

Original Article

Isolation and identification of plant growth-promoting rhizobacteria from *Spinifex littoreus* in Parangkusumo Coastal Sand Dunes, Indonesia

Isolamento e identificação de rizobactérias promotoras de crescimento de plantas de *Spinifex littoreus* em Parangkusumo Coastal Sand Dunes, Indonésia

R. Y. Khusna^a , A. Geraldj^{a,b,c,d*} , A. T. Wibowo^{a,b} , Fatimah^{a,c} , C. Clement^a , Y. S. W. Manuhara^{a,b} , H. Santoso^e , F. N. Fauzia^a , Y. K. Putro^a , R. N. Arsad^a , R. Setiawan^a , A. Luqman^{f,g}  and S. Hariyanto^{a*} 

^aUniversitas Airlangga, Faculty of Science and Technology, Department of Biology, Surabaya, Indonesia

^bUniversitas Airlangga, Biotechnology of Tropical Medicinal Plants Research Group, Surabaya, Indonesia

^cUniversitas Airlangga, University CoE-Research Center for Bio-Molecule Engineering, Surabaya, Indonesia

^dUniversitas Airlangga, Institute of Life Science, Technology and Engineering – LIHTR, Surabaya, Indonesia.

^eGenerasi Biologi Indonesia (Genbinesia) Foundation, Gresik, Indonesia

^fInstitute Teknologi Sepuluh Nopember, Department of Biology, Surabaya, Indonesia

^gJulius Maximilians University of Wuerzburg, Institute for Molecular Infection Biology – IMIB, Wuerzburg, Germany

Abstract

Utilizing coastal land for agriculture presents challenges such as low water content, high soil salinity, and low organic compound content. To support plant growth under these conditions, biofertilizers composed of plant growth promoting Rhizobacteria (PGPR), especially those inhabiting coastal areas, are needed. The Parangkusumo sand dunes on the southern coast of Java, Indonesia, is a unique coastal ecosystem characterized by arid conditions, high temperatures, and high soil salinity. To date, no studies have reported the isolation of PGPR from this ecosystem. This study is the first to isolate and identify PGPR associated with *Spinifex littoreus*, a dominant plant species in the Parangkusumo sand dunes, which are adapted to the harsh condition of Parangkusumo sand dunes. Ten rhizobacterial isolates were obtained, with five identified as members of the Bacillaceae family. All isolates demonstrated phosphate solubilization activity, while seven exhibited cellulolytic activity. One isolate, *Priestia aryabhatai* strain 2, notably showed phosphate solubilization and nitrogen fixation activities. The findings of this PGPR activity screening offer valuable insights for developing biofertilizers tailored for coastal agricultural applications.

Keywords: plant growth promoting Rhizobacteria, coastal sand dunes, *Spinifex littoreus*, biofertilizer, sustainable agriculture.

Resumo

A utilização de terras costeiras para a agricultura apresenta desafios, como o baixo teor de água, a alta salinidade do solo e o baixo teor de compostos orgânicos. Para apoiar o crescimento das plantas nessas condições, são necessários biofertilizantes compostos por rizobactérias promotoras do crescimento das plantas (PGPR), especialmente as que habitam as áreas costeiras. As dunas de areia de Parangkusumo, na costa sul de Java, Indonésia, são um ecossistema costeiro único caracterizado por condições áridas, altas temperaturas e alta salinidade do solo. Até o momento, nenhum estudo relatou o isolamento de PGPR desse ecossistema. Este estudo é o primeiro a isolar e identificar a PGPR associada à *Spinifex littoreus*, uma espécie de planta dominante nas dunas de areia de Parangkusumo e adaptada às condições adversas dessas dunas. Foram obtidos dez isolados de rizobactérias, sendo que cinco foram identificados como membros da família Bacillaceae. Todos os isolados demonstraram atividade de solubilização de fosfato, enquanto sete apresentaram atividade celulolítica. Um isolado, a cepa 2 de *Priestia aryabhatai*, demonstrou notavelmente atividades de solubilização de fosfato e fixação de nitrogênio. As descobertas dessa triagem de atividade de PGPR oferecem informações valiosas para o desenvolvimento de biofertilizantes adaptados para aplicações agrícolas costeiras.

