Vietnam Journal of Science and Technology ***61*** *(6) (2023) 1050-1061*

doi:10.15625/2525-2518/18867

Assessment of the role of ACC deaminase, IAA, and siderophore-producing bacteria isolated from Spratly Islands on the growth of *Brassica juncea* on the coral sand

Vu Thi Quynh Chi[1, *], Nguyen Viet Hiep[2], Le Thi My Hao[2], Nguyen Quang Hai[2], Do Thi Kim Anh[1], Pham Mai Huong[1], Than Van Hau[1], Nguyen Cong Viet[1], Ngo Thi Xinh[1], Nguyen Thi Loi[1], Bui Dang Thanh[1]

[1]*Centre of Marine Environmental Monitoring and Analysis, 9 Mac Quyet, Anh Dung, Duong Kinh, Hai Phong, Viet Nam*

[2]*Soils and Fertilizers Institute, 10 Duc Thang, Bac Tu Liem, Ha Noi, Viet Nam*

[*]Email: *vuthiquynhchi73@gmail.com*

Received: 10 September 2023; Accepted for publication: 11 October 2023

Abstract. Root endophytic and rhizosphere bacteria play a significant role in enhancing plant tolerance and promoting growth by producing 1-aminocyclopropane-1-carboxylate (ACC) deaminase, indole-3-acetic acid (IAA), and siderophore. These bacteria have been isolated and screened from 45 plants' roots and rhizosphere soil samples of 19 types of plants which are growing on 8 floating islands in Spratly Islands, Viet Nam. In which, 3 root endophytic bacteria strains were selected and identified to be *Agrobacterium tumefaciens*, *Pseudomonas plecoglossicida*, and *Klebsiella aerogenes* from roots of *Scaevola taccada*, *Barringtonia asiatica* (L.) Kurz, and *Carica papaya*, respectively. Correspondingly, 3 rhizosphere bacteria strains were selected and identified to be *Bacillus velezensis*, *Bacillus aryabhattai*, and *Bacillus velezensis* from rhizosphere soils of *Canavalia maritima* (Aubt) Thouars (C. *obtusifolia* DC.), *Cucumis sativus*, *Heliotropium foertherianum*, respectively. These strains were found to increase the germination rate of *Brassica juncea*'s seeds and 20-day-old fresh biomass, compared to the control treatment in the same condition of 10 ‰ saline watering. When these bacterial strains were combined as an inoculant, they indicated the same result on coral sand. Thus, the addition of selected endophytic and rhizosphere bacteria is efficient in improving the coral sand environment to enhance food crops' development in the Spratly Islands.

Keywords: Root endophytic bacteria, rhizosphere bacteria, ACC deaminase, IAA, Spratly Islands, coral sand.

Classification numbers: 3.1.2; 3.4.3; 3.4.4.

1. INTRODUCTION

Root endophytic and rhizosphere bacteria play a significant role in enhancing plant tolerance and promoting growth by producing ACC deaminase, IAA, and siderophore. The ACC

is normally secreted largely by the root endophytic bacteria; and rhizosphere bacteria also secrete this enzyme, however, it often produces lower quantities. Both of them often produces ACC deaminase, IAA, and siderophore at the same time [1]. Many studies have confirmed that the interaction between two bacteria groups (endophytic bacteria and rhizosphere bacteria) can help plants well grown in extreme environmental conditions such as sandy soil salinization in islands or drought coastal areas [2].

The Spratly Islands of Viet Nam are large coral archipelago located at latitude 6°30' to 12°00'N, longitude 111°20' to 117°20' E. Soil in Spratly Islands are dominated by coral sand belonging to sandy soil group. According to FAO classification, coral sand is divided into nine types, including Endo-Salic Arenosol Humic Arenosol (HuA), Humi Skeletic Arenosol (HuSA), Umbri Skeletic Arenosol (USA), Hapli Skeletic Arenosol (HaSA), Humi Duric Arenosol (HuDA), Humi Haplic Arenosol (HuHA), Umbri Haplic Arenosol (UHA), and Haplic Arenosol (HaA) [3]. Despite these soils having a light texture, poor nutrients and salinization, these soils play an important role in vegetable production to serve for people who are living on these islands due to cultivation land limitation. The aim of this study is to create the inoculant containing beneficial bacteria producing ACC deaminase, IAA and siderophore, which are isolated from native plant root and rhizosphere soils in islands to improve the coral sand and to support for vegetable growing toward self-sufficiency green vegetable for the daily mean of people in the islands.

2. MATERIALS AND METHODS

2.1. Materials

The roots and rhizosphere soil samples were collected from different islands such as An Bang, Nam Yet, Truong Sa Dong, Sinh Ton, Son Ca, Song Tu Tay, and Truong Sa, in Truong Sa district, Khanh Hoa province, Viet Nam in 2020. Total of 45 samples of roots and rhizosphere soils were collected from 19 species of plants existing on the above islands, including: *Coccoloba uvifera*, *Heliotropium foertherianum*, *Barringtonia asiatica* (L.) Kurz, *Terminalia catappa* L., *Casuarina equisetifolia*, *Cocos nucifera*, *Scaevola taccada*, *Morinda citrifolia* L., *Ipomoea pes-caprae* (L.) Sweet, *Canavalia maritima* (Aubt). Thouars (*C. Obtusifolia* DC.), *Cynodon dactylon*, *Eleusine indica*, *Cyperus rotundus*, *Mimosa pudica* L., *Carica papaya*, *Musa paradisiaca* L., *Mentha arvensis* L., *Cucumis sativus*, and *Cucurbita moschata* Duchesne. The root and rhizosphere soil samples were used as the original materials to isolate beneficial bacteria, to produce inoculant to improve the soil fertility as well as to support for plant growth in sanlinization soil in islands and drought sandy soil in costal provinces.

