). A fully grown *Fusarium* culture on PDA was supplemented with 10 mL of sterile water, and conidia were extracted by scraping the surface with a sterile scalpel. One mL of the conidial suspension was added to a sterile Petri plate, followed by 15 mL of PDA medium, which was rotated for uniform distribution. After solidification, wells were created with a sterile 5 mm cork borer on the plate's sides. Crude antibiotics from the endophyte isolates were added to the wells at 50, 75, 100, and 150 µl.

Each treatment was replicated three times. Mycelial growth and inhibition were assessed after 72 hours of incubation at 28 ± 2 °C, and the diameters of the inhibition zones were measured. Sterile water without crude antibiotics served as a control.

Phylogenetic analysis of potent isolates of endophytes

The best-performing isolates in terms of biocontrol ability and plant growth promotion were selected for species identification using the 16S-23S rDNA genome sequencing. Nucleotide sequencing of PCR products was done at Bioserve Biotechnologies Pvt. Ltd, Hyderabad, India. The BLAST program through the internet server at the National Centre for Biotechnology Information (National Institutes of Health, Bethesda, USA) GenBank database (http://www.ncbi.nlm.nih.gov/) was used to perform rDNA homology searches. Sequences were selected and established an evolutionary relationship based on a maximum identity score of > 97 %. The MEGA X with maximum likelihood (ML) analyses was used to construct the phylogenetic tree to infer evolutionary trees for nucleotide base sequence along with the downloaded sequence from NCBI (40).

Field evaluation of potent bacterial endophytes

The field experiment was conducted in ICAR Research farm, Dimapur, Nagaland, using the potent isolates of endophytes to assess the suppression of fungal diseases of King chilli. The chilli seeds were sown in a raised seedbed, spaced 5 cm apart in a straight line. Around 40 to 45 days old seedlings were used for transplanting and bacterial endophytes were treated during transplanting in the main field. The main field was prepared and divided into 21 blocks of 5 x 5 m size to accommodate the 7 treatments with 3 replications. Freshly prepared 3 days old culture was used for preparation of liquid formulation of each endophyte containing 10^8 CFU mL^{-1} microbial suspension and used for inoculation with seedling dip (10 %) for 30 min duration at transplanting and foliar application (2 %) two times at 30 days intervals. Three replications for each treatment were maintained using a randomized block design (RBD). Along with biometric data, the observation of disease incidence was documented for each treatment.

Measurement of disease incidence

Per cent wilt and die-back incidence of King chilli was calculated by counting the number of affected plants out of the total plants. The observation of infected plants in each treatment was recorded and per cent disease incidence was calculated using a formula.

$$PDI = \frac{Total\ No.of\ infected\ plants}{Total\ no.of\ plants\ observed}\ X\ 100$$

The severity of anthracnose was measured by using the disease scoring scale given in Table 1 (41).

Statistical analysis

The results of each experiment were analyzed independently. Treatment means were compared using Duncan's Multiple Range Test (DMRT) (42). The analysis was conducted using the IRRISTAT analysis package version 92-1, developed by the Biometrics Unit of the International Rice Research Institute in the Philippines.

Table 1. Anthracnose disease severity scores, resistance levels and symptom descriptions of chili fruit.

Score	Resistance level	Symptom description
0	HR, highly resistant	No infection
1	R, resistant	1-2 % of the fruit with necrotic lesions or a larger water-soaked lesion surrounding the infection site
3	MR, moderately resistant	2 to 5 % of the fruit with necrotic lesions, possibly acervuli may be present
5	MS, moderately susceptible	5 to 10 % of the fruit with necrotic lesions, possibly acervuli
7	S, susceptible	10 to 25 % of the fruit is covered with necrotic lesions with acervuli
9	HS, highly susceptible	More than 25 % of the fruit showed necrosis

Results

Survey for the occurrence of fungal diseases in Naga King Chilli

The survey was carried out in various potential growing regions of Nagaland, including Dimapur, Kohima, Peren, Wokha, Phek, and Mon, where the incidence of diseases was recorded across different locations (Table 2). The investigation revealed several diseases affecting King chilli, including damping off, anthracnose, dieback, Fusarium wilt, and fruit rot. Among the fungal diseases, anthracnose was identified in all surveyed areas, while Fusarium wilt and damping off were only observed in Zhadima and Moalvam, respectively. Notably, anthracnose and fruit rot emerged as the most significant diseases, contributing to economic yield losses during the ripening stage across all locations (Table 3).

Bacterial endophytes and pathogenic cultures of King chilli

A total of 20 bacterial endophytes were isolated from samples collected across various growing regions of Nagaland, designated as KEB1 to KEB20. The identification of these bacterial cultures was performed using gram staining and morphological characteristics. The results indicated that all isolated endophytic bacteria were Gram-negative (Table 5). Additionally, three pathogenic cultures *viz*., *Pythium*, *Fusarium*, and *Colletotrichum* were isolated from infected plant samples collected during the survey for further investigation.

Molecular characterization of bacterial endophytes

Amplification of 20 isolates from the specific region of the 16S-23S rRNA intervening sequence using specific primers produced an expected amplicon of 560 bp, indicating that the isolates belong to the *Pseudomonas* genus.

In-vitro screening of endophytes against the pathogens of King chilli

To evaluate bio-efficacy, the bacterial endophytes were tested *in-vitro* against the Naga King chilli pathogens *Pythium sp.*, *Fusarium*, and *Colletotrichum*. Among the twenty

isolates, KEB15, KEB5, and KEB7 demonstrated percent inhibitions of 66.67 %, 69.26 %, and 66.30 %, respectively, in the mycelial growth of *Pythium*, *Fusarium*, and *Colletotrichum* when compared to the control (Table 4).

