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Diversity of Antimicrobial Peptide Genes in *Bacillus* from the Andaman and Nicobar Islands: Untapped Island Microbial Diversity for Disease Management in Crop Plants

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Abstract

Taxonomic and functional characterization of a total of 90 bacterial isolates representing bulk and rhizosphere soils of diverse niches of Andaman and Nicobar Islands, India were carried out. Twelve bacterial isolates were found promising for the biological suppression of agriculturally important fungal and bacterial plant pathogens such as *Ralstonia solanacearum, Xanthomonas oryzae* pv. *oryzae, and Colletotrichum gloeosporioides.* The 16S rRNA gene sequence analysis revealed their identity as belonging to *Bacillus subtilis, Bacillus amyloliquefaciens,* and *Lysinibacillus sphaericus.* The isolates were positive for plant growth promotion (PGP) traits including siderophore production, and nutrient solubilization especially phosphorous, zinc, and potassium. Interestingly, the PCR test confirmed the presence of 62 antimicrobial peptides (AMP) biosynthesis genes specific to the genus *Bacillus.* Whilst all tested species of *Bacillus* harboured the *bacD* biosynthesis gene, the *B. subtilis* (Ba_Abi), and *B. amyloliquefaciens* (Ba_Abi) harboured the maximum AMP biosynthesis genes analysed in the study. Upon *in planta* evaluation, the biocontrol potential of the bacterial isolates against leaf spot disease of chilli was observed. The study culminated in the isolation and identification of diverse *Bacillus* species for exploitation as bioinoculants for plant health management programmes.

Introduction

Bacillus species—the key member of the phylum Firmicutes are ubiquitous in diverse environments as well as extreme niches. The ecological adaptation of *Bacillus* is mainly attributed to the production of resting spores for survival in adverse conditions [28]. In agriculture, *Bacillus* species have been exploited as microbial inoculants for crop production and protection against pests and pathogens in particular [10, 12, 36, 38, 40]. The biological control potential of *Bacillus* species is due to their ability to secrete metabolites especially antimicrobial peptides (AMP) that are also reported as anti-microbial, anti-viral, and anti-tumour activities [9, 43]. Over 70 different antibiotics are produced by *Bacillus* against taxonomically diverse phytopathogenic microorganisms [4, 41]. Recently, biosynthetic genes involved in the production of AMP have been identified as bmyB, fenD, ituC, and *srfA* which are involved in the production of cyclic lipopeptides such as bacillomycin, fengycin, iturin, and surfactin A displaying direct antimicrobial activities [7, 41, 44]. In addition, *Bacillus* is well known for its ability to contribute to plant growth by the production of phytohormones and plant nutrient mobilization [2, 17, 21] and abiotic stress [28].

The Andaman and Nicobar group of Islands situated in the Bay of Bengal are well-separated landmass that is stretching from Myanmar in the north to Sumatra in the south between 6° and 14° North latitudes and 92° and 94° East longitudes. The total geographical area of Andaman and Nicobar Islands is 8249 sq. km which is covering of

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86% forest area and 14% cleared for habitation and agriculture purposes. The substantial undisturbed island clusters are unique and well known for their rich biodiversity including microbial wealth that is yet to be explored and harnessed for the benefit of humankind. In the present study, we attempted to explore, isolate, and characterize *Bacillus* and the closely related genera from diverse locations of the Bay islands. We further conducted a series of functional assays to harness them for plant health management in agriculture.

Materials and Methods

Soil Sampling and Isolation of Bacteria

Bulk soil and rhizosphere soil samples from agricultural, forest, and non-agricultural ecosystems of Bay islands were collected (S Table 1). Whilst the agricultural samples represented the rhizosphere of major crops like tomato and rice, the non-agricultural samples represented Forest soil, Coastal soil, Barren Island, Saddle Peak, and Termite colonies. The bacterial isolation was performed by serial dilution technique. Briefly, 1.0 g of soil was suspended in 9.0 ml sterile water and was decimally diluted up to 10^{-7}. The 100 µl of suspension from 10^{-5}–10^{-7}dilutions were spread onto nutrient agar plates and incubated at 28 ± 2 °C. The morphologically different and dominant colonies obtained after 24–48 h of incubation were sub-cultured and maintained as glycerol stock at – 20 °C.

Activity Screening for Antimicrobial Activity

For functional screening, we evaluated the antimicrobial activities of *Bacillus* and the related bacterial genera isolated from the Andaman Islands on *Ralstonia solanacearum* (GenBank Accession: KJ010182), *Xanthomonas oryzae* pv. *oryzae* (GenBank Accession: KU533760), and *Colletotrichum gloeosporioides* (GenBank Accession: KX449536), causing diseases on cereals and vegetables on the island, and are a major threat to the expansion of agricultural activities in Andaman. In brief, antibacterial activity on *R. solanacearum* and *Xanthomonas oryzae* pv. *oryzae* was tested by adopting the agar diffusion method on King's Medium B [31]. Here, 200 ml of King's B medium seeded with freshly grown 48 h old bacterial cells (1×10^8 CFU ml^{-1}) was allowed to solidify in a Petri plate, followed by drop inoculation of exponential growing *Bacillus* (1×10^8 CFU ml^{-1}; 20 µl) in a 7.0 mm wells; the plates were incubated at 28 °C for 24 h. Sterile water was used as the negative control. Data on the inhibition zone was recorded at 48 hpi.