Palavras-chave: Rizobactérias promotoras de crescimento de plantas, dunas de areia costeiras, *Spinifex littoreus*, biofertilizante, agricultura sustentável.

*e-mail: almando.geraldi@fst.unair.ac.id; sucipto-h@fst.unair.ac.id

Received: March 26, 2024 – Accepted: July 17, 2024



This is an Open Access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

1. Introduction

The need for agricultural land is increasing due to the rising demand and consumption of agricultural products (Bengochea Paz et al., 2020). With one of the world's longest coastlines, Indonesia has coastal areas with potential for agriculture (Sui et al., 2020). However, high salinity, low organic matter, and limited freshwater availability hinder agricultural use in these areas (Park et al. 2022; Rumanti et al., 2018; Ismail et al., 2022). Staple crops such as corn, wheat, and rice are sensitive to salinity, negatively impacting their yield (Hussain et al., 2019; Aftab and Hakeem, 2020).

Biofertilizers composed of plant growth-promoting Rhizobacteria (PGPR) can enhance crop growth and yield in coastal regions (Hoque et al., 2023). For example, *Bacillus aryabhatai* isolated from saline areas in Bangladesh has increased salt-responsive gene expression in rice (*Oryza sativa* L.) (Sultana et al., 2020). Similarly, *B. aryabhatai* from salt-affected rice fields in Malaysia increased rice grain weight (Shultana et al., 2020), and *Pseudomonas putida* enhanced wheat growth in saline soils in Pakistan (Ullah et al., 2022). Halotolerant *Bacillus safensis* improved corn's physiological response to saline conditions and increased growth (Azeem et al., 2022).

The Parangkusumo sand dunes on Java's southern coast is the only barchan-type sand dunes in a humid tropical climate region characterized by an arid, saline, and low-nutrient environment that can be a source of PGPR for biofertilizers (Putro and Prasetyowati, 2019; Nicolla et al., 2021). Previous metagenomic studies detected various PGPR species in these dunes (Vu et al., 2022). One way to discover rhizobacteria's potential to enhance nutrient availability for plants is by testing their abilities on nitrogen fixation, phosphate solubilization, and organic compound hydrolysis. Rhizobacteria isolates showing positive results for activities mentioned is a potential candidate of PGPR.

This study explores rhizospheric bacteria with PGPR activity from *Spinifex littoreus*, the dominant plant found in the Parangkusumo sand dunes. The bacterial isolates obtained were tested for their nitrogen fixation, phosphate solubilization, and organic compound hydrolysis abilities. These isolates could be used to formulate biofertilizers for crops cultivated in coastal areas.

2. Materials and Methods

2.1. Isolation and morphological characterization of the bacterial isolates

Soil samples were collected aseptically from the area surrounding the roots of *S. littoreus* within the Parangkusumo coastal sand dunes. Ten grams of soil were mixed with 90 ml of sterile saline solution (0.85% NaCl) in 250 ml conical flasks and agitated on an orbital shaker at 180 rpm for 30 minutes to create a homogeneous soil suspension. One milliliter of each suspension was spread onto Reasoner's 2A (R2A) agar (Himedia, India) plates and incubated at 37°C for 24 hours. Colonies displaying diverse morphological characteristics (i.e., shape, color, elevation, margin) were selected and subjected to Gram staining.

2.2. Molecular identification of bacterial isolates

The genomic DNA of isolates was extracted and purified using the Wizard® Genomic DNA Purification Kit (Promega, USA). The target region of the 16S rRNA gene was amplified using primers 27F (5'-AGA GTT TGA TCM TGG CTC AG-3') and 1492R (5'-TAC GGY TAC CTT GTT ACG ACT T-3') (Satyapal et al., 2018). The PCR product was purified using the DNA Clean & Concentrator™-5 kit (Zymo Research, USA). Purified PCR products were sent to First Base (Singapore) for bidirectional Sanger sequencing. Sequences were compared for homology against the NCBI database of 16S ribosomal RNA sequences using Nucleotide BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) (Camacho et al., 2009) and confirmed with 16S-based identification from EZBioCloud (<https://www.ezbiocloud.net/>) (Yoon et al., 2017).