Screening of endophytes for multiple plant growth-promoting activities

The isolates were evaluated for their plant growth-promoting (PGP) activities, including HCN, ammonia, and

Table 2. Place of collection of Naga chilli for endophytes isolation.

Sl. No.	District	Place of collection	GPS location	Designation
1	Dimapur	Piphema	25°44'58.3"N 93°57'36.2"E	KEB1
2	Dimapur	Jharnapani	25°45'14.4"N 93°50'40.0"E	KEB2
3	Dimapur	Moalvam	25°44'02.2"N 93°51'09.8"E	KEB3
4	Dimapur	Kukidolong	25°46'06.0"N 93°49'08.7"E	KEB4
5	Dimapur	PiphemaNew	25°43'20.2"N 93°55'44.5"E	KEB5
6	Peren	Athibung	25°29'37.9"N 93°36'35.4"E	KEB6
7	Peren	Jaluki	25°37'47.0"N 93°40'35.0"E	KEB7
8	Peren	Tening	25°20'52.2"N 93°39'34.8"E	KEB8
9	Mon	Mon town	26°43'56.9"N 95°03'37.6"E	KEB9
10	Mon	Shangnyu	26°76'56.3"N 95°10'80.5"E	KEB10
11	Mon	Tekuk	26°74'19.7"N 94°97'39.6"E	KEB11
12	Mon	Longwa	26°39'57.1"N 95°10'49.7"E	KEB12
13	Mon	Totok Chinglem	26°66'19.3"N 94°98'68.5"E	KEB13
14	Mon	Tizit	26°53'52.1"N 95°04'42.1"E	KEB14
16	Mon	Lampongsheangah	26°43'28.6"N 95°02'00.5"E	KEB16
17	Kohima	Tseisebasa	25°45'17.2"N 94°04'50.4"E	KEB17
18	Kohima	Nerhema	25°46'03.3"N 94°05'40.6"E	KEB18
19	Kohima	Chiephobozon	25°47'00.4"N 94°05'50.2"E	KEB19
20	Kohima	Zhadima	25°46'59.6"N 94°07'29.5"E	KEB20

Table 3. Survey for the occurrence of fungal diseases of Naga King Chilli in Nagaland.

Sl. No.	District	Place of collection	Disease occurrence
1	Dimapur	Piphema	Anthracnose and fruit rot
2	Dimapur	Moalvam, Kukidulong	Damping off, Anthracnose and fruit rot
3	Dimapur	Jharnapani	Damping off, Anthracnose, fruit rot, Die back, Fusarium wilt
4	Kohima	Zhadima, Tseisebasa, Nerhema, Chiephobozon	Anthracnose
5	Peren	Athibung	Anthracnose and Fruit rot
6	Peren	Jalukie, Tening	Fruit rot
7	Wokha	Wokha village	Anthracnose, and Fruit rot
8	Mon	Mon town, Longwa, Shangnyu, Tekuk, Totak Chinglem, Lampongsheangah, Tizit	Anthracnose and Fruit rot
9	Phek	Phek	Anthracnose/fruit rot

Table 4. Evaluation of King chilli endophytic bacteria against the pathogens of King chilli under *in-vitro.*

Sl No.	Isolates	*Pythium*		*Fusarium*		*Colletotrichum*	
		Mycelial growth (mm)*	% inhibition over control	Mycelial growth (mm)*	% inhibition over control	Mycelial growth (mm)*	% inhibition over control
1	KEB1	56.67	37.04	55.67	38.15	48.67	45.93
2	KEB2	45.00	50.00	35.67	60.37	33.00	63.33
3	KEB3	59.67	33.70	52.33	41.85	58.67	34.81
4	KEB4	64.33	28.52	63.00	30.00	57.00	36.67
5	KEB5	32.33	64.07	27.67	69.26	39.33	56.30
6	KEB6	67.67	24.81	56.33	37.41	60.33	32.96
7	KEB7	57.33	36.30	51.67	42.59	30.33	66.30
8	KEB8	37.00	58.89	49.33	45.19	46.67	48.15

9	KEB9	54.33	39.63	63.00	30.00	76.33	15.19
10	KEB10	56.33	37.41	68.67	23.70	71.00	21.11
11	KEB11	66.00	26.67	61.33	31.85	72.00	20.00
12	KEB12	55.67	38.15	66.67	25.93	56.33	37.41
13	KEB13	54.67	39.26	63.33	29.63	65.67	27.04
14	KEB14	59.67	33.70	55.33	38.52	57.00	36.67
15	KEB15	30.00	66.67	42.33	52.96	45.00	50.00
16	KEB16	57.00	36.67	54.00	40.00	51.00	43.33
17	KEB17	52.67	41.48	56.00	37.78	74.33	17.41
18	KEB18	46.00	48.89	61.67	31.48	75.00	16.67
19	KEB19	65.33	27.41	59.67	33.70	71.33	20.74
20	KEB20	60.00	33.33	61.67	31.48	66.67	25.93
21	Control	90.00	0.00	90.00	0.00	90.00	0.00
	CD		17.715		15.556		18.555
	SE(m)		6.185		5.431		6.479

*Values are the mean of three replications.

IAA production. Each isolate exhibited varying levels of these activities (Table 5). The results indicated that five out of the twenty isolates tested positive for HCN production, while sixteen were positive for ammonia production. Additionally, seventeen isolates showed IAA production, with nine exhibiting higher levels of production. Notably, four isolates *viz*., KEB3, KEB5, KEB7 and KEB14 were positive for all three activities of HCN, ammonia, and IAA production.

Table 5. Gram staining and plant growth substances produced by endophytes.

