



Isolation and characterization of halotolerant bacteria from Indonesian sand dunes ecosystem and their plant growth-promoting properties

Almando Gerald^{1,2,3,4} · Devida Thalia Salsabella¹ · Inggrit Tyautari¹ · Rama Ali Sadikin¹ · Roni Setiawan¹ · Yusag Abdullah¹ · Billy Valerian¹ · Heri Prabowo⁵ · Christopher Clement¹ · Tri Nurhariyati¹ · Fatimah^{1,4} · Junairiah¹ · Elisa Azura Azman⁶ · Anjar Tri Wibowo^{1,2}

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Abstract

The Parangkusumo sand dune is a tropical coastal ecosystem in Indonesia with unique characteristics, such as high salinity, high temperatures, and limited nutrient availability. This study isolated and characterized halotolerant plant growth-promoting rhizobacteria (PGPR) from the rhizosphere and soil of the halophyte *Calotropis gigantea* in this unique environment. Ten bacterial isolates dominated by genus *Bacillus* were identified. Functional assays revealed significant properties among the isolates, including phosphate solubilization, nitrogen fixation, cellulolytic activity, and anti-phytopathogenic. Three *Bacillus* species, *B. aryabhatai*, *B. subtilis*, and *B. subtilis subsp. spizizenii* exhibited superior salt tolerance and plant growth-promoting attributes under normal and high-salinity conditions. The three bacterial isolates demonstrated synergistic activity through antagonistic testing, indicating their potential for biofertilizer consortium. Notably, a consortium of these strains significantly enhanced sesame (*Sesamum indicum*) seed germination and biomass under salt stress, outperforming single-strain inoculations. These findings highlight the potential of these halotolerant PGPR as biofertilizers for sustainable agriculture in saline or nutrient-poor environments. The results of this study suggested further possibilities of field applications to optimize these strains for broader agricultural use.

Keywords Plant growth promoting rhizobacteria · Coastal sand dunes · *Calotropis gigantea* · Biofertilizer · Sustainable agriculture

1 Introduction

Plant microbiomes are microbial communities, such as bacterial, fungal, archaeal, and viral communities, that are associated with plants and their growth and development (Lundberg et al. 2012; Vu et al. 2022). Based on their niche, physiological and ecological roles, these microbial communities can be divided into endophytic and epiphytic microbes. Endophytic bacteria live inside plant tissues and support plant growth by improving nutrient uptake, producing growth-promoting compounds, and increasing disease resistance. Conversely, epiphytic bacteria colonize the external surface of plants, contributing to plant growth and development by inhibiting pathogens and performing various beneficial interactions and activities (Gerald et al. 2024; Khoa et al. 2022; Kumar et al. 2017).

In the tropical region, one of the most extreme environments for plants is the sand dune ecosystem, characterized

✉ Anjar Tri Wibowo
anjar.tri@fst.unair.ac.id

¹ Department of Biology, Faculty of Science and Technology, Universitas Airlangga, Surabaya 60115, Indonesia

² Biotechnology of Tropical Medicinal Plants Research Group, Airlangga University, Surabaya 60115, Indonesia

³ Institute of Life Science, Technology and Engineering, Surabaya 60115, Indonesia

⁴ University CoE-Research Center for Bio-Molecule Engineering, Airlangga University, Surabaya 60115, Indonesia

⁵ Indonesian Sweetener and Fiber Crops Research Institute, Malang 65152, Indonesia

⁶ Faculty of Agriculture, Department of Crop Science, Universiti Putra Malaysia, 43400 Serdang, Malaysia

by high temperatures, high salinity, and low soil nutrient availability (Gilbert et al. 2008). The daytime temperature in this type of ecosystem ranges between 25 to 35 °C but can further increase up to 65 °C when exposed to direct sunlight (Martínez et al. 2022). Plants thriving in this extreme environment are adapted to sand and wind abrasion, high heat, saline environment, and low nutrient availability (Gilbert et al. 2008; Puijenbroek et al. 2017). Plant microbiomes might support plant growth and survival by mitigating the harsh conditions of high salinity and limited nutrients. It was reported that various soil and endophytic bacteria found in environments with high salinity can support plant growth by facilitating nutrient uptake and salt resistance (Vu et al. 2022; Kearl et al. 2019; Nautiyal et al. 2013; Qin et al. 2016). While most sand dunes are located in arid climate, Parangkusumo sand dune located in Bantul, Indonesia stands out as a unique sand dune ecosystem that exists in the wet climate. Diverse plants inhabited this ecosystem, including *Spinifex littoreus*, *Hibiscus tiliaceus*, and *Calotropis gigantea* (Laksono et al. 2020; Nicolla et al. 2021). Diverse microorganisms also adapted to this harsh environment through their specific adaptation mechanism, it was reported that bacterial diversity found in this area correlates with soil physicochemical factors, including salinity and nutrient content, especially calcium, copper, zinc, and titanium (Vu et al. 2022). Despite growing interest in plant growth-promoting microorganisms, studies on bacterial diversity in tropical coastal dunes remain limited, particularly regarding their functional potential as plant growth promoters. Most existing research has focused on temperate or arid coastal systems (Kearl et al. 2019; Nautiyal et al. 2013; Qin et al. 2016), leaving a significant knowledge gap for tropical ecosystems where unique environmental conditions may select for distinct microbial communities.

Plants living in harsh conditions with limited soil nutrients, high salinity, and limited water retention capacity might survive due to the presence of Plant Growth Promoting Rhizobacteria (PGPR). These bacteria can support plant growth through various mechanisms, including nitrogen fixation, degradation of organic matter, solubilization of phosphate, and enhancing plant resistance to pathogens (Khusna et al. 2024; Kuan et al. 2016; Qingwei et al. 2023; Tang et al. 2020). However, current understanding of PGPR from tropical coastal dunes remains limited, with studies typically focusing either on diversity surveys (Vu et al. 2022) or examining individual strain growth-promoting traits (Kearl et al. 2019; Nautiyal et al. 2013; Qin et al. 2016; Khusna et al. 2024), while lacking comprehensive functional characterization of bacterial consortia in plant systems. This highlights the need for systematic evaluation of multiple PGPR traits and their synergistic effects on plant growth under salt stress. This study aims to isolate, identify, and characterize bacteria from the Parangkusumo sand dune ecosystem, specifically

from the soil and roots of *Calotropis gigantea*, a predominant plant in this area. *C. gigantea* thrives in these harsh ecosystem, making it an ideal model to study plant–microbe interactions in saline and nutrient poor condition. However, despite its ecological dominance, the microbial communities associated with *C. gigantea* and their functional roles in mitigating abiotic stress have never been explored before. This study also evaluates bacterial salt tolerance and their biochemical activities to unravel their roles in plant adaptation to abiotic stress. Additionally, antagonistic tests and inoculation assay to sesame seeds are conducted to determine whether the bacterial isolates can be cultivated together and applied to plants as biofertilizer. Overall this work provided valuable information regarding the characteristics of coastal sand dune bacteria and their potential use as biofertilizer for crops grown in coastal or salt-affected areas.