The coral sand, namely HaSA, collected from Truong Sa island and used for experiment on *Brassica juncea* with inoculant is a biological product containing a mixture of ACC deaminase and IAA-producing bacteria which was isolated from the Spratly Islands of Viet Nam. The researched bacterial strains were cultured separately by shaking in Luria Bertani broth medium on a horizontal shaker at a temperature of 40 ^{o}C, shaking speed of 200 rpm, shaking culture time of 48 hours. Bacterial biomass after shaking culture was used to add to the coral sand (the density of beneficial microorganisms in the final coral sand reaches a minimum of 10^6 CFU/g) or to create biological products by taking 100 ml of each type, then adding 1000 grams of carrier (sterilized peat) and then freeze-drying to give the density of beneficial microorganisms of each type in 1 gram of the inoculant reaches a minimum of 10^8 CFU/g.

2.2. Methods

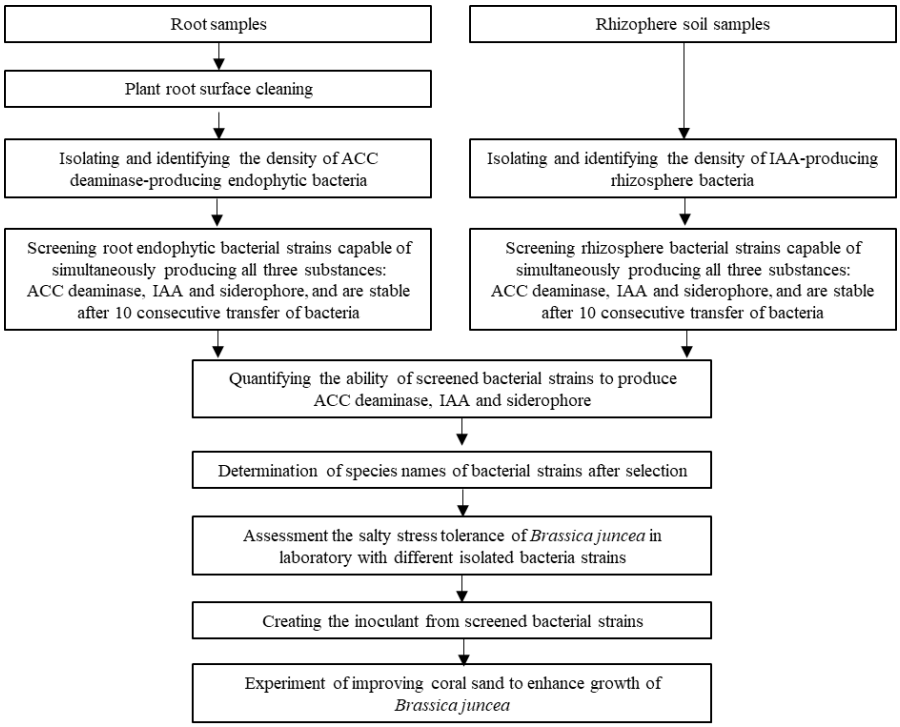

Figure 1. Structuring the method of experiments.

Plant root cleaning: The collected plant roots were cleaned by sterile distilled water; cut into smaller pieces (1 - 2 cm) and dried by desiccant paper and the chemical residue was removed from root pieces by alcohol (70 %) within 3 minutes, and hypochloride (1 %) in 30 seconds, and finally rinsed by sterile distilled water 4 times [4].

Isolating and identifying the density of bacteria

ACC deaminase-producing endophytic bacteria: Using the dilution method and inoculated on petri containing DF-agar medium supplemented with 3 mM ACC and DF-agar medium to identify density of bacteria [5]. IAA-producing rhizosphere bacteria: Using the dilution method, inoculates on agar petri plate containing Luria Bertani medium to account the bacteria. The bacterial colonies were found by the red halo around the bacterial colonies in the presence of Salkowski reagent and penetrating the nitrocellulose membrane under the effect of UV light [6].

Determining the ability producing siderophores: By the modified Chrome Azurol S (CAS) [7].

Quantification of ACC deaminase and IAA

Determination of amount of IAA produced by bacterial strains: Take a bacterial biomass ring of the glass stirring rod into 100 mL of the Luria Bertani broth medium and then shake centrifuge and finally measured by spectrometer at 530 nm [8].

The amount of ACC deaminase activity produced by bacterial strains was estimated by monitoring the amount of α-ketobutyric acid produced from cleavage of ACC [9].

Determination of amount of siderophore produced by bacterial strains: After 48 h of culture, determine the amount of siderophore formed in the culture by measuring the absorbance at 400 nm using a UV-Vis spectrometer on the standard curve described by Gupta *et al*. [10].

Classification and identification of genera: The bacteria genera after isolating was classified and identified following the detailed instructions by Schaad *et al.* [11].

Determination of species names of bacterial strains after selection: Sequence 16S rRNA gene segments with 27F forward primer (5'-AGAGTTTGATCCTGGCTC-3') and 1492R reverse primer (5'– TACGGTTACCTTGTTACGACT-3') [12]. Then, the sequences of the gene fragments were compared with the 16S gene sequences on GenBank. The bacterial strains were identified using the BLAST/NCBI program.