2.3. Phosphate solubilization assay

The phosphate solubilization assay was done by culturing bacteria in Luria Bertani (LB) broth medium (Himedia, India) and incubated in an incubator shaker (120 rpm) at 37°C for 24 hours. Cultures were adjusted to an optical density (OD) of 0.1 at 600 nm by LB dilution. Twenty microliters of bacterial culture were injected onto sterile paper discs (d=6 mm) placed on Pikovskaya's agar medium (Himedia, India) in triplicate and incubated at 37°C for 48 hours. The diameter of the clear zone around the discs was measured to calculate the phosphate solubilization index (Akbar et al., 2021).

2.4. Nitrogen fixing assay

The nitrogen fixation assay used semi-solid Nitrogen Free Bromothymol (NFB) medium (5 g/L malic acid, 4 g/L KOH, 0.5 g/L K_2HPO_4 , 0.1 g/L $MgSO_4 \cdot 7H_2O$, 0.05 g/L $MnSO_4 \cdot H_2O$, 0.02 g/L NaCl, 0.01 g/L $CaCl_2$, 0.05 g/L $FeSO_4 \cdot 7H_2O$, 0.002 g/L $Na_2MoO_4 \cdot 2H_2O$, 1.75 g/L Bacto agar, and 2 mL/L 0.5% Bromothymol blue). Cultures were adjusted to an OD of 0.1 at 600 nm, and 20 µl was inoculated into the NFB medium in triplicate. The inoculated media was incubated at 30°C for 30 days. Positive results were indicated by a color change from yellow-green to blue and pellicle formation (Nafisah et al., 2022).

2.5. Cellulolytic assay

Overnight cultures of bacterial isolates in LB broth medium were adjusted to an OD of 0.1 at 600 nm and injected onto sterile paper discs placed on Bushnell Haas agar supplemented with carboxymethyl cellulose (CMC) in triplicate. Plates were incubated at 37°C for 48 hours, then flooded with 0.1% (weight/volume) Congo red reagent. Plates were incubated again for 30 min at room temperature and washed with 1 mol/L NaCl. The diameter of the clear zone indicating cellulolytic activity was measured (Ni'matuzahroh et al., 2024; Zhang and Dong, 2022).

2.6. Data analysis

The mean of clear zone diameter in phosphate solubilization and cellulolytic assay was calculated. Data was subjected to Analysis of Variance (ANOVA) and post

hoc comparison tests with a confidence level of 95% ($\alpha = 0.05$) using GraphPad Prism software version 9.5. ANOVA was performed to determine the significance of the differences between the means of clear zone diameter in phosphate solubilization and cellulolytic assay. Tukey's HSD post hoc method was applied to identify homogeneous subsets.

3. Results

3.1. Characterization and identification of bacterial isolates

Ten bacterial isolates with diverse colony characteristics were successfully isolated. Gram staining revealed five Gram-positive rod-shaped bacteria, two Gram-positive cocci, and three Gram-negative rod-shaped bacteria (Table 1). Molecular identification using 16S rRNA gene sequencing identified five isolates as members of the Bacillaceae family from genera *Peribacillus*, *Bacillus*, and *Priestia*. The unidentified isolates were labeled as SLA, SLB, SLC, SLD, and SLE (Table 2).

3.2. Phosphate solubilization activities

All ten isolates exhibited phosphate solubilization activity, with phosphate solubilization indices ranging from 0.23 to 0.34 (Figure 1). The highest activity was shown by isolate SLC with an index of 0.34. No significant differences were found among the isolates.

3.3. Nitrogen fixing activities

Of the ten bacterial isolates tested, only *P. aryabhattai* strain 2 exhibited nitrogen-fixing activity, as indicated by a color change in the NFB medium from yellow to blue after 14 days (Figure 2). The remaining isolates showed no nitrogen-fixing activity.

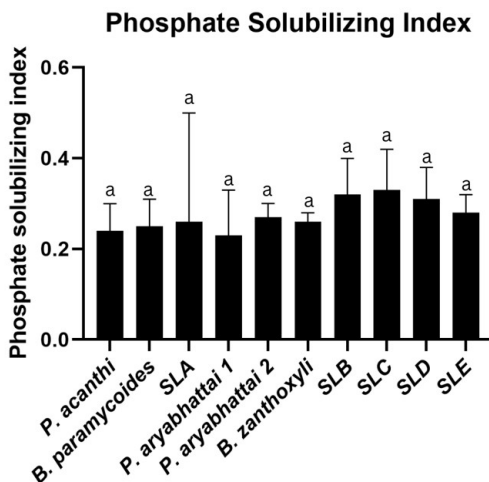


Figure 1. The mean $\pm$ SD of the phosphate solubilization index of the ten bacterial isolates. Letters indicate grouping based on the Anova post-hoc Tukey test.