2 Materials and methods

This study was done in several steps including isolation of bacteria from soil and rhizosphere samples, characterization of bacterial isolates, identification of bacterial isolates, and testing their potential as biofertilizer agents (phosphate solubilization, anti-plant pathogen, nitrogen fixation, cellulytic activity, antagonistic assay for specific bacterial strains, salt tolerance assay, and seed germination assay following bacterial inoculation).

2.1 Sample collection

Samples of soil and rhizosphere soil surrounding *C. gigantea* were collected from nine sites at Parangkusumo sand dune, Bantul Regency, Indonesia. These sampling sites are spread along a latitudinal gradient extending inland from the shoreline and distributed among three distinct sand dune zones: (1) the coastal zone adjacent to the shoreline, (2) the intermediate zone, and (3) the transitional boundary zone where the dunes meet agricultural land. Within each zone, three representative sites were chosen at regular intervals where *C. gigantea* found. Samples were aseptically collected by taking rhizosphere soil from around the roots of *C. gigantea*. Additionally, approximately 100 g of soil was collected from areas around the plants.

2.2 Isolation of bacterial strains

Under sterile conditions, five grams of soil sample were placed in a 50 mL falcon tube, followed by the addition of 45 mL of sterile distilled water. The mixture was vortexed for 1 min and allowed to settle until the soil precipitated. One millilitre of the soil suspension was transferred to 9 mL of 0.85% saline (dilution 10^{-2}) and homogenized

using vortex. The 0.85% saline solution was prepared by dissolving 3.4 g of NaCl in 400 mL of distilled water. After the dilution step, the sample was plated on Luria Bertani (LB) and Reasoner's 2A (R2A) agar media using the pour plate method. The plates were then incubated at 37 °C for 24 to 48 h.

2.3 Characterization and identification of bacterial strains

Macroscopic characterization was performed by observing the colony shape, size, colour, surface texture, margin, and opacity of the bacterial colonies. Microscopic characterization was conducted through Gram staining of the bacterial isolate. Microscopic observation parameters included bacterial cell shape and Gram classification. For molecular identification, bacterial DNA was extracted and purified using the Presto™ gDNA Bacteria Kit Quick. Amplification of the 16S rRNA gene was performed using Go Taq Green Master Mix with universal 16S rRNA primers 27F (5'-AGAGTTTGATCTGGCTCAG-3') and 1492R (5'-GGT TACCTTGTTACGACTT-3'). The amplification protocol included an initial pre-denaturation at 92 °C for 7 min, followed by 30 s of denaturation at 92 °C, 45 s of annealing at 55 °C, and 45 s of extension at 72 °C, with a final extension step at 72 °C for 7 min. The PCR results were subjected to the Sanger sequencing method, and the resulting sequences were then compared to NCBI database for homology (Yoon et al. 2017).

2.4 Phosphate solubilization assay

The phosphate solubilization assay was conducted by culturing the bacterial isolates in LB broth and incubating them in a shaker at 37 °C for 24 h. The bacterial culture was adjusted to an optical density (OD) of 0.1 by diluting it with LB broth. Then, 20 µl of the bacterial culture were inoculated onto sterile paper discs placed on Pikovskaya's agar medium using the disc diffusion method, with three replicates on separate Petri dishes. A negative control was prepared using the same volume of LB broth. The cultures were then incubated at 37 °C for 48 h. After incubation, the clear zone around the bacterial colony and the diameter of the colony were measured. The phosphate solubilization index was then calculated according to the method of Sharon et al. (2016) (Sharon et al. 2016).

2.5 Anti-plant pathogen assay

The anti-plant pathogen assay was performed using the disc diffusion method. Bacterial cultures were grown in LB broth and incubated for 24 h. The cultures were then centrifuged at 14,000 rpm for 1 min to separate the

supernatant from the pellet. The phytopathogenic bacterium *Xanthomonas campestris* was swabbed onto the surface of Mueller Hinton Agar (MHA) medium, which was divided into four areas. In the first and second areas, paper discs soaked with bacterial isolate supernatant were placed. A paper disc soaked in LB broth was used as a negative control in the third area, and a paper disc soaked in gentamicin (40 mg/mL) was placed in the fourth area as a positive control. The plates were incubated at 37 °C for 24 h. Antibacterial activity was determined by the presence of an inhibition zone around the test discs, and the diameter of the inhibition zones was measured using a calliper.

2.6 Nitrogen fixation assay

The nitrogen fixation assay was conducted using Nitrogen Free Bromothymol (NFB) semi-solid medium. The bacterial isolates were cultured in LB broth and incubated in a shaker at 37 °C for 24 h. The bacterial culture was adjusted to an optical density (OD) of 0.1 by diluting it with LB broth. Twenty microliters of the bacterial culture were inoculated into the NFB medium using a micropipette by carefully inserting the pipette into the medium. The cultures were then incubated at 30 °C for 30 days. A positive result for nitrogen fixation was indicated by a color change in the NFB medium from yellow to blue and the formation of a pellicle on the surface of the medium, as described by Baldani et al. (2014) (Baldani et al. 2014).