For antifungal activity, a dual-culture confrontation assay was carried out on *C. gloeosporioides* [43]. In brief, a five-mm mycelial plug from a freshly grown culture of *C. gloeosporioides* was placed at the centre of the potato dextrose agar plate, and the *Bacillus* isolates were streaked at four equidistant sites 2.5 cm from the margin of mycelial plugs. Mock plates were maintained only with *C. gloeosporioides* sans the bacterial streak, and the plates were incubated at 28 °C for seven days. The antagonistic effect of test isolates against *C. gloeosporioides* was observed based on the relative growth of fungi in treatment and control using the formula as follows.

$$\text{Per cent Inhibition} = (C - T) / C \times 100;$$

where $C -$ The growth rate of the pathogen in control and $T -$ Growth of pathogen in treatment

All the experiments were repeated twice with three replications. Twelve putative *Bacillus* isolates showing consistent and broad-spectrum antagonism against both bacterial and fungal pathogens in vitro were selected for further analysis.

Identification of Bacterial Species

Species identity of *Bacillus* and related bacterial isolates were established using 16S rRNA gene sequence analysis. For amplification and sequencing of the 16S rRNA gene, Polymerase Chain Reactions (PCR) methodology suggested by Edwards et al. [14] was used with slight modifications. PCR reactions (50 µl) contained 100 ng genomic DNA, 200 µM concentrations of each deoxynucleotide triphosphate, 10 pmol concentrations of each forward and reverse primers, 1.5 mM $MgCl_2$, 1X Taq buffer A (GeNeI), and 1 U of Taq DNA polymerase. The polymerase chain reaction was performed in a Thermal Cycler (C1000TM, Bio-Rad, Hercules, California, United States) with an initial denaturation of 95˚C for 5.0 min followed by 35–40 cycles of 92 °C for 1.0 min, the annealing temperature of 48 °C for 30 s and 72 °C for 2 min and a final extension step of 72 °C for 6 min. PCR products were resolved using 1.0% agarose gel and purified products were sequenced bi-directionally and analysed. The sequences obtained were assembled in DNA Baser.v4 software, edited, and end trimmed in CLC sequence viewer. Then blast search was performed in the NCBI database (https://blast.ncbi.nlm.nih.gov/Blast.cgi) for genetic identity and the final sequences were submitted to NCBI [26].

Detection of AMP Genes in the Bacterial Isolates

The bacterial strains were PCR screened for the presence of antimicrobial (AMP) biosynthesis genes using a total of 18 primers specific for several AMP genes described by Chung et al. [11] (S Table 2). PCR amplifications were carried out with 50-µl reaction mixtures using a Thermal Cycler

(C1000™, Bio-Rad, Hercules, California, United States) with the following cycle conditions: initial activation at 95 °C for 15 min; 40 cycles of 95 °C for 1.0 min, the annealing temperature of 55 °C or 52 °C for 1.0 min, and 72 °C extensions for 1.5 min; and a final extension at 72 °C for 7.0 min. A total of 5.0 µl of each amplification reaction was analysed by electrophoresis using a 1.5% agarose gel followed by ethidium bromide staining and UV visualization. PCR amplification, sequencing, and sequence submission in the public database were performed as described above.

Screening for Plant Probiotic Bacterial Traits

The bacterial isolates were screened for PGP traits such as production of siderophore [37]; phosphorous (P) solubilization [31]; potassium (K) solubilization [18, 30]; and zinc (Zn) solubilization [39] by adopting standard methods.

Screening for Plant Growth Promotion and Vigour

The potential of *Bacillus* and the related genera was evaluated on chilli (*Capsicum annuum* L)-the emerging vegetable crop on Andaman Island for enhancing the seedling vigour and plant growth. A total of 12 *Bacillus* isolates showing antifungal activity were tested on chilli variety, Co-1. Briefly, the chilli seeds (1.0 g) were soaked for 30 min in 1.0 ml of mid-log phase bacterial cell suspension (~ 1 $X10^7$ cfu/ml), and air-dried in shade before sowing. Bacterized seeds were sown in plastic filled with partially sterilized soil mixture (2:1:1 mixture of laterite soil: fine sand: farmyard manure) and were grown in greenhouse conditions set at 28 ± 2 ℃ for 4 weeks. The seedling vigour and growth promotion were calculated as described below [13]

$$\begin{aligned}&\text{Percent seedling growth promotion (\%)}\\&= \left[\text{total seedling length in treatment}\right]\\&- \left[\text{total seedling length in control}\right]/\\&\text{total seedling length in control]} \times 100\end{aligned}$$