3.4. Cellulolytic activity

Seven isolates demonstrated cellulolytic activity (Figure 3). *P. aryabhattai* strain 1 had the highest activity with an index of 1.18, followed by isolate SLB with an index of 0.70. The cellulolytic activity of these two isolates differed significantly from others (Figure 3a).

4. Discussion

To our knowledge, this study is among the first to isolate PGPR associated with *S. littoreus*, the dominant plant found in the Parangkusumo sand dunes. Ten PGPR isolates were obtained, with five identified as the members of Bacillaceae family. Indeed, a previous study (Geraldi et al., 2022) also reported a high abundance of this bacteria family found in Parangkusumo sand dunes. Geraldi et al. (2022) also reported five species from this family, i.e., genera *Bacillus* and *Priestia*, exhibiting antimicrobial activities. Other studies (Zelaya-Molina et al., 2023; Manetsberger et al., 2023; Tsotetsi et al., 2022; Shahid et al., 2021) also reported the two genera having plant growth-promoting activities, facilitating nutrient acquisition and producing compounds that modulate plant growth and defense mechanisms.

PGPR facilitates nutrient acquisition through activities like phosphate solubilization. In the topsoil, there are approximately 50 to 3000 mg/kg of phosphorus, but only 0.1% is available for plant uptake due to immobilization, adsorption, and precipitation with soil cations (Zhu et al., 2018; Kishore et al., 2015). PGPR, such as genera *Bacillus*, *Pseudomonas*, and *Burkholderia*, can solubilize phosphate through various mechanisms, including the production of enzymes (e.g., phosphatase and phytase), organic acids (e.g., citric acid, oxalic acid, and succinic acid), and ion chelators (e.g., siderophores) (Sarmah and Sarma 2023; Rawat et al., 2021). All ten isolates in this study showed those activities that can enhance plant growth in saline soils. *Bacillus paramycoides* and *P. aryabhattai* harbor genes related to phosphate solubilization activity, e.g., genes encoding alkaline phosphatase (GenBank accession number: NZ_MAO101000075 Region: 31327-

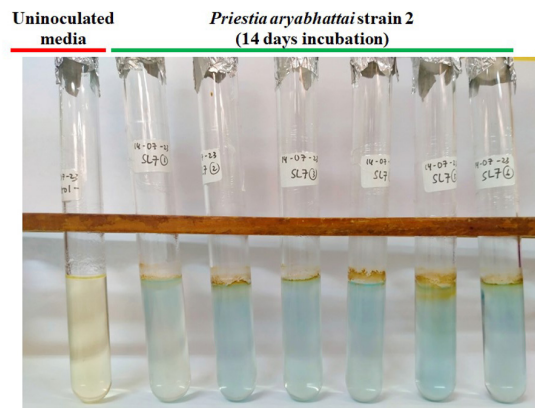


Figure 2. The result of nitrogen-fixing activity assay of ten bacterial isolates. Positive assay result was shown by *Priestia aryabhattai* strain 2.

Table 1. The colony and microscopic characterization of ten bacterial isolates.