2.7 Cellulolytic activity

The cellulolytic activity assay was conducted using selective Bushnell-Haas medium supplemented with carboxymethyl cellulose (CMC). The bacteria were first cultured in LB broth and incubated in a shaker at 37 °C for 24 h. The bacterial culture was then adjusted to an optical density (OD) of 0.1 by dilution with LB broth. Subsequently, 20 µl of the bacterial culture were inoculated onto Bushnell-Haas medium supplemented with CMC using the disc diffusion method, with three replicates on separate Petri dishes. A negative control was prepared by inoculating the same volume of LB broth. The cultures were incubated at 37 °C for 48 h. Cellulolytic activity was assessed using a 0.1% Congo Red solution, which was poured over the cultures and left for 15 min. The solution was then discarded, and the plates were rinsed with NaCl three times for 15 min each to remove unbound Congo Red. The formation of clear zones around the bacterial colonies indicated the degradation of CMC, demonstrating cellulolytic activity (Gupta et al. 2012).

2.8 Antagonistic test for selected strains

The antagonistic activity of the bacterial strains was evaluated using the dual culture method on solid LB media through the streak method. After 24 h of incubation at 37 °C, the antagonistic effect was observed as zones of growth inhibition between the bacterial strains. The inhibition was measured as the distance between the edges of the two bacterial strains tested on the same plate (Zicca et al. 2020).

2.9 Salt tolerance assay for selected strains

Bacterial strains were tested for their tolerance to NaCl using two methods: Total Plate Count (TPC) and bacterial growth rate in LB broth. Isolates were cultured in LB media supplemented with NaCl at concentrations of 0 ppm, 0.1 ppm, 0.2 ppm, 0.5 ppm, 1 ppm, 2 ppm, 3 ppm, 4 ppm, and 5 ppm, and incubated at 37 °C with for 24 h. After incubation, the isolates were transferred to Nutrient Agar (NA) media for colony enumeration. The TPC method was used to assess the viability of bacterial growth, with the number of colonies on each Petri dish indicating the growth response to NaCl treatment. In the second method, the bacterial strains were cultured in LB broth containing NaCl at two different concentrations, 200 mM and 2 M. After inoculation, the bacterial cultures were incubated at 28 °C for 86 h. Bacterial growth was evaluated by measuring the optical density (OD) at 600 nm every 12 h (Ramadoss et al. 2013).

2.10 Plant germination assay

Germination assay was conducted using sesame (*Sesamum indicum* L.) seeds to evaluate the effects of plant growth-promoting rhizobacteria (PGPR) under saline conditions. Sesame seeds were surface-sterilized by immersion in 5% sodium hypochlorite for 2 min, followed by 70% ethanol for 2 min. The seeds were then rinsed three times with sterile distilled water to remove residual ethanol. After sterilization, seeds were air-dried in a laminar airflow cabinet (LAF). For bacterial inoculation, seeds were soaked in each of five treatments: three single PGPR isolates, one PGPR consortium, and one control (sterile distilled water). The seeds were incubated in bacterial suspensions (1×10^8 CFU/mL) or sterile water for 1.5 h in a shaker incubator at room temperature. After incubation, seeds were separated from the bacterial suspension and air-dried in LAF. Sterile Petri dishes were then prepared by placing three layers of filter paper moistened with 150 mM NaCl or sterile distilled water (control). Each treatment was replicated five times, with thirty treated seeds were evenly distributed on the moistened filter paper in each replicate, which was then sealed with cling film to maintain moisture. The petri dishes were incubated in darkness for three days and then transferred to a constant light

at room temperature. Germination was monitored daily for seven days by counting the number of germinated seeds, indicated by the emergence of radicle. On the final day (Day 7), the fresh weight of ten representative seedlings with emerged radicles and cotyledons was measured for each treatment using an analytical balance (Mahmud et al. 2023).

3 Results

3.1 Macroscopic, microscopic, and molecular identification of bacterial isolates

A total of 10 bacterial isolates were obtained from the Parangkusumo sand dunes. Four isolates were collected from the rhizosphere of *C. gigantea* (isolates Cg1 to Cg4), while six isolates were obtained from the surrounding soil (isolates A to F) (Table 1). Most of the isolates were Gram-positive, with white, opaque colonies, except for Cg4, which exhibited smooth, yellow colonies and was identified as a Gram-negative bacterium.

The molecular identification using 16S rRNA analysis revealed that the most dominant genus isolated in this study was *Bacillus*, with 8 out of the 10 isolates identified as members of this genus (Table 2). The following bacterial isolates were obtained from soil samples: *B. subtilis* subsp. *spizizenii*, *B. cereus*, *B. aryabhattai*, *B. cillus velezensis*, *B. rugosus*, and *B. amyloliquefaciens*. The four bacterial isolates associated with *C. gigantea* rhizosphere were *B. cereus*, *B. rugosus*, *B. amyloliquefaciens*, and *Micrococcus aloeverae*.

3.2 Phosphate solubilization potential of isolated bacterial strains

Bacterial colonies that displayed clear halo zones around their growth on Pikovskaya Agar media were identified as phosphate solubilizers (Fig. 1). All isolates demonstrated phosphate solubilization ability, with *B. subtilis* and *B. subtilis* subsp. *spizizenii*, both isolated from soil, showing the highest phosphate solubilization index (PSI). A high PSI was also observed in *B. aryabhattai* and *B. cereus* strain 1. The average PSI values ranged from 0.17 to 2.56.

3.3 Nitrogen fixation potential of isolated bacterial strains

All isolates were screened for their nitrogen-fixing activity using NFB semi-solid media indicated by a change in color from yellow to bluish hue in the media after 30-days incubation period at 30 °C. The result showed that all isolates showed nitrogen fixation potential with various degrees of activity, with *B. aryabhattai* showing the highest nitrogen

Table 1 Colony morphology and microscopic characters of bacterial strains isolated from the Parangkusumo Sand Dune Ecosystem