Assessment of the salty stress tolerance of Brassica juncea in laboratory with different isolated bacteria strains

Experiment design: The experiment was conducted in the laboratory of the Department of Microbiology, Vietnam Soil and Fertilizer Institute in 2021. Experiment was conducted with 8 treatments: T1: Coral sand was inoculated with strain R4A1, T2: Coral sand was inoculated with strain R8A2, T3: Coral sand was inoculated with strain R15A4, Control.R: Coral sand (control), T4: Coral sand was inoculated with strain S1I2, T5: Coral sand was inoculated with strain S20I6, T6: Coral sand was inoculated with strain S40I14, Control.S: Coral sand (control). The soil subject for the experiments was coral sand (type HaSA). Using *Brassica juncea* for evaluation; plastic trays for growing sprouts with dimensions of 34 × 25 × 7 cm; The experiment was arranged in a completely randomized block design outside the net house, repeated 3 times (each time is 1 tray). Each treatment tray (T1, T2, T3, T4, T5, T6) was added with 5 ml of culture solution of each selected bacterial strain with a density of 10^9 CFU/mL. Sowing 100 *Brassica juncea* seeds in each tray, covered lightly with soil and water with salt concentration 10 ‰ 2 times/day. Monitoring the germination rate by counting the number of germinated seeds out of the total number of seeds sown and weighting the fresh biomass after 20 days of sowing.

Experiment of improving coral sand to enhance growth of Brassica juncea

Experiment design: The experiment was conducted in the greenhouse of the Naval Environmental Monitoring Center, Viet Nam Navy Command in 2022 with carried *Brassica juncea* on coral sand (type HaSA). The experiment was conducted with 5 treatments: Control: Coral sand, TN1: Coral sand was supplemented with 40 g inoculant/kg of coral sand, TN2: Coral sand was supplemented with 80 g/kg, TN3: Coral sand was supplemented with 120 g/kg, TN4: Coral sand was supplemented with 160 g/kg. The experiment used plastic pots with dimensions of 40 cm × 20 cm × 16 cm (length x width x height). Each pot contains 14.4 kg of coral sand and grows 04 *Brassica juncea*. The amount of chemical fertilizer used for each pot is 3 g of N:P:K fertilizer type 16:16:8+TE. All experimental pots were watered twice a day with fresh water. Monitoring criteria include plant height and leaf width at 10, 20, 30, 40, and 50 days after sowing, and fresh biomass at harvest (50 days after sowing).

3. RESULTS AND DISCUSSION

3.1. Density of ACC deaminase-producing endophytic bacteria and IAA-producing rhizosphere bacteria

The total number of samples surveyed for bacterial density was 45 samples. The density of ACC deaminase-producing endophytic bacteria ranged from 1.42×10^2 to 7.46×10^3 (CFU/g root). The density of IAA-producing rhizosphere bacteria ranged from 2.17×10^3 to 6.62×10^4 (CFU/g soil). For each tree the density of IAA-producing rhizosphere bacteria in the rhizosphere soil was higher than the density of ACC deaminase-producing endophytic bacteria in the root. This indicates the rhizosphere soil environment is more favorable for microorganisms to grow and develop than in roots. The characteristics of the rhizosphere and endophytic bacteria were similar to those of previous studies [13]. This study demonstrated the existence of a significant population of ACC deaminase-producing endophytic bacteria and IAA-producing rhizosphere bacteria. These specialized bacteria groups will contribute to the growth and development of plants in limited conditions.

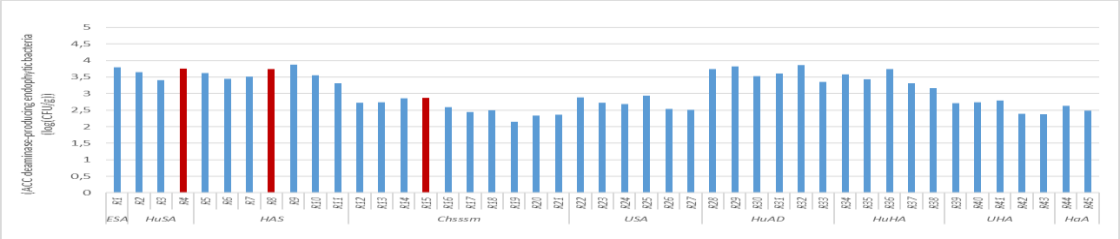

Figure 2: Density of ACC deaminase-producing endophytic bacteria of different types of plant roots.

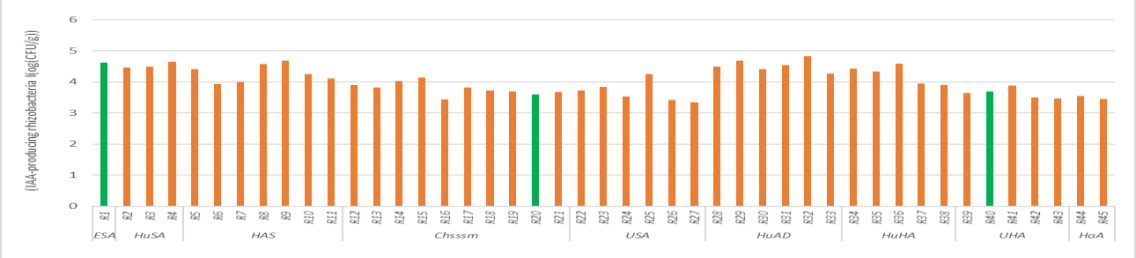

Figure 3. Density of IAA producing rhizosphere bacteria promoting plant growth from different plant rhizosphere samples.

3.2. Isolation and screening of ACC deaminase-producing endophytic bacteria and IAA-producing rhizosphere bacteria

From 45 plant roots samples, a total of 400 strains of endophytic bacteria with ACC deaminase producing activity (colonies grow on DF-agar medium supplemented with 3 mM ACC but not or very weakly on DF-agar) were isolated. Simultaneously, from 45 rhizosphere soil samples, 486 strains of plant growth-stimulating rhizosphere bacteria with IAA-producing activity were also isolated.