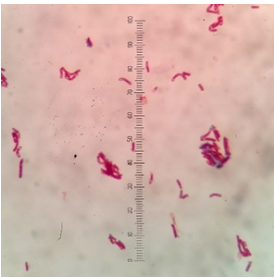
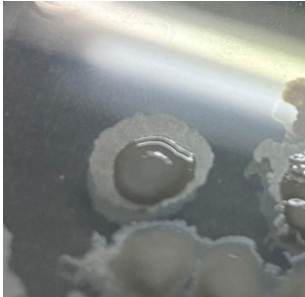
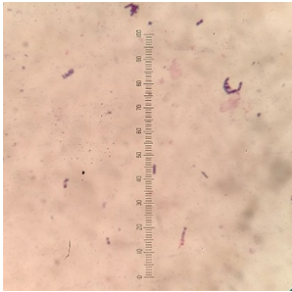
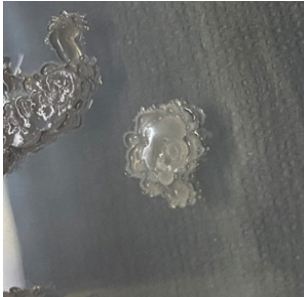
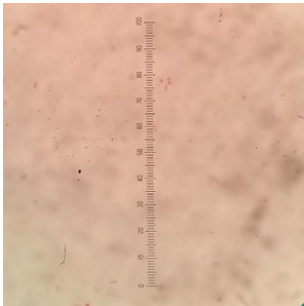
Isolate number	Colony Characterization	Microscopic Characterization	
1	White, opaque, medium-sized, circular, flat, entire margin, and smooth glistening surface		
2	White, translucent, medium-sized, irregular, flat, undulate margins, and smooth glistening surface		
3	White, translucent, medium-sized, irregular, raised, lobate margins, and smooth glistening surface		

Table 1. Continued...

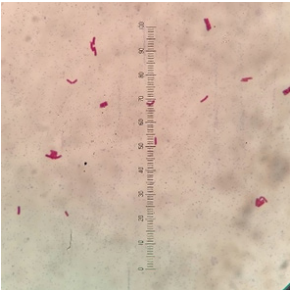
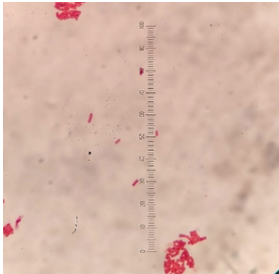
Isolate number	Colony Characterization	Microscopic Characterization
4	White, opaque medium-sized, circular, flat, entire margins, and smooth glistening surface	 Rod-shape, gram positive 
5	White, translucent, medium-sized, irregular, raised, lobate margins, and smooth glistening surface	 Rod-shape, gram positive 
6	Cream, opaque, medium-sized, circular, raised, entire margins, and smooth glistening surface	 Rod-shape, gram positive 

Table 1. Continued...

Isolate number	Colony Characterization	Microscopic Characterization
7	White, opaque, small-sized, circular shape, raised, entire margins, and smooth glistening surface	Rod-shape, gram negative
8	White, opaque medium-sized, circular, flat, entire margins, and smooth glistening surface	Spherical-shape, gram positive
9	White, opaque, medium-sized, circular, flat, entire margins, and smooth glistening surface	Rod-shape, gram negative

Table 1. Continued...

Isolate number	Colony Characterization	Microscopic Characterization
10	Cream, opaque medium-sized, circular, flat, entire margins, and smooth glistening surface	Spherical-shape, gram positive

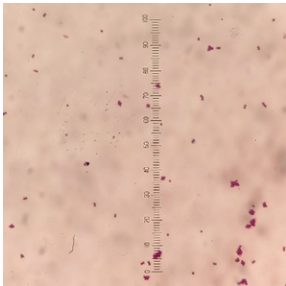


Table 2. Molecular identification results of the isolates.

Isolate Number	Closest Species	Identity (%)	Isolate Code
1	Peribacillus acanthi	99.65	-
2	Bacillus paramycoides	99.71	-
3	-	-	SLA
4	Priestia aryabhatai strain 1	99.86	
5	Priestia aryabhatai strain 2	99.78	
6	Bacillus zanthoxyli	99.57	
7	-	-	SLB
8	-	-	SLC
9	-	-	SLD
10	-	-	SLE