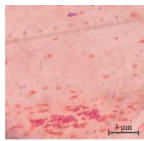
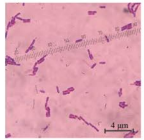
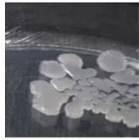
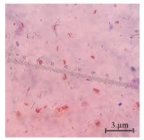
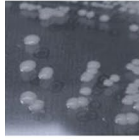
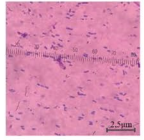
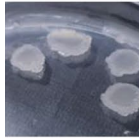
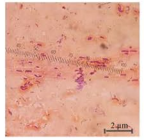
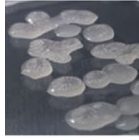
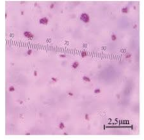
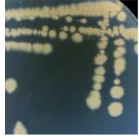
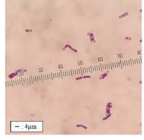
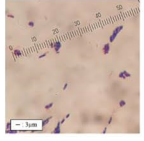
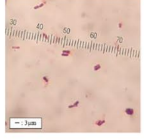
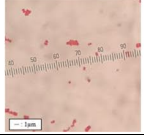
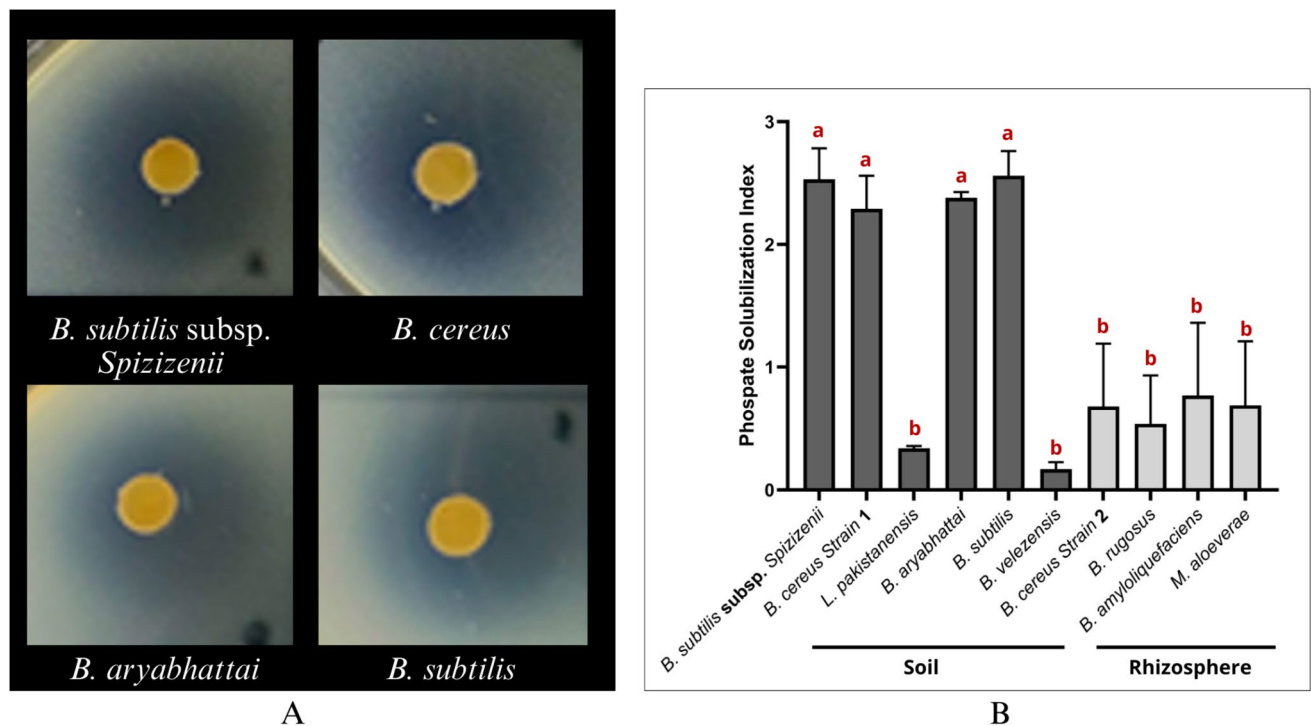
Isolate code	Colony characteristics	Colony morphology	Microscopic characteristic	Cellular morphology
A	Colonies are white and opaque with a round, flat shape, flat edges, and smooth surface.		Rod-shaped, gram-positive	
B	Colonies are white and opaque with irregular shapes, prominent, flat edges, and wrinkled surfaces.		Rod-shaped, gram-positive	
C	Colonies are white and opaque with irregular shapes, convex, wavy edges, and smooth surfaces.		Rod-shaped, gram-positive	
D	Colonies are white and opaque with intact, prominent shapes, flat edges, and smooth surfaces.		Rod-shaped, gram-positive	
E	Colonies are white and opaque with irregular shapes, prominent, wavy edges, and wrinkled surfaces.		Rod-shaped, gram-positive	
F	Colonies are white and opaque with a round, convex shape, flat edges, and a wrinkled surface.		Rod-shaped, gram-positive	
Cg1	Colonies are yellowish-white and opaque with irregular shapes, prominent, wavy edges, and slimy surfaces.		Rod-shaped, gram-positive	
Cg2	Colonies are yellowish-white and opaque with irregular shapes, prominent, wavy edges, and rough surfaces.		Rod-shaped, gram-positive	
Cg3	Colonies are yellowish-white and opaque with irregular shapes, prominent, wavy edges, and rough surfaces.		Rod-shaped, gram-positive	
Cg4	Colonies are yellow with a smooth surface and irregular shape, convex, and flat edges.		Spherical and Gram-negative.	

Table 2 The results of BLASTn based on 16S rRNA sequence

Isolate code	Source	Identification results	Per. ident	Query cover	Acc. number
A	Soil	<i>Bacillus subtilis</i> subsp. <i>spizizenii</i>	99.23%	100%	NR 112686.1
B	Soil	<i>Bacillus cereus</i>	99.58%	100%	NR 115526.1
C	Soil	<i>Lysinibacillus pakistanensis</i>	98.90%	100%	NR 113166.1
D	Soil	<i>Bacillus aryabhatai</i>	99.72%	100%	NR 115953.1
E	Soil	<i>Bacillus subtilis</i>	98.95%	100%	NR 027552.1
F	Soil	<i>Bacillus velezensis</i>	99.71%	100%	NR 112636.1
Cg1	Rhizosphere Soil	<i>Bacillus cereus</i>	99.93%	100%	NR 112636.1
Cg2	Rhizosphere Soil	<i>Bacillus rugosus</i>	100%	100%	NR 181236.1
Cg3	Rhizosphere Soil	<i>Bacillus amyloliquefaciens</i>	99.93%	100%	NR 041455.1
Cg4	Rhizosphere Soil	<i>Micrococcus aloeverae</i>	99.85%	100%	NR 134088.1

**Fig. 1** The phosphate solubilizing activity **A** and phosphate solubilization index (PSI) **B** of bacterial strains isolated from Parangkusumo sand dunes. Data are shown as Mean $\pm$ SD ($n=3$); the mean value

followed by different letters indicate significant differences ($p<0.05$) based on the Tukey test

fixation potential and *B. velezensis* showing the lowest (Table 3).