In this study, 13 root endophytic bacteria strains and 18 rhizosphere bacteria producing simultaneously ACC deaminase, IAA and siderophere activities were isolated and screened from 45 root samples and 45 rhizosphere soil samples. The rhizosphere bacteria strains reached the rate of 23.68 % (18/76), higher than endophytic bacterial strains. The endophytic bacteria reached the rate of 20.31 % (13/64). These results were higher than the those reported byTeja Gaonkar *et al.* (16.93 %) [14]. The root endophytic bacteria strains (13 trains) were found to belong to genera: *Agrobacterium* (1 trains), *Arthrobacter* (1 trains), *Bacillus* (1 trains), *Burkholderia* (2 trains), *Klebsiella* (1 trains), *Halobacillus* (1 trains), *Oceanobacillus* (1 trains), *Pseudomonas* (4 trains), and *Sediminibacillus* (1 trains). The selected strains of rhizosphere bacteria (18 trains) belong to genera: *Acinetobacter* (1), *Agrobacterium* (2), *Achromocbacter* (2),

Bacillus (5), *Enterobacter* (3), *Klebsiella* (3), and *Pseudomonas* (2) (Table 1) [11]. Thus, *Bacillus* and *Pseudomonas* genera were relatively common in both the root endophytic bacteria group as well as the rhizosphere bacteria group. The remaining bacteria were similar to those previously reported in [15 - 17].

Table 1. Endophytic and rhizosphere bacteria strains isolated from root and rhizosphere soil.

No.	Types of plant/crop	Island	Types of soil	Number of bacterial strains *						Bacterial strains	Genera name of the strain
				ACC deaminase		IAA		Siderophore			
				R	S	R	S	R	S		
1	*Canavalia maritima* (Aubt). *Thouars* (C. *obtusifolia* DC.)	STD	ESA	9	2	1	6	0	2	S1I1	*Agrobacterium* sp.
										S1I2	*Bacillus* sp.
2	*Coccoloba uvifera*	STT	HuA	11	3	2	13	0	1	S2I3	*Bacillus* sp.
3	*Casuarina equisetifolia*	STT		7	1	0	6	0	0		
4	*Scaevola taccada*	STT		14	2	3	11	1	0	R4A1	*Agrobacterium* sp.
5	*Coccoloba uvifera*	TS	HuSA	13	2	1	9	0	0		
6	*Terminalia catappa* L.	TSD		8	0	2	16	0	0		
7	*Cocos nucifera*	PV		6	1	1	5	0	0		
8	*Barringtonia asiatica* (L.) Kurz	NY		11	2	4	20	1	0	R8A2	*Pseudomonas* sp.
9	*Scaevola taccada*	TSD		13	2	3	15	0	0		
10	*Morinda citrifolia* L.	PV		8	0	1	6	0	0		
11	*Cynodon dactylon*	TS		6	1	0	5	0	0		
12	*Coccoloba uvifera*	TS	USA	9	3	2	7	0	0		
13	*Barringtonia asiatica* (L.) Kurz	TS		14	4	3	11	1	2	R13A3, S13I4, S13I5	*Arthrobacter* sp. *Acinetobacter* sp. *Klebsiella* sp.
14	*Heliotropium foertherianum*	TS		16	4	3	17	0	0		
15	*Scaevola taccada*	TS		17	2	4	9	1	0	R15A4	*Klebsiella* sp.
16	*Ipomoea pes-caprae* (L.) *Sweet*	TS		6	1	0	4	0	0		
17	*Musa paradisiaca* L.	TS		8	2	1	10	0	0		
18	*Carica papaya*	TS		8	0	1	7	1	0	R18A5	*Bacillus sp.*
19	*Mentha arvensis* L.	TS		5	1	0	18	0	0		
20	*Cucumis sativus*	TS		7	3	1	9	0	1	S20I6	*Bacillus* sp.
21	*Cucurbita moschata Duchesne*	TS		5	1	2	17	0	0		
22	*Heliotropium foertherianum*	TS	HaSA	12	6	2	18	0	2	S22I7 S22I8	*Klebsiella* sp. *Enterobacter* sp.
23	*Coccoloba uvifera*	SC		13	2	3	11	1	0	R23A6	*Halobacillus sp*
24	*Casuarina equisetifolia*	ST		10	2	1	13	0	0		
25	*Scaevola taccada*	STT		13	1	3	7	2	0	R25A7 R25A8	*Oceanobacillus sp.*, *Pseudomonas* sp.
26	*Ipomoea pes-caprae* (L.) *Sweet*	STT		3	0	0	6	0	0		
27	*Eleusine indica*	ST		4	2	0	8	0	1	S27I9	*Agrobacterium* sp.
28	*Coccoloba uvifera*	TS	HuDA	9	1	1	11	0	0		
29	*Heliotropium foertherianum*	STT		7	0	2	10	1	0	R29A9	*Pseudomonas* sp.
30	*Terminalia catappa* L.	STT		7	0	1	13	0	0		
31	*Casuarina equisetifolia*	TS		8	2	1	9	1	0	R31A10	*Sediminibacillus sp.*
32	*Scaevola taccada*	TS		11	0	2	7	0	0		
33	*Cynodon dactylon*	TS		7	3	1	8	0	2	S33I10 S33I11	*Enterobacter* sp. *Pseudomonas* sp.
34	*Coccoloba uvifera*	STT	HuHA	9	0	2	7	1	0	R34A11	*Burkholderia* sp.
35	*Casuarina equisetifolia*	STT		8	2	1	16	0	1	S35I12	*Bacillus* sp.
36	*Heliotropium foertherianum*	STT		13	1	3	13	0	0		
37	*Mimosa pudica* L.	STT		6	2	0	9	0	0		
38	*Ipomoea pes-caprae* (L.) *Sweet*	STT		5	0	0	14	0	0		
39	*Coccoloba uvifera*	NY	UHA	8	3	0	12	0	1	S39I13	*Klebsiella* sp.
40	*Heliotropium foertherianum*	ST		10	3	1	11	1	2	S40I14	*Bacillus* sp.
										R40A12 S40I15	*Pseudomonas* sp. *Enterobacter* sp.
41	*Scaevola taccada*	ST		11	5	0	15	0	2	S41I16 S41I17	*Achromobacter* sp. *Pseudomonas* sp.