Screening for Plant Disease Suppression

The disease suppressive potential of *Bacillus* and the related genera was evaluated against the emerging foliar disease of chilli caused by *Colletotrichum* [16]. Actively growing cell suspension of *Bacillus* (~ 10^7 CFU ml^{-1}) was prophylactically foliar sprayed using a hand sprayer and challenged with a conidial suspension of *Colletotrichum* (~ 1×10^8 CFU ml^{-1}) sprayed on the leaf surfaces. For comparison, carbendazim (50 WP) and sterile water served positive and negative control, respectively. All the experimental plants were incubated at 28 ± 2 °C and 90% RH under the greenhouse. Three replications were maintained for all treatments. Data on the disease severity on five randomly selected leaves in each treatment was recorded and scored as prescribed [22]; here, **0.0:** no leaf spot symptom; **1.0:** up to 1% leaf area covered by symptoms; **3.0:** 1–10% leaf area covered by symptoms; **5.0:** 11–25% leaf area covered by symptoms**; 7.0:** 26–50% leaf area covered by symptoms; **9.0:** > 50% leaf area covered by symptom, and the disease severity was calculated as follows.

$$\begin{aligned}&\text{Disease severity (Percent disease index)}\\&= \left[\text{Sum of all disease ratings}/\right.\\&\left(\text{Total number of ratings} \times \text{Maximum}\right.\\&\left.\text{disease grade}\right)\left.\right] \times 100\end{aligned}$$

$$\begin{aligned}&\text{Biocontrol efficacy (\%)} = \left[\text{Disease severity in control}\right]\\&- \left[\text{Disease severity in treatment}\right]/\text{Disease severity in control]}\\&\times 100\end{aligned}$$

Statistical Analysis

The statistical analysis was carried out using online WASP-Web Agri Stat Package 2.0 (http://www.ccari.res.in/wasp2.0/index.php) developed by ICAR-Central Coastal Agricultural Research Institute, Goa, India. All the results were expressed as the mean ± SE of different independent replicates. The values of $P \leq 0.05/0.01$ were considered statistically significant.

Submission of Gene Sequences to Databases

All curated sequences were submitted to GenBank database [26], and accession number were derived as follows;**16S rRNA gene sequences:** *Bacillus amyloliquefaciens* Ba_Abi **-KP864633**; *Bacillus subtilis* Bs_Ahv **-KP864634**; *Bacillus subtilis* Bs_Adg **-KP864635**; *Bacillus subtilis* Bs_Ane-**KP864636**; *Bacillus subtilis* Bs_Asi **-KP864637**; *Bacillus subtilis* SM4 **-MH988460**; *Bacillus subtilis* Sp_Fs-**MH427072**; *Bacillus subtilis* Cc_Ss -**MH427073**; *Bacillus subtilis* Mh_Fs-**MH427071**; *Bacillus subtilis* Wn_Ss-**MH427074**; *Lysinibacillus sphaericus* Ls_Agu **-KP864632**; *Lysinibacillus sphaericus* NS2 **-MH988459. AMP gene sequences:** i. **Iturin**- ***ituC***: KY560273, KY560274; -***ituD***: KY560275, KY560276; ii. **Bacillomycin -*bamC***: KY560263; iii. **Bacilysin-*bacAB***: KY560298 **to** KY560300; ***bacD*** -KY560251**to**KY560262; iv. **Mersacidin- *mrsA***: KY560309; v. **Fengycin- *fenB*:** KY560302 **to** KY560308;

fenCEA: KY560264 **to** KY560266; **vi. Surfactin-*srfA***: KY560288 **to** KY560298; ***Sfp***: KY560280 **to** KY560283; vii. **Subtilin-*spaB-erib*:** KY560284, KY560285; ***spacS*:** KY560286, KY560287; viii. **Subtilosin- *albF***: KY560245 **to** KY560250; ***albA***: KY560267 **to** KY560272.

Results

Isolation and Identification of Bacteria

A total of ninety bacterial isolates obtained from diverse locations and niches in six different islands of the Andaman & Nicobar archipelago (Fig. 1; S Table 1) were characterized. The bacterial isolates represented crop rhizosphere (21), bulk agricultural soil (35), marine environments (13), forest environments (16), and unique niches like Barren Island, Mud volcano, Saddle, Peak, and Termite soil (6). Functional screening of the bacterial isolates revealed excellent antifungal and antibacterial activities of twelve bacterial isolates (Fig. 1) on three important phytopathogens (Table 1; S Table 1). The 16S rRNA gene sequence analysis revealed their species identity as *Bacillus subtilis* (9), *Bacillus amyloliquefaciens* (1), and *Lysinibacillus sphaericus* (2). The bacterial isolates were assigned accession numbers and deposited in NCBI.

Screening for Antimicrobial Activity

Activity screening performed on selected plant pathogens revealed their excellent antibacterial activity. Whilst *Bacillus amyloliquefaciens* (Ba_Abi), and *Bacillus subtilis* (Bs_Ahv) were found promising for suppression of bacterial wilt pathogen *R. solanacearum,* the isolates *Bacillus subtilis* (Bs_Ane) and *Lysinibacillus sphaericus* (Ls_Agu) inhibited bacterial blight pathogen, *Xanthomonas oryzae* pv. *oryzae.* Three of the isolates, *Bacillus subtilis* (Bs_Ane), *Bacillus subtilis* (Bs_Adg), and *Bacillus subtilis* (Wn_S) displayed antifungal activity on the fungal pathogen *Colletotrichum gloeosporioides* (Table 2).