3.4 Cellulolytic activity of isolated bacterial strains

The cellulolytic activity was assessed using CMC agar plates followed by Congo-red staining. The presence of clear zones around microbial growth indicated cellulolytic activity (Fig. 2). Eight of the ten isolates demonstrated positive cellulolytic activity, with *L. pakistanensis* and *B. subtilis* exhibiting the highest cellulolytic indices. The assay also

revealed that *B. subtilis* subsp. *spizizenii* and *B. aryabhatai* did not display any cellulolytic activity.

3.5 Anti-phytopathogenic potential of isolated bacterial strains

The anti-phytopathogenic activity was evaluated on LB agar against the bacterial pathogen *X. campestris*. Eight of the ten isolates demonstrated anti-phytopathogenic activity, with *B. velezensis* and *M. aloeverae* showing the strongest inhibition (Fig. 3). In contrast, *B. cereus* strain 1 and

Table 3 The nitrogen fixation activity of bacterial strains isolated from soil and rhizosphere soil of *C. gigantea* at Parangkusumo sand dunes

Bacterial species	Nitrogen fixing
<i>B. subtilis</i> subsp. <i>spizizenii</i>	+++
<i>B. cereus</i> strain 1	+++
<i>L. pakistanensis</i>	+++
<i>B. aryabhattai</i>	+++
<i>B. subtilis</i>	+
<i>B. velezensis</i>	+
<i>B. cereus</i> strain 2	+++
<i>B. rugosus</i>	+++
<i>B. amyloliquefaciens</i>	+++
<i>M. aloeverae</i>	+++

B. amyloliquefaciens did not exhibit anti-phytopathogenic activity against *X. campestris*.

3.6 Salt tolerance assay of selected bacterial isolates

Three bacterial strains with the highest overall PGPR potential (*B. subtilis* subsp. *spizizenii*, *B. aryabhattai*, and *B. subtilis*) were tested for their salt tolerance, with *B. cereus* serving as a comparative control. All strains were able to grow

during 84 h of incubation under high (2 M NaCl) salt stress conditions. *B. subtilis* subsp. *spizizenii* exhibited superior growth under high salt stress conditions, while *B. cereus* showed the lowest growth rate (Fig. 4).

3.7 Antagonistic test between selected bacterial isolates

An antagonistic test was conducted between *B. subtilis* subsp. *spizizenii*, *B. aryabhattai*, and *B. subtilis* to assess whether these isolates could inhibit each other's growth. The strains were paired and co-cultured using the streak method, followed by incubation at 34 °C for 24 h. The results showed no antagonistic effect between any pair combination, as seen by the absence of inhibition zones at the contact points between isolates (Fig. 5). This indicates that none of the strains produced compounds or mechanisms that inhibit the growth of the others. Thus, these isolates have the potential to be cultured together as a consortium, which is valuable for various biotechnological or industrial applications, such as biofertilizer development.

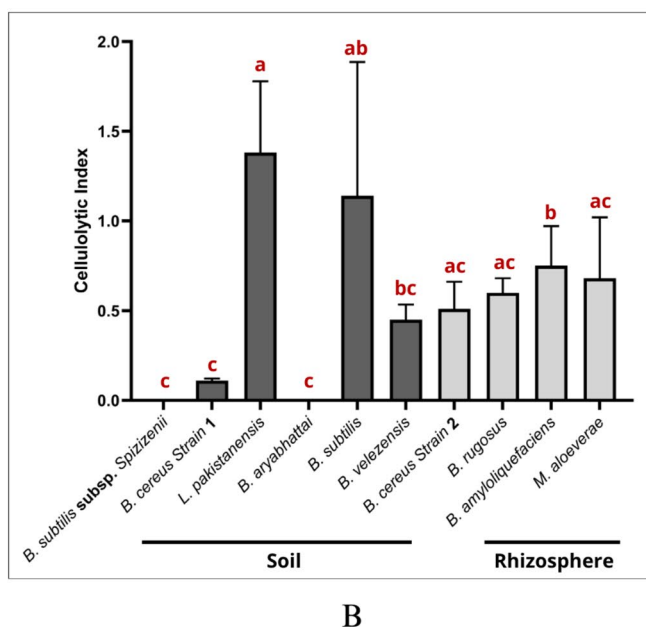
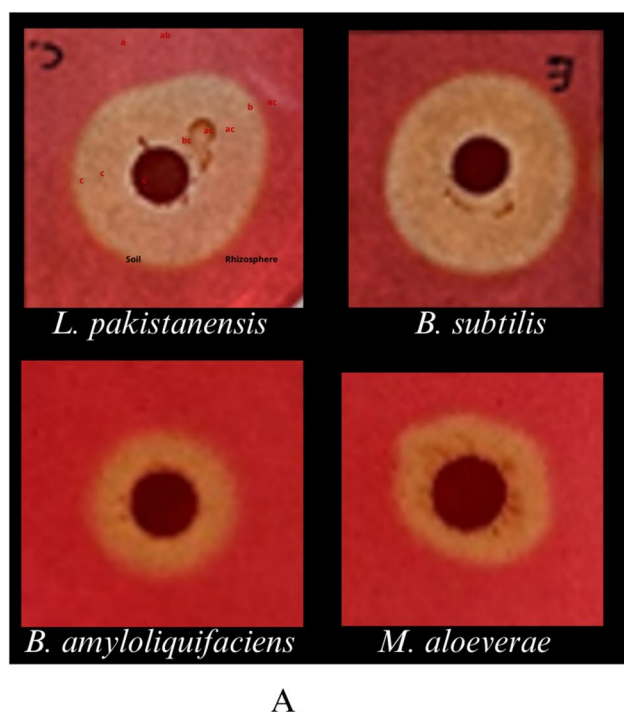


Fig. 2 The cellulolytic activity **A** and cellulolytic index **B** of bacterial strains isolated from Parangkusumo sand dunes. Data are shown as Mean ± SD ($n = 3$); mean values followed by different letters indicate significant differences ($p < 0.05$), according to the Tukey test