Sl No.	Isolates	Gram staining	HCN	Ammonia	IAA
1	KEB1	G^{-ve}	-	+	+
2	KEB2	G^{-ve}	-	+	++
3	KEB3	G^{-ve}	+	+	++
4	KEB4	G^{-ve}	-	+	++
5	KEB5	G^{-ve}	+	+	++
6	KEB6	G^{-ve}	-	-	+
7	KEB7	G^{-ve}	+	+	+
8	KEB8	G^{-ve}	-	+	++
9	KEB9	G^{-ve}	-	-	+
10	KEB10	G^{-ve}	-	+	+
11	KEB11	G-ve	+	-	-
12	KEB12	G^{-ve}	-	+	+
13	KEB13	G^{-ve}	-	+	++
14	KEB14	G^{-ve}	+	+	++
15	KEB15	G^{-ve}	-	+	+
16	KEB16	G^{-ve}	-	+	++
17	KEB17	G^{-ve}	-	+	+
18	KEB18	G^{-ve}	-	+	-
19	KEB19	G^{-ve}	-	+	++
20	KEB20	G^{-ve}	-	-	-
21	Control	-	-	-	-

***Ammonia, HCN production Positive = **+**, Negative = **-** , IAA production No colour = **-**, Light pink = **+**, Dark pink = **++**.

Bioassay of crude antibiotics of potent endophytes

The crude antibiotics from the five best-performing antagonistic endophytic isolates—KEB2, KEB5, KEB6, KEB7, and KEB11-were tested for their efficacy against the Fusarium wilt pathogen using the agar diffusion well method (Table 6, Fig. 2). The bacterial endophytic isolates effectively suppressed the linear growth of *Fusarium*. Notably, the crude antibiotics from KEB11 and KEB2 exhibited the largest inhibition zones, measuring 35.14 mm and 34.16 mm, respectively.

Table 6. Inhibition zone of *Fusarium* culture by crude antibiotics of bacterial endophytes

Sl. No.	Endophytes	Inhibition zone (mm)
1.	KEB2	34.16
2.	KEB5	32.36
3.	KEB6	32.75
4.	KEB7	25.87
5.	KEB11	35.14
6.	Control	00.00

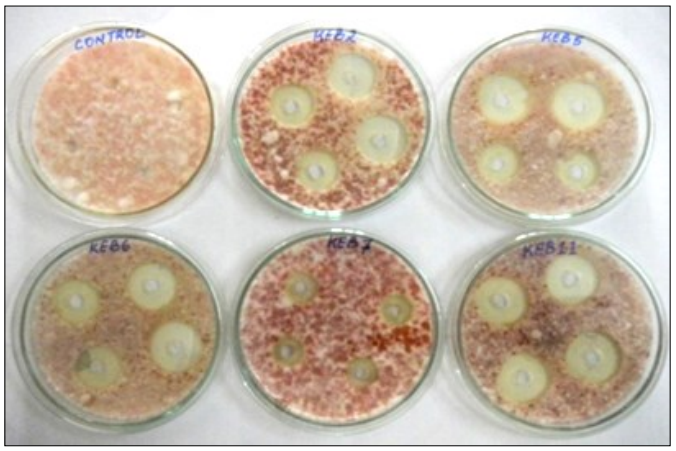

Fig. 2. Bioassay of crude antibiotics of bacterial endophytes by agar plate method. Label (left to right from top) **1**.Control, **2**. KEB2, **3**. KEB5, **4**.KEB6, **5**.KEB7, **6**.KEB11,

Phylogenetic analysis of the potent isolates of endophytes

Sequencing of the 16S-23S rRNA intervening specific region revealed that the nucleotide sequence exhibited over 97 % pairwise similarity with *Pseudomonas spp*. The phylogenetic tree constructed using the Maximum Likelihood method indicated a close relationship, forming two distinct clusters with *P. putida* and *P. fluorescens* (Fig. 3). Based on BLAST and phylogenetic analysis, the isolates KEB5 and KEB7 were identified as *P. putida*, while KEB15 was identified as *P. fluorescens*.