No.	Types of plant/crop	Island	Types of soil	Number of bacterial strains *						Bacterial strains	Genera name of the strain
				ACC deaminase		IAA		Siderophore			
				R	S	R	S	R	S		
42	*Cocos nucifera*	NY		6	0	0	8	0	0		
43	*Cyperus rotundus*	ST		4	2	1	9	0	1	S43I18	*Achromobacter* sp.
44	*Casuarina equisetifolia*	SC	HaA	8	0	3	14	1	0	R44A13	*Burkholderia* sp.
45	*Barringtonia asiatica* (L.) Kurz	SC		7	2	1	16	0	0		
	Total			**400**	**76**	**64**	**486**	**13**	**18**		

Note: TS: Truong Sa; TSD: Truong Sa Dong; STT: Song Tu Tay; ST: Sinh Ton; STD: Sinh Ton Dong; NY: Nam Yet; SC: Son Ca; PV: Phan Vinh

* Number of endophytic and rhizosphere bacterial strains isolated from roots (R) and rhizosphere soil (S) which capable of producing ACC deaminase, IAA and Sidophere.

Table 2. Morphological characteristics of the 6 selected bacterial strains.

No.	Sample name	Genera name	Morphological characteristics
1	R4A1	*Agrobacterium* sp.	Gram (-). Grows anaerobically (-). Grows aerobically (+). Colonies yellow or orange on YDC, or NBY (-). Colonies mucoid on YDC at 30 °C (+). Colonies grew quickly. Colony size is average. Fluorescent pigment on KB (-). Diffusible non-fluorescent pigment on KB (-). Urease (-). Oxidase (-). Growth at 40 ^{o}C (-). Grow on DIM agar (+). Spores formed (-). Aerial mycelium (-)
2	R8A2	*Pseudomonas* sp.	Gram (-). Grows anaerobically (-). Grows aerobically (+). Colonies yellow or orange on YDC, or NBY (-). Colonies mucoid on YDC at 30 °C (-). Fluorescent pigment on KB (+). Diffusible non-fluorescent pigment on KB (-). Urease (-). Oxidase (-). Growth at 40 ^{o}C (-). Grow on DIM agar (-). Spores formed (-). Aerial mycelium (-)
3	R15A4	*Klebsiella* sp.	Gram (-), non-motile. Grows anaerobically (+). Grows aerobically (-). Colonies were round, small, convex, clear white, without edges. Catalase (+), urease (+), oxidase (+), H_2S (-), indol (-), MR (-), VP (Voges Proskau) (+). Spores formed (-). Aerial mycelium (-)
4	S1I2	*Bacillus* sp.	Gram (+). Grows anaerobically (+). Grows aerobically (+). Colonies yellow or orange on YDC, or NBY (-). Colonies mucoid on YDC at 30 °C (-). Colonies were root-shaped, large, irregular, not smooth, flat, with a nucleus in the middle, gray-cream color, with clear edges. Fluorescent pigment on KB (-). Diffusible non-fluorescent pigment on KB (-). Urease (-). Oxidase (-). Growth at 40 ^{o}C (+). Grow on DIM agar (-). Spores formed (+). Aerial mycelium (-)
5	S20I6	*Bacillus* sp.	Gram (+). Grows anaerobically (+). Grows aerobically (+). Colonies yellow or orange on YDC, or NBY (-). Colonies mucoid on YDC at 30 °C (-). Colonies were round or root-shaped, large, irregular, non-smooth, flat, with a central nucleus, gray, with clear edges. Fluorescent pigment on KB (-). Diffusible non-fluorescent pigment on KB (-). Urease (-). Oxidase (-). Growth at 40 ^{o}C (+). Grow on DIM agar (-). Spores formed (+). Aerial mycelium (-).
6	S40I14	*Bacillus* sp.	Gram (+). Grows anaerobically (+). Grows aerobically (-). Colonies yellow or orange on YDC, or NBY (-). Colonies mucoid on YDC at 30 °C (+). The colonies were small, round, even, glossy, convex, milky white, without edges. Fluorescent pigment on KB (-). Diffusible non-fluorescent pigment on KB (-). Urease (+). Oxidase (-). Indol (-). Fermenting lactose with gas production during a 48 - hour incubation at 35 – 37 °C in the presence of bile salts and detergents. Fermenting malonate, citrate, sucrose. Voges-Proskauer (+). Growth at 40 ^{o}C (-). Grow on DIM agar (-). Spores formed (+). Aerial mycelium (-)

Note: (+) means yes, (-) means no.

The results of evaluation of the stability of 3 qualitative activities such as producing ACC deaminase, IAA and siderophore against 13 strains of selected root endophytic bacteria showed that: At the third culture transfer, only 11 bacterial strains remained of stable activity, in the 5th culture transfer, the number of bacterial strains that remained stable was 7, from the 8th culture transfer onward, only 03 strains of bacteria remained stable. For the selected 18 strains of rhizobacteria, it was found that: In the 3rd culture transfer, only 14 strains remained stable activity, in the 5th culture transfer, the number of strains keeping stable activity was 6, from the second inoculation. After the 8th transmission, only 3 strains remained stable. As the results, 6 strains were found as of stable activity, including *Agrobacterium* sp. (R4A1), *Pseudomonas* sp. (R8A2), *Klebsiella* sp. (R15A4), and *Bacillus* sp. (S1I2, S20I6, S40I14) (Table 2).