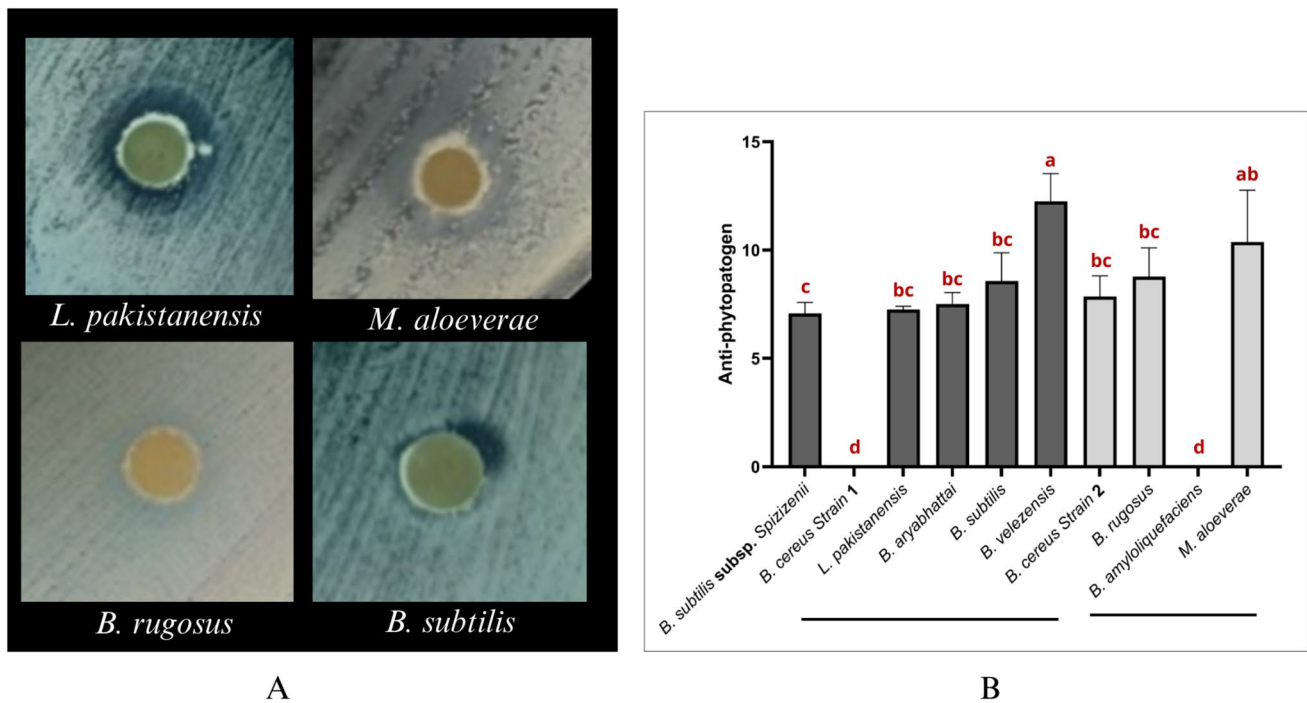


Fig. 3 The anti-phytopathogen activity **A** and diameter of inhibition zone **B** of bacterial strains isolated from Parangkusumo sand dunes. Data are shown as Mean $\pm$ SD ($n=3$); mean values followed by different letters indicate significant differences ($p < 0.05$), according to the Tukey test

Fig. 4 The growth curve of four bacterial strains grown under high salinity (2 M NaCl)

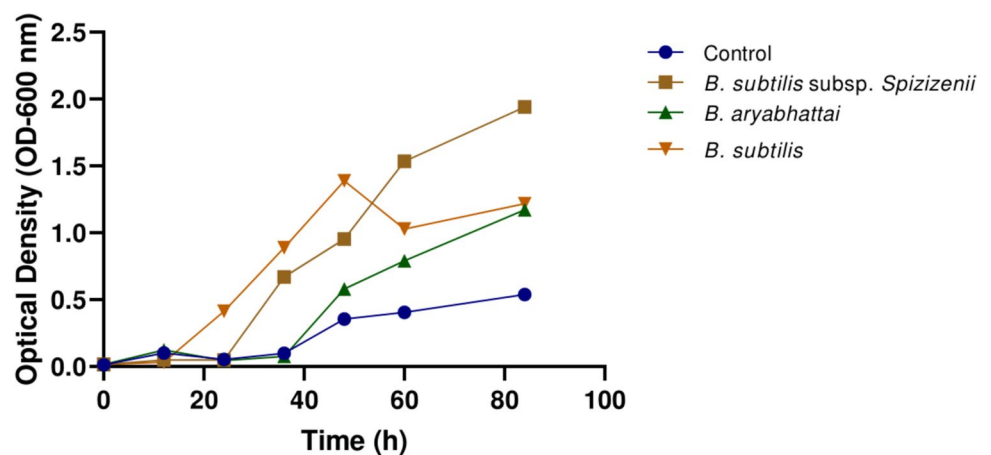
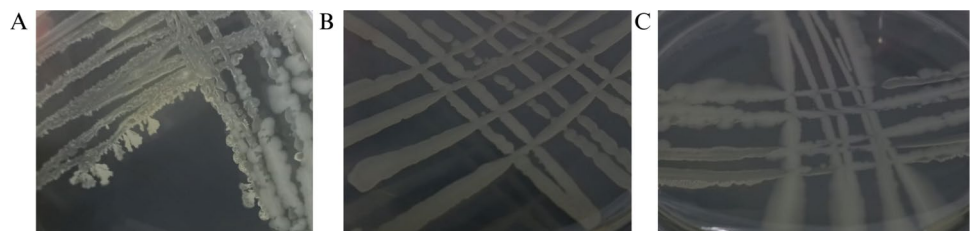


Fig. 5 Antagonistic test between selected bacterial isolates (**A**: *B. subtilis* subsp. *spizizenii* vs. *B. aryabhattai*; **B**: *B. subtilis* subsp. *spizizenii* vs. *B. subtilis*; **C**: *B. subtilis* vs. *B. aryabhattai*)



3.8 Inoculation of bacterial consortium enhances germination and biomass of salt-stressed sesame seeds

This study evaluated the sesame seed germination rate and biomass under various treatments: single-strain bacterial inoculation, a bacterial consortium inoculation, and a

non-inoculated control, both under normal and salt stress conditions. The results showed that bacterial consortium significantly promoted germination and biomass of sesame seeds under salt stress (150 mM), exceeding single-strain inoculation and control (Fig. 6). Seeds inoculated with the consortium showed a significant increase in germination rate and biomass accumulation compared to control, while single-strain inoculations showed similar or lower than control.