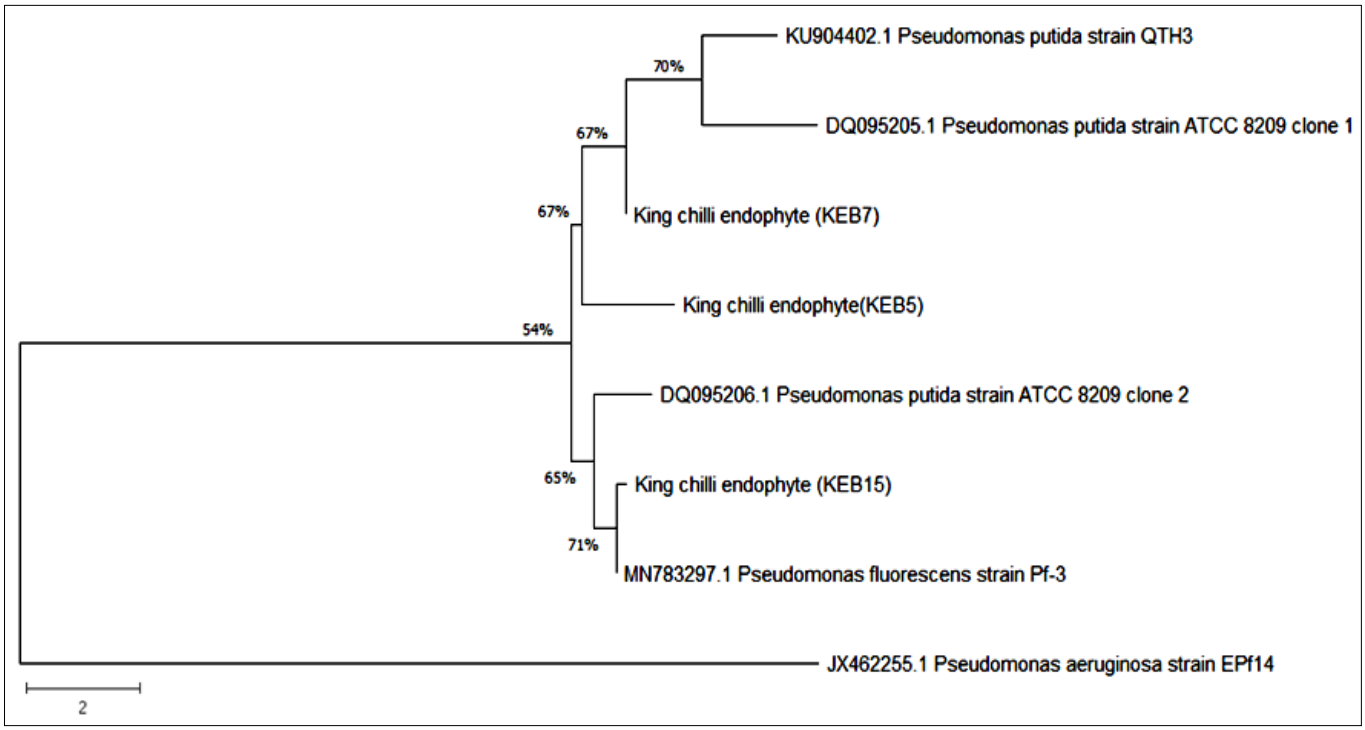

Fig. 3. Evolutionary analysis by Maximum Likelihood method. The evolutionary history was inferred by using the Maximum Likelihood method and Tamura-Nei model (Tamura and Nei 1993). The tree with the highest log likelihood (-5451.86) is shown. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Tamura-Nei model, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. This analysis involved 8 nucleotide sequences. There were a total of 754 positions in the final dataset. Evolutionary analyses were conducted in MEGA X as per Kumar et al. (40).

Table 7. Management of fungal diseases of King chilli by bacterial endophytes.

Treatment	Percent Disease Incidence (%)			Plant height (cm)	No. of fruits/ plant	Yield/ plot (in kg)	Yield (kg/ha)
	Wilt	Anthracnose	Die back				
Control (T0)	7.5	15	30	80.1	36	3.32	2072.58
KEB2 (T1)	7.5	0	17.5	80.2	54	4.04	2523.51
KEB5 (T2)	7.5	7.5	10	68.5	36	4.22	2634.69
KEB6 (T3)	7.5	12.5	15	70.5	24	4.47	2795.23
KEB7 (T4)	0	0	10	85.1	57	5.89	3683.67
KEB15 (T5)	0	5	15	77.0	65	3.97	2481.72
Mancozeb (T7)	2.5	12.5	22.5	73.9	54	5.43	3391.64
CD	0.702	0.878	15.350	7.465	10.781	0.719	449.329
SE(m)	0.225	0.282	5.102	2.396	3.461	0.231	144.22

Evaluation of effective bacterial endophytes against major soil borne diseases of King chilli under field condition

Tests were conducted to evaluate the biocontrol efficacy of five elite bacterial endophytes (KEB2, KEB5, KEB6, KEB7, and KEB15) of King chilli, alongside chemical control, against major chilli diseases and their impact on disease control and yield. The bacterial biocontrol agents were applied under field conditions using soil application, seedling drench, and foliar application. The incidence of bacterial wilt, dieback, and anthracnose was recorded at regular intervals in the field. Among the treatments, isolates KEB7 and KEB15 exhibited the lowest disease incidence compared to the chemical control. Notably, KEB7 was the most effective isolate, achieving a maximum plant height of 85.10 cm and a yield of 3683.67 kg/ha (Table 7).

Discussion

The survey aimed to investigate the diseases affecting King chilli and their impact on crop production. We gathered insights into the occurrence of various pathogens across different growing regions. Fungal diseases were found to be the primary cause of yield loss, with anthracnose and

fruit rot being the most prevalent during the ripening stage. The shelf life of King chilli fruits was significantly reduced, making them highly susceptible to these diseases (43). Anthracnose emerged as a major contributor to economic loss in nearly all growing regions of Nagaland. Similar findings were reported by other authors regarding diseases such as die-back, stem rot, wilt, collar rot, and leaf spot in King chilli fields in Assam (44).

The study focused on isolating endophytes to demonstrate their antagonistic activity against pathogens. Although previous studies primarily examined endophytes in other types of chillies, this research isolated 20 bacterial endophytes from King chilli fruits across various locations in Nagaland. Most of these isolates were identified as Gram-negative bacteria (45) belonging to the genus *Pseudomonas* through analysis of the 16S-23S rRNA intervening sequence. Several findings reported bacterial antagonists showing antagonistic properties were identified by using 16S rDNA Sequence into different genera in chilli. Molecular characterization further identified 18 out of 40 isolates using the 16S rRNA gene (46).

Endophytes colonize the same ecological niche as plant pathogens and establish a close relationship with their host plants (47), making them valuable candidates for biological control. In our *in-vitro* evaluations, these endophytes effectively suppressed pathogens such as *Pythium*, *Fusarium*, and *Colletotrichum*. However, the effectiveness observed *in-vitro* may not directly translate to field conditions. During dual culture screenings, various endophytes demonstrated significant inhibition of pathogens, specifically, endophytes A40F2, A20F2, A26F3, and 20F2 showed impressive results, inhibiting the growth of *Macrophomina phaseolina* by 65 %, *Fusarium oxysporum* by 46 %, *Fusarium solani* by 72 %, and *Cercospora nicotianae* by 70 % (48). Additionally, endophytic bacteria isolated from chilli fruit demonstrated strong inhibition of *Colletotrichum gloesporioides*, *Sclerotium rolfsii, F. oxysporum, C. capsici*, and *Pythium* sp. (46, 49).