Screening for AMP Genes

PCR-based screening confirmed the presence of a total of 62 AMP gene sequences in the 12 *Bacillus* strains that showed antimicrobial activity on bacterial and fungal pathogens. The results revealed a high-frequency occurrence of AMP genes in bacterial isolates obtained from the island (Fig. 2). Whilst *Bacillus amyloliquefaciens* Ba_Asi showed nine AMP biosynthesis gene sequences, Ba_Abi recorded eight of them. Whereas the bacilysin-specific *bacD* gene was found in all the twelve stains tested; the surfactin-specific *srfA* gene was detected in ten of the isolates. Other AMPs such as fengycin (*fenB*), subtilosin (*albF* and *albA*), and bacillomycin (*bamC*) gene were also detected. Nucleotide sequence analysis

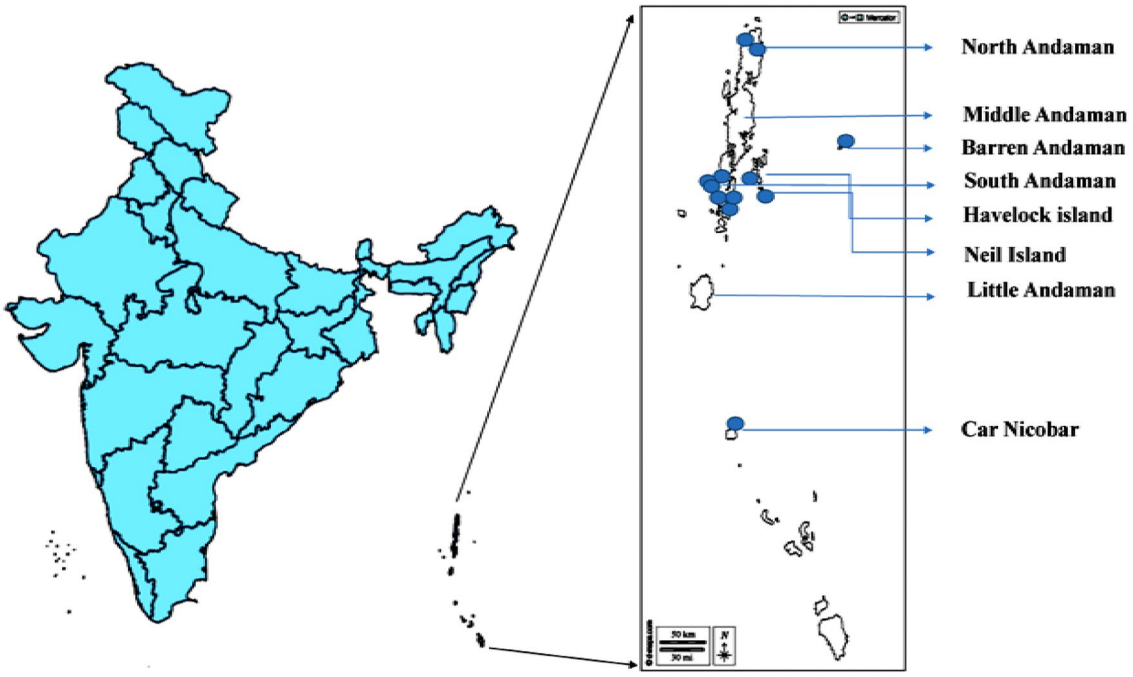

Fig. 1 Sampling sites and isolates obtained from in various locations at Andaman & Nicobar Islands. Details of samples and sampling sites: Refer to Supplementary Table 1

Table 1 Diversity of *Bacillus* and *Bacillus* -derived genera representing various sites of Andaman and Nicobar Islands

Strains	Place of Collection	Niche	Crop	16S rRNA gene sequence based identification	Colony morphology
Ba_Abi	Barren Island	Soil	Volcano	*Bacillus amyloliquefaciens*	
Bs_Ahv	Havelock Island	Rhizosphere	Tomato	*Bacillus subtilis*	
Bs_Adg	Diglipur, North Andaman	Rhizosphere	Brinjal	*Bacillus subtilis*	
Bs_Ane	Neil Island	Rhizosphere	Chilli	*Bacillus subtilis*	
Bs_Asi	Sippighat, South Andaman	Rhizosphere	Coconut	*Bacillus subtilis*	
SM4	Shoalbay, South Andaman	Manure pit	Nil	*Bacillus subtilis*	
Sp_Fs	Saddle Peak, North Andaman	Forest soil	Nil	*Bacillus subtilis*	
Cc_Ss	Carbyn's Cove, South Andaman	Seashore soil	Coconut	*Bacillus subtilis*	

Table 1 (continued)

Strains	Place of Collection	Niche	Crop	16S rRNA gene sequence based identification	Colony morphology
Mh_Fs	Mount Harriet	Forest soil	Nil	*Bacillus subtilis*	
Wn_Ss	Wandoor, South Andaman	Sea shore soil	Mangrove	*Bacillus subtilis*	
Ls_Agu	Guptapara, South Andaman	Rhizosphere	Brinjal	*Lysinibacillus sphaericus*	
NS2	Nicobar island	Sand	Nil	*Lysinibacillus sphaericus*	

revealed a high identify 99–100% match with sequences available in the NCBI database. All sequences were submitted to the NCBI database and GenBank numbers were assigned (Table 3).