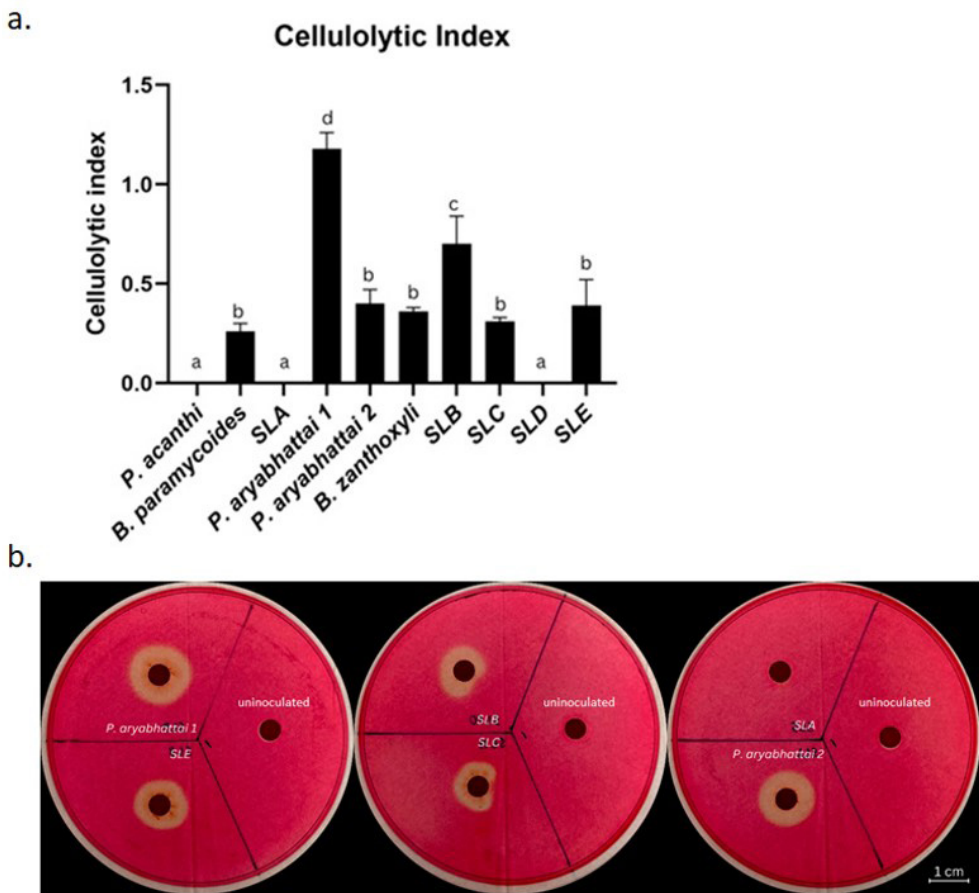


Figure 3. The results of the cellulolytic assay of the ten isolates: a. The mean $\pm$ SD of the cellulolytic index. Letters indicate grouping based on the Anova post-hoc Tukey test; b. The positive result of selected isolates in Bushnell Haas agar with CMC.

33000 and NZ_CP024035 Region: 1201855-1203510) and genes encoding phosphate signaling complex protein (GenBank accession number: NZ_MAOI01000066 Region: 141474-142130 and NZ_CP024035 Region: complement (4276578-4277237)) (Khalifa and Alsowayeh 2023;

Yang et al., 2024). Furthermore, these two species can increase phosphate accumulation and wheat growth in saline soil conditions (Li et al., 2023; Shahid et al., 2022). Meanwhile, *Bacillus zanthoxyli* has been reported to exhibit phosphate solubilization activity and enhance the growth

of tall fescue (*Lolium arundinaceum*) under saline stress conditions (Li et al., 2022). Interestingly, in this study, phosphate solubilization activity from *Peribacillus acanthi* is reported for the first time.

Nitrogen is another nutrient with low bioavailability in soil (Hamane et al., 2020; Nag et al., 2020). PGPR can facilitate nitrogen acquisition through nitrogen fixation via the nitrogenase enzyme (Aasfar et al., 2021; Mahmud et al., 2020). In this study, the only isolate showing nitrogen fixation activity was *P. aryabhatai* strain 2. This species harbors *nifU* gene (GenBank accession number: NZ_CP024035 Region: 4713040-4713270) linked to nitrogen fixation (Santos et al., 2012; Patil et al., 2017; Khalifa and Alsowayeh, 2023). Previous studies (Mehmood et al., 2021; Deng et al., 2022) also indicated that *P. aryabhatai* can fixate atmospheric nitrogen, supporting wheat and maize growth.

The last evaluated activity in this study was cellulolytic activity, which is critical for providing glucose nutrients to PGPR in rhizospheric soil by breaking down cellulose from plant litter, the most abundant biomass in soil (Tang et al., 2020). Prior reports have indicated that *P. aryabhatai* produces cellulase enzyme (Wen et al., 2015; Paz et al., 2016; Pandiarajan and Revathy, 2020). However, studies reporting cellulase production by *B. paramycoides* and *B. zanthoxyli* are limited. Instead, both species have been reported as producers of protease and lipase (Ren et al., 2022; Oliveira et al., 2022).