The degrading ACC activity of endophytic bacteria was higher than that of rhizosphere bacteria. The R4A1 endophytic bacteria strain was of highest activity (732.3 ± 4.19 nmoL α-ketobutyrate mg protein^{-1} h^{-1}). The biosynthesis of IAA activity of rhizosphere bacteria was higher than that of endophytic bacteria, S1I2 was the highest activity (259.47 ± 2.66 µg/ml). The biosynthesis of siderophere activity of S1I2 rhizosphere bacteria was the highest 62.71 ± 0.94 µg/ml) (Table 3).

Table 3. ACC deaminase, IAA, and siderophore-producing activities of each selected bacterial strains was measured at 48 hours of culture.

No.	Bacterial strains	Degrading ACC (nmoL α-ketobutyrate mg protein^{-1} h^{-1})	Biosynthesis of IAA in culture solution (µg/ml)	Biosynthesis of siderophore in culture solution (µg/ml)
1	R4A1	**732.3 ± 4.19**	42.57 ± 0.78	31.75 ± 0.57
2	R8A2	541.5 ± 5.72	34.76 ± 0.75	33.49 ± 0.68
3	R15A4	454.2 ± 6.34	30.15 ± 0.64	22.36 ± 0.41
4	S1I2	116.37 ± 1.49	**259.47 ± 2.66**	53.95 ± 0.87
5	S20I6	89.15 ± 1.32	177.38 ± 2.07	48.67 ± 0.82
6	S40I14	136.54 ± 1.89	226.53 ± 2.43	**62.71 ± 0.94**

These 6 selected strains were identified as the following: The strain R4A1, isolated from roots of *Scaevola taccada* growing on HuA soil, has a closely related species name, *Agrobacterium tumefaciens*; the strain R8A2, isolated from the roots of *Barringtonia asiatica* (L.) Kurz growing on HuSA soil, has a closely related species name, *Pseudomonas plecoglossicida*; the strain R15A4, isolated from roots of *Scaevola taccada* grown in the USA, has a closely related species name *Klebsiella aerogenes*; the strain S1I2, isolated from *Canavalia maritima (*Aubt*).* Thouars (*C. obtusifolia* DC.) rhizosphere grown on the ESA soil, has a closely related specie name *Bacillus velezensis*; the train S20I6, isolated from the rhizosphere of *Cucumis sativus* grown on USA soil, has a closely related species name, *Bacillus aryabhattai*; the strain S40I14, isolated from the rhizosphere of *Heliotropium foertherianum* grown on UHA soil, has the closely related species names *Bacillus velezensis* (Table 4).

Table 4. Identification results of selected strains with significant activity.

No.	Strain	Length of comparison (Nucleotides)	Similarity (%)	Close species name	Reference gene bank code	Registered gene bank code	Risk group
1	R4A1	1383	99.86	*Agrobacterium tumefaciens*	KP050793.1	OR523265	1
2	R8A2	1359	99.63	*Pseudomonas plecoglossicida*	NR_024662.1	OR523284	1
3	R15A4	1463	99.66	*Klebsiella aerogenes*	CP045870.1	OR523285	2
4	S1I2	1435	99.72	*Bacillus velezensis*	MF662463.1	OR523288	1
5	S20I6	1361	99.56	*Bacillus aryabhattai*	MN543816.1	OR523292	1
6	S40I14	1333	99.78	*Bacillus velezensis*	NR_116240.1	OR523293	1

Thus, the isolation and selection of rhizosphere bacteria with the 3 biological characteristics mentioned above are similar to previous publications. The SB1.ACC2 (*Alcaligenes* sp.), SB1.ACC3 (*Bacillus* sp.), and SB2.ACC2 (*Ochrobactrum* sp.) were isolated from the rice rhizosphere grown on sandy coastal soils of India [18] and the *Pseudomonas aeruginosa* species also isolated from coastal dune soil with all three activities simultaneously [14].

The biosafety subgroups of isolated and screened bacterial strains (according to the TRBA 466. Technical Rules for Biological Agents. Classification of Prokaryotes (Bacteria and Archaea into Risk Groups. 2010)), all trains were as risk groups 1 and 2: 5/6 strains belong to risk group 1 (allowed for widespread use in agriculture, industry and environment); 1/6 strains belong to risk group 2 (priority used in research and teaching, restricted release to the environment but not banned) (Table 4). Based on this level of biosecurity, all selected trains were further evaluated of the effectiveness of the strains on the growth and development of plants grown on coral sand.

3.3. Evaluation of the effectiveness of selected bacterial strains on the salty tolerance and promoting growth of *Brassica juncea*

The results indicated that, under stressful conditions of salt concentration (10 ‰) the test on coral sand contaminated the endophytic bacteria or the rhizosphere bacteria strain with the growth of *B. juncea* was better than that of control test. The germination rate and fresh biomass of *Brassica juncea* in the experiment of adding rhizosphere bacteria (S1I2) was the highest at 94.67 % and 157.33 g. The experiment with adding endophytic bacteria (R8A2) was the highest at 92.67 % and 149.33 g (Table 5).

Table 5. Germination and fresh biomass of *Brassica juncea* at 20 days after sowing.

Treatments	**The group of endophytic bacteria**		**Treatments**	**The group of rhizosphere bacteria**	
	Germination (%)	**Fresh weight after 20 days (gram)**		**Germination (%)**	**Fresh weight after 20 days (gram)**
T1	89.67^{a}	129.00^{b}	**T4**	**94.67^{a}**	**157.33^{a}**
T2	**92.67^{a}**	**149.33^{a}**	T5	90.00^{b}	131.33^{b}
T3	86.00^{b}	124.33^{b}	T6	86.67^{c}	125.00^{b}
Control.R	80.33^{c}	114.33^{c}	Control.S	79.67^{d}	114.67^{c}
$LSD_{0.05}$	*3.12*	*6.17*		*3.26*	*7.59*
CV %	*1.90*	*2.54*		*1.97*	*3,05*

Note: In the same column, different letters following the data represent the difference between the means of the treatments at the 5 % statistical significance level.