Screening for Plant Probiotic Traits

Bacterial isolates displayed diverse function traits contributing towards plant growth promotion and seedling vigour. Significantly, the *Bacillus* and related genera isolate from Andaman island displayed siderophore secretion (12 isolates), phosphate solubilization (9 isolates), zinc solubilization (10 isolates), and potassium solubilization (5 isolates). Notably, *Bacillus subtilis* Bs_Asi was found prolific for all the PGP traits tested (Table 2). In tune with the observation of plant growth conferring bacterial traits, *Bacillus amyloliquefaciens* Ba_Abi contributed to growth promotion in chilli seedlings up to 60.8%; the isolates such as *Bacillus subtilis* Bs_Ahv (52.7%) and *Bacillus subtilis* Bs_Asi (52.68%) also showed promise for growth promotion and vigour in chilli seedling (Table 4).

Screening for Plant Disease Suppression

The *Bacillus* and the related genera isolated from the diverse niches showed leaf spot suppressive effects upon delivery to the chilli phyllosphere as a foliar spray. In particular, the bacterial isolates, *B. amyloliquefaciens* Ba_Abi; *B. subtilis* Bs_Asi; Bs_Ane, and Bs_Ahv significantly reduced the leaf spot severity; over 70% reductions in leaf spot were recorded on chilli (Table 4).

Discussion

Microbial diversity on the planet earth is one of the under-explored and unexploited natural resources for sustainable agriculture. The Andaman and Nicobar Islands situated in the Bay of Bengal are regarded as one of the most important biodiversity hot spots in the world. Our bacterial diversity exploration on diverse niches and soils in the remote and undisturbed Andaman Island revealed a high-frequency occurrence of antimicrobial *Bacillus* that can be potentially utilized as microbial agents of crop protection. In recent years, public outcry

Table 2 Activity screening of *Bacillus* and *Bacillus*-derived genera for antimicrobial and plant growth promotion traits

Strains	Biocontrol traits			PGP traits			
	R. solanacearum	*X.oryzae*	*C. gloeosporioides*	Siderophore	P-solubilization	K-solubilization	Zn-solubilization
	Zone of inhibition (mm)		Mycelial inhibition (%)	(mm)	(mm)	(mm)	(mm)
B. amyloliquefaciens Ba_Abi	15.8 ± 0.4	13.5 ± 0.3	42.4 ± 1.4	23 (+++)	7 (++)	–	9 (++)
B. subtilis Sp_Fs	9.7 ± 0.2	10.7 ± 0.4	36.4 ± 0.9	20 (+++)	–	3 (+)	5 (+)
B. subtilis SM4	6.4 ± 0.3	12.9 ± 0.6	41.0 ± 0.6	24 (+++)	6 (++)	–	10 (++)
B. subtilis Bs_Adg	9.39 ± 0.3	12.0 ± 0.6	44.4 ± 0.6	26 (+++)	11 (+++)	–	10 (+)
B. subtilis Bs_Ahv	12.9 ± 0.2	11.4 ± 0.9	36.9 ± 1.0	24 (+++)	12 (+++)	–	8 (++)
B. subtilis Bs_Ane	10.39 ± 0.3	28.6 ± 0.9	47.8 ± 0.1	26 (+++)	6 (++)	–	9 (++)
B. subtilis Bs_Asi	8.7 ± 0.1	11.2 ± 0.1	22.0 ± 1.2	28 (+++)	9 (++)	3 (+)	8 (++)
B. subtilis Cc_Ss	10.2 ± 0.1	10.20.1	33.4 ± 0.9	25 (+++)	–	3 (+)	9 (++)
B. subtilis Mh_Fs	10.2 ± 0.2	9.2 ± 0.1	41.6 ± 0.9	22 (+++)	–	4 (+)	4 (++)
B. subtilis Wn_Ss	8.6 ± 0.2	8.4 ± 0.3	44.4 ± 0.3	23 (+++)	6 (++)	–	10 (++)
L. sphaericus NS2	9.1 ± 0.3	12.3 ± 0.2	21.1 ± 0.6	9 (++)	8 (++)	3 (+)	–
L. sphaericus Ls_Agu	10.5 ± 0.0	15.2 ± 0.1	29.2 ± 0.2	9 (++)	9 (++)	–	–

All the numerical values indicated in table are mean of three replications; ± indicates Standard Error of three independent determinations; Plant growth promoting (PGP) traits

'–' no production

'+'—medium (0.3–0.5 cm)

'++'—strong (0.6–0.9 cm)