The positive effects of inoculating endophytic bacteria stem from their synergistic combination of various PGP traits (50). Endophytes are increasingly recognized for their ability to enhance plant growth and provide antifungal and antibacterial benefits against numerous pathogens (51). In our study, we assessed the mechanisms of plant growth promotion by measuring the production of ammonia, IAA, and HCN. IAA production is particularly significant, as it directly influences plant growth (52), especially in low-fertility soils (53), and enhances root development, thereby improving mineral and water uptake (54-56). Notably, all isolates produced a substantial amount of IAA, except for three. The ability of endophytes to produce IAA plays a crucial role in promoting growth and maintaining plant health. Previous research has similarly highlighted the IAA biosynthetic potential of endophytic bacterial isolates (57, 58). Additionally, ammonia production by these endophytes has been identified as another important PGP trait, acting as a signaling molecule that facilitates interactions between plants and bacteria (59, 60).

Inoculation with HCN-producing bacteria typically does not harm the host plant, and certain rhizobacteria can function effectively as biological control agents (61). Many bacterial endophytes enhance plant growth indirectly by suppressing phytopathogens through the production of antimicrobial substances like HCN, utilizing various mechanisms. In our study, several isolates exhibited strong HCN activity. Notably, 90 % of the isolates were capable of producing HCN, with *Bacillus* and *Pseudomonas* species showing the highest levels of production (62). Almost all isolates demonstrated one or more plant growth-promoting activities, indicating their potential role in supporting plant growth when applied externally. These findings suggest that these endophytes could significantly contribute to enhancing plant health and productivity.

Plant-associated endophytes are significant sources of secondary metabolites with antimicrobial properties, coexisting with their host plants without causing any disease or infection (63). These metabolites are being recognized as effective agents for suppressing plant pathogens. Many of these compounds have demonstrated antibacterial, antifungal, antidiabetic, antioxidant, and immune-suppressive activities (64). In our study, we evaluated the antimicrobial properties of selected endophytic isolates against various pathogens. The strains exhibited strong antagonistic activities, highlighting the potential of endophytes as a valuable source of bioactive antibiotics for disease suppression. For instance, 16 endophytic strains isolated from the wild ethnomedicinal plant *Glycyrrhiza uralensis* (liquorice) effectively controlled common fungal pathogens affecting various crops (65).

The identification of bacterial endophytes was confirmed using 16S-23S rRNA gene sequencing (66). The results indicated that the endophytic bacterial isolates were Gram-negative. Molecular studies classified these isolates within the genus *Pseudomonas*, known for their biocontrol and plant growth-promoting properties. Phylogenetic analysis revealed the selected isolates to be *P. putida* and *P. fluorescens*, both recognized for their strong biocontrol and plant growth-promoting abilities. Several studies have shown that *P. fluorescens* (67, 68) and *P. putida* (69, 70) can quickly colonize plant tissues and effectively utilize plant resources. These species produce a wide array of bioactive metabolites, including antibiotics, siderophores, and growth-promoting substances, which allow them to compete effectively with other microorganisms. Additionally, some *Pseudomonas* species are associated with soilborne pathogens, highlighting their dual role in plant health (68).

Bacteria associated with plants that possess biocontrol and plant growth-promoting (PGP) properties have been suggested as environmentally friendly solutions for sustainable agriculture. However, their effectiveness in field conditions has not been extensively evaluated (67). In this study, we assessed the top-performing bacterial isolates for their potential application in sustainable agriculture, focusing on reducing yield losses caused by diseases. We screened each isolate for disease suppression, taking into account various biocontrol and PGP mechanisms.

Under field conditions, the isolates significantly enhanced plant growth, development, yield, and disease resistance. Among the five most effective endophytic isolates tested in the field trial, KEB7 demonstrated notable disease suppression and yield improvement. For example, in *Brassica napus*, the application of a bacterial consortium containing endophytic *P. fluorescens* led to increased economic yields (67). Additionally, utilizing endophytic bacteria may facilitate the development of biocontrol agents that can self-perpetuate by colonizing host plants and being passed on to their progeny, thereby enhancing both biocontrol and plant growth promotion (71).

Conclusion

The characterization of the diverse community of bacterial endophytes from King chilli has provided valuable insights into biocontrol strategies for suppressing various pathogens and promoting plant growth. Selected elite strains exhibited significant antagonistic activity through mechanisms such as the production of antimicrobial compounds, resource competition, and the induction of systemic resistance in the host plant. The combined biocontrol and growth-promoting capabilities of these endophytes highlight their potential as multifunctional agents in sustainable agriculture. Leveraging these beneficial microbes could enhance disease control and support overall plant health and productivity.

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Authors' contributions

RG and CBKS carried out the isolation of endophytes, molecular genetic studies and lab experiments. TA and PTKJ participated sequence alignment, analysis and correction of manuscript. CR participated in design, statistical analysis and correction of manuscript. DBC conceived of the study and participated in its design and coordination.

Compliance with ethical standards

Conflict of interest: No conflict of interest among the authors

Ethical issues: None.