Inoculation with ACC deaminase-producing endophytic bacteria will promote root growth of seedlings even in the early development stage in different crops [19]. Meanwhile, infection with rhizosphere bacteria with IAA and ACC deaminase activity will lead to better root system development, active shoot growth [15, 20]. Research on the effect of ACC deaminase-producing bacteria on 15-day-old rice seeds under salt stress conditions showed that, when infected with experimental strains of bacteria, all the growth parameters of the rice plants were higher than that of the control, the roots and shoots were long. The fresh and dry weight of the roots was especially high when inoculated with *Alcaligenes* sp. The direct action of ACC-deaminase results in root elongation [18]. In this study, when evaluating the ability to improve the tolerance and promote plant growth of promising strains, the initial test results showed that under stressful conditions of salt concentration (10 ‰), both groups of bacteria had better resistance to as well

as growth promotion at the early stage (20 days of age) than the uninfected controls. We continued to use combinations of these 6 strains to evaluate their potency in off-grid conditions.

The effect of inoculant containing 2 groups of selected bacteria (endophytic bacteria and rhizosphere bacteria) on the growth of *Brassica juncea* on coral sands, the results showed that the stem height, leaf width, and fresh weight at harvest had statistically significant differences compared with the control (at 50 days after sowing). The stem height of *Brassica juncea* TN1, TN2, TN3, and TN4 increased from 20.8 to 57.6 % compared to the control. The maximum leaf width of *Brassica juncea* in the trials of inoculants also increased from 24.3 to 68.0 %, compared to that of control. The fresh weight of *Brassica juncea* in these treatments increased the lowest by 41.7 % and the highest by 76.9 % compared to that of control (Table 6). Thus, when using a combination of 6 strains of bacteria selected as inoculants added to coral sand with dosage of 40 and 160 g/kg (inoculant/coral sand), *Brassica juncea* significantly grow.

Table 6. Growth of *Brassica juncea*.

Treatments	Stem height after sowing (dates)						Maximum leaf width (cm)						Harvest weight (kg)	
	10	20	30	40	50	C* (%)	10	20	30	40	50	C* (%)	Fresh biomass (g/plant)	C* (%)
Control	1.7^{a}	8.0^{c}	17.9^{c}	23.2^{c}	24.2^{c}	-	0.7^{a}	4.0^{b}	5.6^{c}	11.3^{c}	11.2^{d}	-	30.8^{c}	-
TN1	1.6^{a}	11.8^{b}	22.7^{b}	27.5^{b}	29.3^{b}	20.8	0.6^{ab}	4.3^{b}	6.7^{b}	11.2^{c}	14.0^{c}	24.3	43.7^{b}	41.7
TN2	1.6^{a}	10.9^{b}	22.1^{b}	29.7^{b}	29.4^{b}	21.3	0.5^{b}	4.3^{b}	7.0^{b}	13.0^{b}	16.9^{b}	50.7	45.0^{b}	46.1
TN3	1.6^{a}	14.3^{a}	27.9^{a}	33.5^{a}	36.9^{a}	52.3	0.6^{ab}	5.4^{a}	10.9^{a}	17.3^{a}	20.2^{a}	**79.5**	54.5^{a}	**76.9**
TN4	1.5^{a}	13.6^{a}	27.7^{a}	33.1^{a}	38.2^{a}	**57.6**	0.7^{a}	5.0^{a}	11.0^{a}	17.5^{a}	18.9^{a}	68.0	54.5^{a}	**76.9**
$LSD_{0.05}$	0.58	1.81	2.29	3.18	3.21	-	0.14	0.52	0.77	1.29	1.58	-	6.25	-
CV%	19.9	8.5	5.3	5.9	5.6	-	12.9	6.2	5.1	5.1	5.3	-	7.5	-

Note: In the same column, different letters following the data represent the difference between the means of the treatments at the 5% statistical significance level; * It is compared to the control.

The inoculant produced with selected endophytic and rhizosphere bacteria was applied to improve the soil (coral sand) on Truong Sa and Sinh Ton islands. Food vegetables grown on coral sands have been improved to grow and develop well, with green and delicious sensory quality.

4. CONCLUSIONS

Isolating bacteria from root plants and rhizosphere soil from Spratly Island of Viet Nam has found 400 endophytic bacteria strains producing ACC deaminase and 486 rhizobacteria strains producing IAA. After screening, it was selected of 3 endophytic bacteria strains (R4A1, R8A2, R15A4) and 3 rhizobacteria strains (S1I2, S20I6, S40I14) which were identified correspondingly as: (*Agrobacterium tumefaciens, Pseudomonas plecoglossicida, Klebsiella aerogenes) and (Bacillus velezensis, Bacillus aryabhattai, Bacillus velezensis).* These are beneficial bacteria strains for soil fertility improvement and enhancing the plant tolerance ability.

The positive effectiveness of six bacteria strains was found through laboratory experiment, so that the germination rate of *Brassica juncea* on coral sand increased from 7.06 - 18.83 % and fresh biomass at 20 days after sowing also increased from 8.75 - 37.20 % in comparing to control under irrigation of salty water 10 ‰.

Applied inoculant containing 6 above bacteria strains for *Brassica juncea* on coral sand in green house with different rate which has improved vegetable height from 20.8 to 57.6 % and

leaf width increased from 24.3 to 79.5 %, and fresh biomass of *Brassica juncea* has increased from 41.7 to 76.9 % in comparing to control. The optimum rate of inoculant applied for *Brassica juncea* on coral sand ranges from 120 gram to 160 gram per kg.