'+++'—very strong (> 1.0 cm)

over the environmental impact arising out of the use of agrochemicals has necessitated the search for alternative plant health management strategies. We isolated and characterized ninety bacterial isolates from diverse niches including agricultural and forest soils. Most of the isolates showed excellent biocontrol potential against agriculturally important fungal and bacterial pathogens such as *R. solanacearum* (Vascular wilt pathogen), *X. oryzae* pv. *oryzae* (Leaf blight pathogen), and *C. gloeosporioides* (Leaf spot pathogen). Taxonomic identification exploiting conserved 16S rRNA gene sequence confirmed all twelve isolates belonged to *Bacillus* and the related genera. At the species level, *Bacillus subtilis* (nine isolates) dominated our collection over other species such as *Lysinibacillus sphaericus* (two isolates) and *Bacillus amyloliquefaciens* (one isolate). Various *Bacillus* strains especially *B. subtilis,* and *B. amyloliquefaciens* have been widely reported as biocontrol agents of crop pathogens and plant growth promoters worldwide [17, 25, 27, 38]. In addition to out-competing the pathogens for nutrients and space, the *Bacillus* species are known for the production of secondary metabolites [5, 9, 11]. *Bacillus* species are well-known producer of antimicrobial peptides such as iturins, surfactins, fengycins, bacilysin, bacillomycin, mersacidin, and subtilin [4, 33, 46] that is often correlated with their biocontrol potential against fungal and bacterial pathogens. A total of 62 gene sequences involved in the biosynthesis

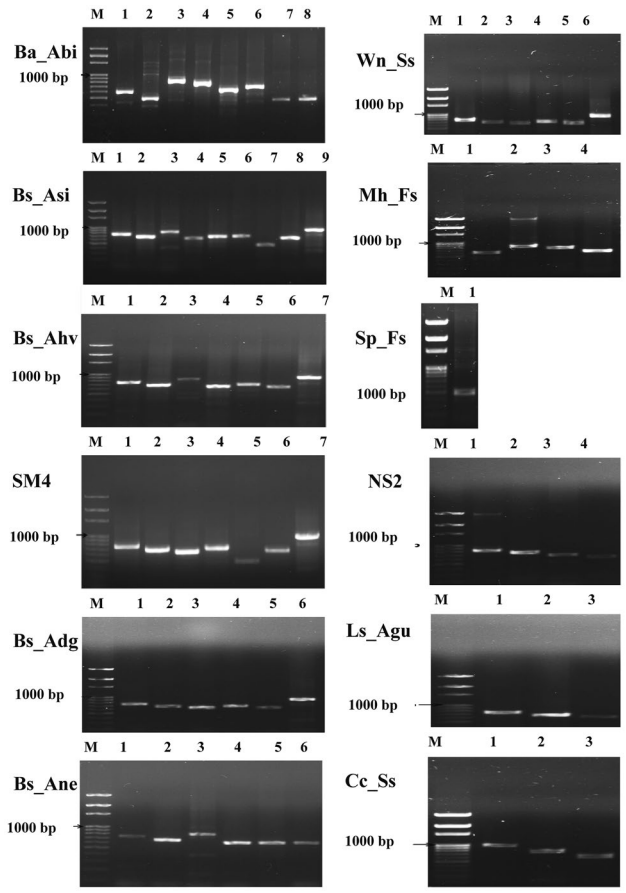

Fig. 2 Detection of AMP genes in *Bacillus* and its derived genera isolated from the Andaman Islands by PCR; M: 100 bp ladder DNA size marker. **i. Ba_Abi: Lanes** 1*: ituC* (594 bp); **2:** *ituD* (482 bp); ***3:*** *bacAB* (815 bp); ***4:*** *bacD* (749 bp); ***5:*** *srfA* (626 bp); ***6:*** *Sfp* (675 bp); ***7:*** *spaB* (688 bp); ***8:*** *spacS* (460 bp) **ii. Wn_Ss: Lanes** 1: *bacD* (749 bp); **2:** *fenB* (670 bp); **3:** *srfA* (626 bp); **4:** *Sfp* (675 bp); **5:** *albA* (625 bp) **iii. Bs_Asi: Lanes** 1:*bacD* (749 bp); **2:***fenB* (670 bp); **3:***fenCEA* (820 bp); **4:***srfA* (626 bp); **5:***Sfp* (675 bp); **6:***spaB* (688 bp); **7:** *spacS* (460 bp); **8:***albA*; **9:** *albF* (888 bp) **iv. Mh_Fs: Lanes** 1: *ituC* (594 bp); **2:** *bacAB* (815 bp); **3:** *bacD* (749 bp); **4:** *srfA* (626 bp) **v. Bs_Ahv: Lanes** 1: *bacD* (749 bp); **2:** *fenB* (670 bp); **3:** *fenCEA* (820 bp); **4:** *srfA* (626 bp); **5:** *Sfp* (675 bp); **6:** *albA* (625 bp); **7:** *albF* (888 bp) **vi. Sp_Fs: Lane** 1: *bacD* (749 bp) **vii. SM4:** Lanes 1: *bacD* (749 bp); **2:** *fenB* (670 bp); **3:** *srfA* (626 bp); **4:** *Sfp* (675 bp); **5:** *spacS* (460 bp); **6:** *albA* (625 bp); **7:** *albF* (888 bp) **viii. NS2: Lanes** 1**:** *bacAB* (815 bp); **2:** *bacD* (749 bp); **3:** *fenB* (670 bp); **4:** *srfA* (626 bp) **ix. Bs_Adg: Lanes** 1: *bacD* (749 bp); 2: *fenB* (670 bp); 3: *srfA* (626 bp); 4: *Sfp* (675 bp); 5: *albA* (625 bp); 6: *albF* (888 bp) **x. Ls_Agu: Lanes** 1**:** *bacD* (749 bp); **2:** *fenB* (670 bp); **3:** *srfA* (626 bp) **xi. Bs_Ane: Lanes** 1: *bacD* (749 bp); **2:** *fenB* (670 bp); **3:** *fenCEA* (820 bp); **4:** *srfA* (626 bp); **5:** *albA* (625 bp); **6:** *albF* (888 bp) **xii. Cc_Ss: Lanes** 1: *bamC* (957 bp); **2:** *bacD* (749 bp); **3:** *mrsA* (597 bp); **6:** *albF* (888 bp)