4 Discussion

This study reports the isolation and characterization of plant growth-promoting rhizobacteria (PGPR) from a unique wet tropical coastal sand dune ecosystem. The harsh conditions of this environment, characterized by high salinity, extreme temperatures, and low nutrient availability, make it an ideal site for identifying microbes with specialized adaptations for promoting plant growth under stress. Among the isolated strains, the dominant genera belonged to *Bacillus*, which is consistent with previous studies reporting the PGPR potential of halotolerant *Bacillus* (Ayaz et al. 2022; Nawaz et al. 2020; Wang et al. 2018). Previous studies on PGPR in saline and tropical environments have identified various genera with plant growth-promoting traits, including *Bacillus*, *Pseudomonas*, and *Acinetobacter* (Kearl et al. 2019; Nautiyal et al. 2013; Qin et al. 2016). However, in this study,

Bacillus was found to be the dominant genus, reinforcing its established role as a key halotolerant PGPR in extreme environments. The identification of these halotolerant PGPR, particularly those with the ability to solubilize phosphate, fix nitrogen, degrade cellulose, and exhibit anti-phytopathogenic activity, highlights their potential for use in sustainable agriculture, especially in saline or nutrient-poor soils.

The ability of PGPR to solubilize phosphate is particularly important since phosphate forms insoluble complexes in soil with high salinity (Dey et al. 2021). Phosphorus in soil exists in organic and inorganic forms, with the latter often bound to iron and calcium. Its release from minerals is slow, requiring microbial activity for solubilization. Microorganisms achieve this by producing organic acids and enzymes like citric acid, gluconic acids, phytase, and phosphatase which lower soil pH, or by releasing H^+ ions during cation assimilation. The resulting acidic conditions enhance phosphorus dissolution, improving its bioavailability for plant uptake (Singh and Satyanarayana 2011). This study shows that the isolated *Bacillus* (*B. subtilis*, *B. subtilis* subsp. *Spizizenii*, *B. aryabhatai*) can solubilize phosphate and The PSI values of these three species were higher than *B. paramycoides* and *B. aryabhatai*, which previously reported as PGPR that enhance plant phosphorus uptake in saline soils (Li et al. 2023; Lu et al. 2023).

Another limiting factor in plant growth is the lack of nitrogen in soil due to erosion and volatilization (Islam

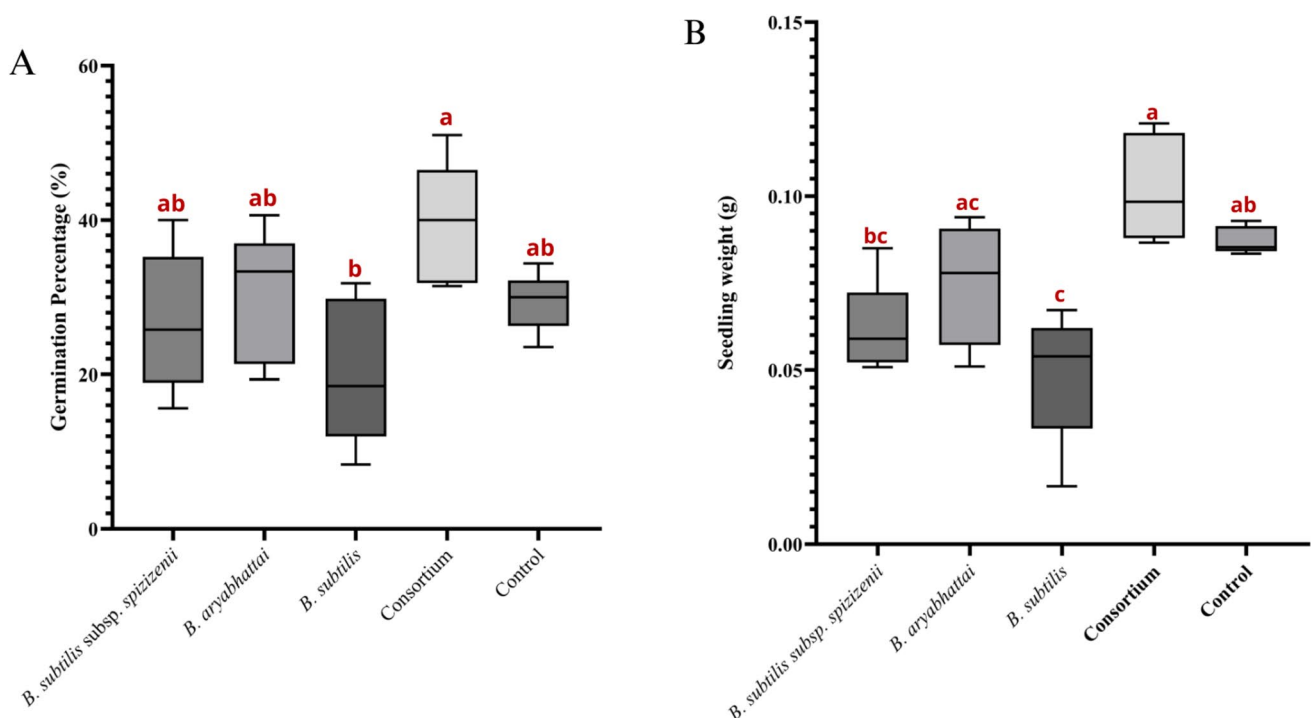


Fig. 6 The germination rate **A** and fresh weight of 7-day-old sesame (*S. indicum* L.) seeds **B** in 150 mM NaCl following inoculation with different bacterial strains