References

1. Bosland PW, Baral JB. 'Bhut Jolokia'—the world's hottest known chile pepper is a putative naturally occurring interspecific hybrid. HortScience.2007;42:222–224. https://doi.org/10.21273/hortsci.42.2.222
2. Sanatombi K, Sen-Mandi S, Sharma G. DNA profiling of Capsicum landraces of Manipur. Scientia Horticulturae. 2010;124:405–408.https://doi.org/10.1016/j.scienta.2010.01.006
3. Bhagowati RR, Changkija S. Genetic variability and traditional practices in Naga King Chili landraces of Nagaland. Asian Agri-History. 2009;13:171–180.
4. Sanatombi K, Sharma G. In-vitro propagation of Capsicum chinense Jacq. Biologia Plantarum. 2008;52:517–520.https://doi.org/10.1007/s10535-008-0100-x
5. Kumar S, Kumar R, Kumar S, Kumar Singh A, Singh M, Bahadur Rai A, Rai M. Incidence of leaf curl disease on Capsicum germplasm under field conditions. Indian Journal of Agricultural Science. 2011;81:187.
6. Verma PK, Rawat KK, Das N, Pradhan B. A botanical enigma of India's hottest chilli Bhoot Jolokia (Capsicum chinense Jacq). New York Science Journal. 2013;6:49–51.
7. Liu Y, Nair MG. Capsaicinoids in the hottest pepper Bhut Jolokia and its antioxidant and anti-inflammatory activities. Natural Product Communications.2010;5:1934578X1000500122. https://doi.org/10.1177/1934578x1000500122
8. Sarwa KK, Mazumder B, Rudrapal M, Debnath M, Kumar A, Verma VK, Jangdey MS. Capsaicinoids content of some indigenous capsicum varieties of Assam, India. Journal of Natural Sciences Research. 2013;3:112–116.
9. Purkayastha J, Alam S, Gogoi H, Singh L, Veer V. Molecular characterization of 'Bhut Jolokia' the hottest chilli. Journal of Biosciences. 2012;37:757–768.https://doi.org/10.1007/s12038-012-9249-8
10. Bosland PW, Votava EJ, Votava EM. Peppers: vegetable and spice capsicums. CABI; 2012.https://doi.org/10.1079/9781845938253.0000
11. Kempaiah R, Manjunatha H, Srinivasan K. Protective effect of dietary capsaicin on induced oxidation of low-density lipoprotein in rats. Molecular and Cellular Biochemistry. 2005;275:7–13.https://doi.org/10.1007/s11010-005-7643-3
12. Fisher P, Petrini O. Location of fungal endophytes in tissues of Suaeda fruticosa: a preliminary study. Transactions of British Mycological Society. 1987;89:246–249.https://doi.org/10.1016/s0007-1536(87)80161-4
13. Strobel GA. Endophytes as sources of bioactive products. Microbes and Infection. 2003;5:535–544.https://doi.org/10.1016/s1286-4579(03)00073-x
14. Berg G, Hallmann J. Control of plant pathogenic fungi with bacterial endophytes. In: Schulz B, Boyle C, Sieber T, editors. Microbial root endophytes. Springer; 2006. p. 53–69.https://doi.org/10.1007/3-540-33526-9_4
15. Hallmann J, Quadt-Hallmann A, Mahaffee WF, Kloepper JW. Bacterial endophytes in agricultural crops. Canadian Journal of Microbiology. 1997;43:895–914.https://doi.org/10.1139/m97-131
16. Ramamoorthy V, Viswanathan R, Raguchander T, Prakasam V, Samiyappan R. Induction of systemic resistance by plant growth promoting rhizobacteria in crop plants against pests and diseases. Crop Protection. 2001;20:1–11.https://doi.org/10.1016/s0261-2194(00)00056-9
17. Sturz A, Matheson B. Populations of endophytic bacteria which influence host-resistance to Erwinia-induced bacterial soft rot in potato tubers. Plant Soil. 1996;184:265–271.https://doi.org/10.1007/bf00010455
18. Duijff BJ, Gianinazzi-Pearson V, Lemanceau P. Involvement of the outer membrane lipopolysaccharides in the endophytic colonization of tomato roots by biocontrol Pseudomonas fluorescens strain WCS417r. New Phytologists. 1997;135:325–334. https://doi.org/10.1046/j.1469-8137.1997.00646.x
19. Krishnamurthy K, Gnanamanickam SS. Biological control of sheath blight of rice: Induction of systemic resistance in rice by plant-associated Pseudomonas spp. Current Science. 1997;331–334.

20. Hallmann J, Quadt-Hallmann A, Rodríguez-Kabana R, Kloepper JW. Interactions between Meloidogyne incognita and endophytic bacteria in cotton and cucumber. Soil Biology and Biochemistry. 1998;30:925–937.https://doi.org/10.1016/s0038-0717(97)00183-1

21. Azevedo JL, Maccheroni Jr W, Pereira JO, De Araújo WL. Endophytic microorganisms: a review on insect control and recent advances on tropical plants. Electronic Journal of Biotechnology. 2000;3:15–16.https://doi.org/10.2225/vol3-issue1-fulltext-4

22. Chanway C. Inoculation of tree roots with plant growth promoting soil bacteria: an emerging technology for reforestation. Forest Science. 1997;43:99–112.https://doi.org/10.1093/forestscience/43.1.99

23. Bent E, Chanway CP. The growth-promoting effects of a bacterial endophyte on lodgepole pine are partially inhibited by the presence of other rhizobacteria. Canadian Journal Microbiology. 1998;44:980–988.https://doi.org/10.1139/w98-097

24. Opoku I, Assuah M, Aneani F. Management of black pod disease of cocoa with reduced number of fungicide application and crop sanitation. African Journal of Agriculral Research. 2007;2:601–604.

25. Rangaswami G. An agar block technique for isolating soil microorganisms with special reference to Pythiaceous fungi. Science and Culture. 1958;24:85.

26. Rangaswami G, Mahadevan A. Diseases of crop plants in India. PHI Learning Pvt. Ltd.; 1998.

27. Pleban S, Ingel F, Chet I. Control of Rhizoctonia solani and Sclerotium rolfsii in the greenhouse using endophytic Bacillus spp. Eurapean Journal of Plant Pathology. 1995;101:665–672.https://doi.org/10.1007/bf01874870

28. Shi Y, Lou K, Li C. Isolation, quantity distribution and characterization of endophytic microorganisms within sugar beet. African Journal of Biotechnology. 2009a;8.https://doi.org/10.5897/ajb12.1403

29. McInroy JA, Kloepper JW. Population dynamics of endophytic bacteria in field-grown sweet corn and cotton. Canadian Journal of Microbiology. 1995;41:895–901.