of eight AMP such as iturin, bacillomycin, fengycin, bacilysin, surfactin, mersacidin, subtilin, and subtilosin (Tables 3 and 4) were detected in the *Bacillus* isolates. The AMP biosynthesis gene diversity was found maximum in *B. subtilis* Bs_Asi (9 genes) followed by *B. amyloliquefaciens* Ba_Abi (8 genes). Interestingly an isolate *Bacillus subtilis* Cc_Ss from Carbyn's Cove from coconut soil collected from the seashore tested positive for Mersacidin, a lantibiotic [8]. Our results are also in agreement with many recent reports providing evidence for the production of antimicrobial peptides against multiple plant pathogenic microbes [24, 34]. Bacillomycin secreted by *B. amyloliquefaciens* disturbed the plasma membrane of *Rhizoctonia solani* and induces abnormalities in conidia and mycelia [40]. Interestingly, all twelve antagonistic *Bacillus* analysed showed the presence of the *bacD* gene that code for metabolic machinery for bacilysin biosynthesis. Hyphal abnormalities were observed on the mycelia of *Pestalotiopsis euginae* treated with cell-free extracts of *B. subtilis* that contained iturin and surfactin [23]. *B. amyloliquefaciens* $MEP_2 18$ produces fengycin isoforms which induce alterations in the bacterial surface topography and cell damage in bacterial pathogens *Xanthomonas axonopodis* pv. *vesicatoria* and *Pseudomonas aeruginosa* [24] and fengycin-produced *Bacillus subtilis* kill the plant-pathogenic fungus *Magnaporthe grisea* by inducing reactive oxygen species production and chromatin condensation [45].

In addition to antimicrobial antibiosis, *Bacillus* strains are widely considered plant growth-promoting bacteria. Inoculation of plants with *Bacillus* showed increased root biomass presumably due to the production of phytohormones like IAA, gibberellic acid, and cytokinins [21]. The *Bacillus* strains from hitherto unexplored Bay Islands were prolific for siderophore production. Siderophore production is an important antagonistic trait in many bacterial biocontrol agents against plant pathogens. Siderophore production by *Bacillus* favours easy iron uptake of plants from soils and in addition, siderophores increase the antagonistic potential of *Bacillus* species [20]. It is well known that the constant use of chemical fertilizers mainly phosphorous, nitrogenous, and potassic fertilizers has harmful effects on the environment [1]. Under this scenario, enhancing crop productivity and soil sustainability with Plant Growth Promoting Microorganisms can be an ideal option in the

Table 3 Detection of antimicrobial peptide biosynthesis genes in *Bacillus* and *Bacillus* -derived genera

AMP		Isolates	*Amplicon (bp)
Iturin	***ituC***	*B. amyloliquefaciens* Ba_Abi; *B. subtilis* Mh_Fs	594
	ituD	*B. amyloliquefaciens* Ba_Abi; *B. subtilis* Mh_Fs	482
Bacillomycin	***bamC***	*B. subtilis* Cc_Ss	957
Bacilysin	***bacAB***	*B. amyloliquefaciens* Ba_Abi; *L. sphaericus*NS2; *B. subtilis* Mh_Fs	815
	bacD	*B. amyloliquefaciens* Ba_Abi; *B. subtilis* SM4; *L. sphaericus* NS2; *L. sphaericus* Ls_Agu; *B. subtilis* Bs_Asi; *B. subtilis* Bs_Ane; *B. subtilis* Bs_Adg; *B. subtilis* Bs_Ahv; *B. subtilis* Mh_Fs; *B. subtilis* Sp_Fs; *B. subtilis* Cc_Ss; *B. subtilis* Wn_Ss	749
Mersacidin	***mrsA***	*B. subtilis* Cc_Ss	597
Fengycin	***fenB***	*B. subtilis* SM4; *L. sphaericus* NS2; *L. sphaericus* Ls_Agu; *B. subtilis* Bs_Asi; *B. subtilis* Bs_Ane; *B. subtilis* Bs_Adg; *B. subtilis* Bs_Ahv; *B. subtilis* Wn_Ss	670
	fenCEA	*B. subtilis* Bs_Asi; *B. subtilis* Bs_Ane; *B. subtilis* Bs_Ahv	820
Surfactin	***srfA***	*B. amyloliquefaciens* Ba_Abi; *B. subtilis* SM4; *L. sphaericus* NS2; *L. sphaericus* Ls_Agu; *B. subtilis* Bs_Asi; *B. subtilis* Bs_Ane; *B. subtilis* Bs_Adg; *B. subtilis* Bs_Ahv; *B. subtilis* Mh_Fs; *B. subtilis* Wn_Ss	626
	Sfp	*B. amyloliquefaciens* Ba_Abi; *B. subtilis* Bs_Asi; *B. subtilis* Bs_Adg; *B. subtilis* Bs_Ahv; *B. subtilis* Wn_Ss	675
Subtilin	***spaB-erib***	*B. amyloliquefaciens* Ba_Abi; *B. subtilis* Bs_Asi	688
	spacS	*B. amyloliquefaciens* Ba_Abi; *B. subtilis* Bs_Asi	460
Subtilosin	***albF***	*B. subtilis*SM4; *B. subtilis* Bs_Asi; *B. subtilis* Bs_Ane; *B. subtilis* Bs_Adg; *B. subtilis* Bs_Ahv; *B. subtilis* Wn_Ss	888
	albA	*B. subtilis*SM4; *B. subtilis* Bs_Asi; *B. subtilis* Bs_Ane; *B. subtilis* Bs_Adg; *B. subtilis* Bs_Ahv; *B. subtilis* Wn_Ss	625