et al. 2024). Plants can obtain readily available nitrogen through the help of nitrogen-fixing bacteria in the rhizosphere that fixate nitrogen in air into ammonia (Mahmud et al. 2020). Nitrogen-fixing microorganisms possess the enzyme nitrogenase, which is highly sensitive to oxygen. The presence of oxygen acts as an inhibitor of nitrogenase activity, thereby suppressing nitrogen fixation. This enzyme is encoded by the Mo-nitrogenase protein complex, which consists of MoFe protein and Fe protein. During the nitrogen fixation process, the N_2 molecule is bound by FeMo-co (Fe-Mo cofactor) within the MoFe protein. The Fe protein accepts electrons from electron donors such as ferredoxin transfer these electrons to the MoFe protein. The transferred electrons gradually weaken the triple bond of N_2 , facilitating the interaction of H^+ from the surrounding environment with N_2 to form ammonia (Mahmud et al. 2020). Among the bacterial isolates identified in this study, *B. aryabhatai* showed excellent nitrogen fixation ability. In accordance, this bacterial species was previously reported as PGPR that is able to increase nitrogen availability and support the growth of wheat and corn (Deng et al. 2022; Mehmood et al. 2021). Moreover, *B. aryabhatai* is known to possess nitrogen-fixing genes, such as *nifU* (Deng et al. 2022). The cellulose degradation in the rhizosphere is a complex process that results in improved soil nutrient availability. This study showed that *L. pakistanensis* and *B. subtilis* have the highest cellulolytic activity among eight isolates. The ability of isolates to decompose organic matter can indirectly affect soil fertility and plant growth. Cellulolytic activity is particularly important in saline soils, where decomposition of organic matter is slow because microbial activity is inhibited by salt stress (Xie et al. 2020). The results are in line with previous studies that demonstrated *Bacillus*' ability to produce cellulase enzymes in saline environments (Cai et al. 2024, 2023). Another desired quality of PGPR is its ability to suppress plant pathogens, thus reducing the use of chemical pesticides. Among bacterial isolates, *B. velezensis* and *M. aloeverae* showed the highest anti-phytopathogenic activity against *X. campestris*. Members of genus *Bacillus* were previously reported to produce various antimicrobial compounds, such as lipopeptides and bacteriocins, that could suppress the growth of plant pathogens (Etesami et al. 2023; Maksimov et al. 2020).

Salt tolerance is another crucial trait for PGPR in coastal ecosystems, since salinity is an important constraint to plant growth. Three *Bacillus* species tested in this study showed salinity resistance, with *B. subtilis subsp. spizizenii* showed the highest tolerance. Indeed, several studies on halotolerant PGPR reported *Bacillus* as a highly adaptive genus that is able to produce osmoprotectant and stress-response regulation compounds that can improve plant growth under salt stress (Hasanuzzaman et al. 2022; Valencia-Marin et al. 2024). This study also suggests that these isolates can be beneficial for

plants due to their ability to be cultured together as a bacterial consortium. Microbial consortium was shown to be more effective than a single-strain inoculant in improving soil quality and promoting plant growth (Molina-Romero et al. 2017; Singh et al. 2014; Sundaramoorthy et al. 2011). Different isolates gave complementary benefits for plant growth by producing diverse compounds, such as IAA (Indole-3-Acetic Acid), ACC deaminase, and exopolysaccharides (Mahmood et al. 2016). Bacterial consortium can also enhance the efficiency of bacterial colonization on plants. When multiple compatible strains are cultured together, they can form a more stable biofilm structure to support bacterial growth on the surface of seeds and plant roots, thus supporting the plant-microbe interaction and association (Bhattacharyya and Jha 2012). In this study, single strain treatment did not have a significant effect on plant germination and growth, indicating that individual strains lack the ability to mitigate salt stress. The synergistic interaction among bacterial isolates in the consortium might be responsible for improving sesame seed germination and seedling growth in saline conditions. This emphasizes the importance of selecting compatible strains that can work synergistically when developing microbial consortia as biofertilizers in agriculture. Compared to previous studies on PGPR in saline environments, our study highlights a unique consortium of PGPR strains with complementary mechanisms for enhancing plant growth under saline stress. While earlier research identified salt-tolerant *Bacillus* species like *B. paramycoides* and *B. aryabhatai* and their PGPR potential as single strain (Ayaz et al. 2022; Wang et al. 2018; Li et al. 2023; Lu et al. 2023), we demonstrate their synergistic effects when applied as a consortium. These findings deepen our understanding of PGPR adaptations in coastal sand dunes and identify promising candidates for biofertilizer development.

Our findings demonstrate that the isolated *Bacillus* consortium shows strong potential for alleviating salt stress in crops. However, translating these laboratory results to agricultural settings requires systematic field validation. Future studies should focus on: (1) evaluating the consortium's performance in field trials with economically important crops (e.g., sesame and maize) across varying salinity and nutrient gradients, (2) optimizing application methods (seed coating vs. soil inoculation), and (3) assessing long-term effects on soil health and microbial ecology. These steps are essential for developing this consortium into a practical biofertilizer solution for nutrient poor and saline-affected agricultural lands, particularly in tropical coastal regions.

5 Conclusion

Isolation and characterization of halotolerant PGPR from Parangkusumo sand dune ecosystem has been successfully conducted, with several isolates demonstrating beneficial

traits including phosphate solubilization, nitrogen fixation, cellulolytic activity, anti-phytopathogenic activity, salinity resistance, and plant growth promoting ability. The ability of these isolates to solubilize phosphate and fix nitrogen indicates their potential to reduce the need for synthetic fertilizers, while their cellulolytic and anti-phytopathogenic activities show their important roles to improving soil fertility and protecting plants from disease. The isolates ability to thrive in high salinity conditions shown by *B. subtilis* subsp. *spizizenii*, *B. aryabhattai*, and *B. subtilis*, their compatibility to form consortiums, and their ability to enhance sesame germination under salt stress, suggests their potential utilization in agriculture as biofertilizers, particularly in coastal or salt-affected areas. As a moderately salt-tolerant crop with potential cultivation in coastal agriculture, sesame represents an ideal candidate for immediate application of these PGPR. These PGPR strains could further improve sesame growth and productivity under saline stress and nutrient deficiency, supporting sustainable agriculture in marginal land. Future research should focus on optimizing bacterial consortia for economically important, moderately salt-tolerant crops such as sesame, sorghum, and cotton. It should also assess their long-term effects on soil biological and physico-chemical properties across diverse soil types and environmental conditions. Additionally, biofertilizer formulations should be refined to maximize their economic benefits for farmers.

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Declarations

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