30. Sudhir A, Kumar NP, Audipudi AV, others. Isolation, biochemical and PGP characterization of endophytic Pseudomonas aeruginosa isolated from chilli red fruit antagonistic against chilli anthracnose disease. International Journal of Current Microbiology and Applied Sciences. 2014;3:318–329.

31. Knapp JE, Chandlee JM. RNA/DNA mini-prep from a single sample of orchid tissue. Biotechniques. 1996;21:54–56.https://doi.org/10.2144/96211bm11

32. Melody SC. Plant Molecular Biology - A laboratory manual. Springer-Verlag, New York. 1997;121-154.https://doi.org/10.1007/bf02752266

33. Kumar NR, Arasu VT, Gunasekaran P. Genotyping of antifungal compounds producing plant growth-promoting rhizobacteria, Pseudomonas fluorescens. Current Science. 2002;1463–1466

34. Vicente MRS, Plasencia J. Salicylic acid beyond defence: its role in plant growth and development. Journal of Experimental Botany. 2011;62: 3321-38.https://doi.org/10.1093/jxb/err031

35. Patten CL, Glick BR. Role of Pseudomonas putida indoleacetic acid in development of the host plant root system. Applied and Environmental Microbiology. 2002;68:3795–3801.https://doi.org/10.1128/aem.68.8.3795-3801.2002

36. Miller R, Higgins VJ. Association of cyanide with infection of birdsfoot trefoil by Stemphylium loti. Phytopathology. 1970;60:104–110.https://doi.org/10.1094/phyto-60-104

37. Cappuccino J, Sherman N. Biochemical activities of microorganisms. In: Microbiology: A Laboratory Manual. The Benjamin/Cummings Publishing Co; 1992. p. 188–247.

38. Ramarathnam R, Bo S, Chen Y, Fernando WD, Xuewen G, De Kievit T. Molecular and biochemical detection of fengycin- and bacillomycin D-producing Bacillus spp., antagonistic to fungal pathogens of canola and wheat. Canadian Journal of Microbiology. 2007;53:901–911.https://doi.org/10.1139/w07-049

39. Smania Jr A, Monache FD, Smania E de FA, Cuneo RS. Antibacterial activity of steroidal compounds isolated from Ganoderma applanatum (Pers.) Pat. (Aphyllophoromycetideae) fruit body. International Journal of Medical Mushrooms. 1999;1.https://doi.org/10.1615/intjmedmushr.v1.i4.40

40. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: molecular evolutionary genetics analysis across computing platforms. Molecular Biology and Evolution. 2018;35:1547.https://doi.org/10.1093/molbev/msy096

41. Montri PP, Taylor PWJ, Mongkolporn O. Pathotypes of Colletotrichum capsici the causal agent of chili anthracnose in Thailand. Plant Disease. 2009;93(1):17–20.http://dx.doi.org/10.1094/PDIS-93-1-0017

42. Gomez KA, Gomez AA. Statistical procedures for agricultural research. John Wiley & Sons; 1984.

43. Rajesha G, Bendangsenla, Deka BC, Ngachan SV. Isolation and screening of bacterial endophytes against the fungal pathogens of Naga King Chilli. In: National Seminar on Sustaining Hill Agriculture in Changing Climate: A Compendium of Seminar Papers. Agartala, Tripura, India; December 5-7, 2015. p. 254–255.

44. Talukdar J, Saikia A, Borah P, others. Survey and detection of the diseases of Bhut Jolokia (Capsicum chinense Jacq.) in Assam. Journal of Crop and Weed. 2015;11:186–192.

45. Dowarah B, Agarwal H, Krishnatreya DB, Sharma PL, Kalita N, Agarwala N. Evaluation of seed associated endophytic bacteria from tolerant chilli cv. Firingi Jolokia for their biocontrol potential against bacterial wilt disease. Microbiological Research. 2021;248:126751. https://doi.org/10.1016/j.micres.2021.126751

46. Amaresan N, Jayakumar V, Thajuddin N. Isolation and characterization of endophytic bacteria associated with chilli (Capsicum annuum) grown in coastal agricultural ecosystem. Indian Journal of Biotechnology. 2014;1:123–130.

47. Melnick RL, Zidack NK, Bailey BA, Maximova SN, Guiltinan M, Backman PA. Bacterial endophytes: Bacillus spp. from annual crops as potential biological control agents of black pod rot of cacao. Biological Control. 2008;46:46–56.https://doi.org/10.1016/j.biocontrol.2008.01.022

48. Mmbaga M, Gurung MA, Maheshwari A. Screening of plant endophytes as biological control agents against root rot pathogens of pepper (Capsicum annum L.). Journal of Plant Pathology and Microbiology. 2018;9.https://doi.org/10.4172/2157-7471.1000435

49. Allu S, Kumar NP, Audipudi AV. Isolation, biochemical and PGP characterization of endophytic Pseudomonas aeruginosa isolated from chilli red fruit antagonistic against chilli anthracnose disease. International Journal of Current Microbiology and Applied Sciences. 2014;3(2):318-329.

50. Ribeiro VP, Marriel IE, Sousa SM de, Lana UG de P, Mattos BB, Oliveira CA de, Gomes EA. Endophytic Bacillus strains enhance pearl millet growth and nutrient uptake under low-P. Brazilian Journal of Microbiology. 2018;49:40–46.https://doi.org/10.1016/j.bjm.2018.06.005

51. Kushwaha P, Kashyap PL, Bhardwaj AK, Kuppusamy P, Srivastava AK, Tiwari RK. Bacterial endophyte mediated plant tolerance to salinity: growth responses and mechanisms of action. World Journal of Microbiology and Biotechnology. 2020;36:26.https://doi.org/10.1007/s11274-020-2804-9

52. Shi Y, Lou K, Li C. Isolation, quantity distribution and characterization of endophytic microorganisms within sugar beet. African Journal of Biotechnology. 2009b;8.