Acknowledgements. This paper is done with the support of the project KCB-TS.06. The authors would like to thank the Vietnam People's Navy and Center for Marine Environmental Monitoring and Analysis creating conditions for the authors to participate in field trips to collect data in Truong Sa. The authors acknowledge excellent technical supports from the researchers from the Center for Marine Environmental Monitoring and Analysis and Soils and Fertilizers Research Institute.

CRediT authorship contribution statement. Vu Thi Quynh Chi: Writing-review and editing; Nguyen Viet Hiep: Investigation, Writing-original draft, Le Thi My Hao: Investigation; Do Thi Kim Anh: Formal analysis; Pham Mai Huong: Methodology, Formal analysis; Than Van Hau: Methodology, Investigation, Supervision; Nguyen Cong Viet: Methodology, Investigation; Ngo Thi Xinh: Formal analysis; Nguyen Quang Hai: Investigation, Supervision; Bui Dang Thanh: Supervision.

Declaration of competing interest. We declare that we have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

REFERENCES

1. Fan D., Subramanian S., Smith D. L. - Plant endophytes promote growth and alleviate salt stress in *Arabidopsis thaliana*, Sci. Rep. **10** (2020) 12740.
2. Mekonnen H., Kibret M. - The roles of plant growth promoting rhizobacteria in sustainable vegetable production in Ethiopia, Chem. Biol. Technol. Agric. **8** (2021) Art. 15.
3. Hai N. Q., Hao L. T. M., Hiep N. V., Chi V. T. Q., Loi N. T., Tuan L. A., Lua T. T., Sy D. V., Viet N. C. - Characteristics physical, chemical and biodiversity of sandy soil at the truong sa archipelago, Vietnam Soil Sci. **68** (2002) 13-17.
4. Coombs J. T., Franco C. M. - Isolation and identification of actinobacteria from surface-sterilized wheat roots, Appl. Environ. Microbiol. **69** (2003) 5603-5608.
5. Li Z., Chang S., Lin L., Li Y., An Q. - A colorimetric assay of 1-aminocyclopropane-1-carboxylate (ACC) based on ninhydrin reaction for rapid screening of bacteria containing ACC deaminase, Lett. Appl. Microbiol. **53** (2011) 178-185.
6. Bric J. M., Bostock R. M., Silverstone S. E. - Rapid in situ assay for indoleacetic acid production by bacteria immobilized on a nitrocellulose membrane, Appl. Environ. Microbiol. **57** (1991) 535-538.
7. Milagres A. M., Machuca A., Napoleão D. - Detection of siderophore production from several fungi and bacteria by a modification of chrome azurol S (CAS) agar plate assay. Journal of Microbiological Methods **37** (1999) 1-6.
8. Gordon S. A., Weber R. P. - Colorimetric estimation of indoleacetic acid, Plant Physiol **26** (1951) 192-195.
9. Penrose D. M., Glick B. R. - Methods for isolating and characterizing ACC deaminase-containing plant growth-promoting rhizobacteria, Physiol. Plant. **118** (2003) 10-15.
10. Gupta V., Saharan K., Kumar L., Gupta R., Sahai V., Mittal A. - Spectrophotometric ferric ion biosensor from *Pseudomonas fluorescens* culture, Biotechnol. Bioeng. **100** (2008) 284-296.

11. Schaad N. W., Jones J., W C. - Laboratory guide for Identification of plant pathogenic bacteria, APS Press, St. Paul. Minnesota, 2001.
12. Rajapandi S., Prasad G., Janakiraman K. - Sequence-based identification of microbial contaminants in non-parenteral products, Braz. J. Pharm. Sci. **52** (2016) 329-336.
13. Shaffique S., Khan M. A., Wani S. H., Pande A., Imran M., Kang S. M., Rahim W., Khan S. A., Bhatta D., Kwon E. H., Lee I. J. - A Review on the role of endophytes and plant growth promoting rhizobacteria in mitigating heat stress in plants, Microorganisms **10** (2022).
14. Gaonkar T., Nayak P. K., Garg S., Bhosle S. - Siderophore-producing bacteria from a sand dune ecosystem and the effect of sodium benzoate on siderophore production by a potential isolate, Sci. World J. **2012** (2012) 857249.
15. Belimov A. A., Safronova V. I., Mimura T. - Response of spring rape (*Brassica napus* var. *oleifera* L.) to inoculation with plant growth promoting rhizobacteria containing 1-aminocyclopropane-1-carboxylate deaminase depends on nutrient status of the plant, Can. J. Microbiol. **48** (2002) 189-199.
16. Yadav A. N., Kaur T., Kour D., Rana K. L., Yadav N., Rastegari A. A., Kumar M., Paul D., Sachan S. G., Saxena A. K. Chapter 16 - Saline microbiome: Biodiversity, ecological significance, and potential role in amelioration of salt stress*;* Rastegari, A. A., Yadav, A. N., Yadav, N., Eds.; Elsevier, 2020, pp. 283-309.
17. Egamberdieva D., Wirth S., Bellingrath-Kimura S. D., Mishra J., Arora N. K. - Salt-tolerant plant growth promoting rhizobacteria for enhancing crop productivity of saline soils, Front. Microbiol. **10** (2019) 2791.
18. Bal H., Nayak L., Das S., Adhya T. - Isolation of ACC deaminase producing PGPR from rice rhizosphere and evaluating their plant growth promoting activity under salt stress, Plant Soil, (2012).
19. Zahir Z., Arshad M., Frankenberger W. - Plant growth promoting rhizobacteria: Applications and perspectives in agriculture, Adv. Agron. **81** (2003) 97-168.
20. Glick B. R., Penrose D. M., Li J. - A model for the lowering of plant ethylene concentrations by plant growth-promoting bacteria, J. Theor. Biol. **190** (1998) 63-68.