*Refer to Fig. 2 for PCR amplicons specific for AMP genes

intensive agricultural production system. In our studies, *Bacillus subtilis* Bs_Asi showed promise for solubilization of P, K, and Zn, and other isolated showed positive for solubilization of at least two out of three major nutrients. *Bacillus* mediated crop bio-fertilization for enhanced availability of major nutrients like N [32, 42]; phosphorous [3, 29, 35]; Potassium [15, 30]; and Zinc [6, 19, 39] are earlier reported.

Conclusion

Bacillus amyloliquefaciens Ba_Abi and *Bacillus subtilis* Bs_Asi isolated and characterized from Andaman Islands showed multipronged activity as biocontrol agents against crop diseases and also as plant probiotic agents can be harnessed in microbe-assisted organic vegetable production in future.

Table 4 Activity screening of *Bacillus* and *Bacillus*-derived genera for leaf spot suppression and plant growth promotion

Strains	AMP genes detected	Growth promotion of chilli		Leaf spot suppression (%)	
		Seedling length (cm)	Growth promotion (%)	Per cent disease index (PDI)	Biocontrol efficacy (%)
B. amyloliquefaciens Ba_Abi	Iturin; Bacilysin Surfactin; Subtilin	21.15 ± 2.08^{a}	60.77	5.8 ± 0.34^{j}	87.58
B. subtilis Sp_Fs	Bacilysin	14.16 ± 1.17^{bcd}	3.77	26.0 ± 2.02^{d}	44.32
B. subtilis SM4	Bacilysin; Fengycin Surfactin; Subtilosin	17.99 ± 0.10^{ab}	42.32	24.8 ± 2.3^{e}	46.89
B. subtilis Bs_Adg	Bacilysin;Fengycin; Surfactin;Subtilosin	17.57 ± 1.22^{abc}	30.99	26.4 ± 1.28^{d}	43.46
B. subtilis Bs_Ahv	Bacilysin; Fengycin Surfactin; Subtilosin	19.66 ± 1.24^{a}	52.68	13.6 ± 1.47^{h}	70.87
B. subtilis Bs_Ane	Bacilysin; Fengycin Surfactin; Subtilosin	19.35 ± 1.32^{a}	47.39	10.8 ± 2.0^{i}	76.87
B. subtilis Bs_Asi	Bacilysin; Fengycin Surfactin; Subtilin Subtilosin	19.66 ± 1.52^{a}	48.60	9.8 ± 1.05^{i}	79.01
B. subtilis Cc_Ss	Bacillomycin; Bacilysin; Mersacidin	14.49 ± 1.57^{bcd}	3.25	21.8 ± 0.96^{f}	53.31
B. subtilis Mh_Fs	Iturin;Bacilysin; Surfactin	17.69 ± 1.74^{g}	32.04	35.4 ± 1.08^{b}	24.19
B. subtilis Wn_Ss	Bacilysin;Fengycin; Surfactin; Subtilosin	13.88 ± 1.50^{cd}	6.80	26.2 ± 1.8^{d}	43.89
L. sphaericus NS2	Bacilysin; Fengycin; Surfactin	17.46 ± 1.52^{abc}	30.99	19.4 ± 2.08^{g}	58.45
L. sphaericus Ls_Agu	Bacilysin; Fengycin Surfactin	17.82 ± 0.64^{ab}	33.56	33.4 ± 0.88^{c}	28.47
Control	-	13 ± 0.16^{d}	–	46.7 ± 1.2^{n}	–
Carbendazim	-	–	–	5.2 ± 5.6^{j}	88.86
CD(0.01)		5.257	–	1.513	–
CD(0.05)		3.889	–	1.121	–
CV		13.449	–	3.091	–

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Author Contributions KS and RKG collected samples. KS, MK, RS, and MMD performed the experiments, prepared and analysed the data. KS, VD and RS performed various assays; SKS, NS, and AK supervised the work. AK and KS drafted, and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

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Declarations

Conflict of interest Authors declare that there is no conflict of interest that could have appeared to influence the research work reported in this paper.

Ethics Approval Not applicable.

Consent to Participate All authors have reviewed the manuscript and agree to its publication.

Consent for Publication Not applicable.

Data Availability Not applicable.

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