53. Khan Z, Doty SL. Characterization of bacterial endophytes of sweet potato plants. Plant and Soil. 2009;322:197–207.https://doi.org/10.1007/s11104-009-9908-1

54. Ladha JK, Reddy PM. Steps towards nitrogen fixation in rice. In: Ladha JK, Hauggaard-Nielsen H, editors. The Quest for Nitrogen Fixation in Rice. 2000. p. 33–46.

55. Gyaneshwar P, James EK, Mathan N, Reddy PM, Reinhold-Hurek B, Ladha JK. Endophytic colonization of rice by a diazotrophic strain of Serratia marcescens. Journal of Bacteriology. 2001;183:2634–2645.https://doi.org/10.1128/jb.183.8.2634-2645.2001

56. Rosenblueth M, Martínez-Romero E. Bacterial endophytes and their interactions with hosts. Molecular Plant-Microbe Interactions. 2006;19:827–837.https://doi.org/10.1094/mpmi-19-0827

57. Jasim B, Jimtha CJ, Jyothis M, Radhakrishnan E. Plant growth promoting potential of endophytic bacteria isolated from Piper nigrum. Plant Growth Regulations. 2013;71:1–11.https://doi.org/10.1007/s10725-013-9802-y

58. Khan MS, Gao J, Chen X, Zhang M, Yang F, Du Y, Munir I, Xue J, Zhang X, others. Isolation and characterization of plant growth-promoting endophytic bacteria Paenibacillus polymyxa SK1 from Lilium lancifolium. Biomed Research International. 2020. 1. 8650957. https://doi.org/10.1155/2020/8650957

59. Deepa C, Dastager SG, Pandey A. Isolation and characterization of plant growth promoting bacteria from non-rhizospheric soil and their effect on cowpea (Vigna unguiculata (L.) Walp.) seedling growth. World Journal of Microbiology and Biotechnology. 2010;26:1233–1240.https://doi.org/10.1007/s11274-009-0293-y

60. Hassan SE-D. Plant growth-promoting activities for bacterial and fungal endophytes isolated from medicinal plant of Teucrium polium L. Journal of Advance Research. 2017;8:687–695. https://doi.org/10.1016/j.jare.2017.09.001

61. Saharan B, Nehra V. Plant growth promoting rhizobacteria: a critical review. Life Science Medical Research. 2011;21:30.https://doi.org/25 December 2024

62. 10.4172/2157-7471.1000266

63. Ghodsalavi B, Ahmadzadeh M, Soleimani M, Madloo PB, Taghizad-Farid R. Isolation and characterization of rhizobacteria and their effects on root extracts of Valeriana officinalis. Australian Journal of Crop Science. 2013;7:338–344.

64. Saikkonen K, Wäli P, Helander M, Faeth SH. Evolution of endophyte–plant symbioses. Trends in Plant Sciences. 2004;9:275–280.https://doi.org/10.1016/j.tplants.2004.04.005

65. Christina A, Christapher V, Bhore SJ. Endophytic bacteria as a source of novel antibiotics: an overview. Pharmacognosy Review. 2013;7:11.https://doi.org/10.4103/0973-7847.112833

66. Mohamad OA, Li L, Ma J-B, Hatab S, Xu L, Guo J-W, Rasulov BA, Liu Y-H, Hedlund BP, Li W-J. Evaluation of the antimicrobial activity of endophytic bacterial populations from Chinese traditional medicinal plant licorice and characterization of the bioactive secondary metabolites produced by Bacillus atrophaeus against Verticillium dahliae. Frontiers in Microbiology. 2018;9:924.https://doi.org/10.3389/fmicb.2018.00924

67. Rebotiloe FM, Eunice U-J, Mahloro HS-D. Isolation and identification of bacterial endophytes from Crinum macowanii Baker. African Journal of Biotechnology. 2018;17:1040–1047. https://doi.org/10.5897/AJB2017.16350

68. Lally RD, Galbally P, Moreira AS, Spink J, Ryan D, Germaine KJ, Dowling DN. Application of endophytic Pseudomonas fluorescens and a bacterial consortium to Brassica napus can increase plant height and biomass under greenhouse and field conditions. Frontiers in Plant Sciences. 2017;8:2193.https://doi.org/10.1007/s10535-008-0100-x

69. Raghuveer Singh, Tiameren Ao N, Valenta Kangjam, Rajesha G, Susanta Banik. Plant growth promoting microbial consortia against late blight disease of tomato under natural epiphytotic conditions. Indian Phytopathology. 2022;75:527–539. https://doi.org/10.1007/s42360-022-00464-1

70. Compant S, Duffy B, Nowak J, Clément C, Barka EA. Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and future prospects. Applied and Environmental Microbiology. 2005;71:4951–4959.https://doi.org/10.52783/eel.v14i2.1549

71. Sheoran N, Nadakkakath AV, Munjal V, Kundu A, Subaharan K, Venugopal V, Rajamma S, Eapen SJ, Kumar A. Genetic analysis of plant endophytic Pseudomonas putida BP25 and chemo-profiling of its antimicrobial volatile organic compounds. Microbiological Research. 2015;173:66–78. https://doi.org/10.21273/hortsci.42.2.222

72. Boddey RM, Urquiaga S, Alves BJ, Reis V. Endophytic nitrogen fixation in sugarcane: present knowledge and future applications. Plant and Soil. 2003;252:139–149.https://doi.org/10.1023/a:1024152126541