Further studies are needed to confirm the effects of these isolates on plant growth under coastal conditions. A possible strategy is utilizing *P. aryabhatai* strain 2 as a single isolate in biofertilizer formulations due to its phosphate solubilization, nitrogen fixation, and cellulolytic activities. Another strategy is combining isolates with the highest phosphate solubilization, nitrogen fixation, and cellulolytic activities, such as isolates SLC, *P. aryabhatai* strain 2, and *P. aryabhatai* strain 1.

5. Conclusions

Ten rhizospheric bacterial isolates from *S. littoreus* in the Parangkusumo sand dunes were successfully isolated. Five isolates were identified as Bacillaceae, known for plant growth-promoting activities. All isolates exhibited phosphate solubilization activity, one (*P. aryabhatai* strain 2) showed nitrogen fixation, and seven demonstrated cellulolytic activity. These isolates could be used as biofertilizers for sustainable agriculture, aiding in achieving Sustainable Development Goal 2, “zero hunger”.

Acknowledgements

This research was funded by Direktorat Riset dan Pengabdian Masyarakat, Kementerian Riset, Teknologi, dan Pendidikan Tinggi Republik Indonesia via Pendanaan Penelitian Skema Penelitian Dasar, No: 789/UN3.15/PT/2022.

References

- AASFAR, A., BARGAZ, A., YAAKOUBI, K., HILALI, A., BENNIS, I., ZEROUAL, Y. and MEFTAH KADMIRI, I., 2021. Nitrogen fixing azotobacter species as potential soil biological enhancers for crop nutrition and yield stability. *Frontiers in Microbiology*, vol. 12, pp. 1-19. <http://doi.org/10.3389/fmicb.2021.628379>.
- AFTAB, T. and HAKEEM, K.R., 2020. *Plant micronutrients: deficiency and toxicity management*. Cham: Springer. <https://doi.org/10.1007/978-3-030-49856-6>.
- AKBAR, M.R., PURWOKO, B.S., DEWI, I.S., SUWARNO, W.B., SUGIYANTA, S. and ANSHORI, M.F., 2021. Agronomic and yield selection of doubled haploid lines of rainfed lowland rice in advanced yield trials. *Biodiversitas (Surakarta)*, vol. 22, no. 7, pp. 3006-3012. <http://doi.org/10.13057/biodiv/d220754>.
- AZEEM, M.A., SHAH, F.H., ULLAH, A., ALI, K., JONES, D.A., KHAN, M.E.H. and ASHRAF, A., 2022. Biochemical Characterization of halotolerant *Bacillus safensis* PM22 and its potential to enhance growth of maize under salinity stress. *Plants*, vol. 11, no. 13, pp. 1721. <http://doi.org/10.3390/plants11131721>.
- BENGOCHEA PAZ, D., HENDERSON, K. and LOREAU, M., 2020. Agricultural land use and the sustainability of social-ecological systems. *Ecological Modelling*, vol. 437, pp. 109312. <http://doi.org/10.1016/j.ecolmodel.2020.109312>.
- CAMACHO, C., COULOURIS, G., AVAGYAN, V., MA, N., PAPADOPOULOS, J., BEALER, K. and MADDEN, T.L., 2009. BLAST+: architecture and applications. *BMC Bioinformatics*, vol. 10, pp. 421.
- DENG, C., ZHANG, N., LIANG, X., HUANG, T. and LI, B., 2022. *Bacillus aryabhatai* LAD impacts rhizosphere bacterial community structure and promotes maize plant growth. *Journal of the Science of Food and Agriculture*, vol. 102, no. 14, pp. 6650-6657. <http://doi.org/10.1002/jsfa.12032>. PMID:35603593.
- GERALDI, A., FAMUNGHUI, M., ABIGAIL, M., SIONA SARAGIH, C.F., FEBITANIA, D., ELMARTHENEZ, H., PUTRI, C.A., PUTRI MERDEKAWATI, U.A.S., SADILA, A.Y. and WIJAYA, N.H., 2022. Screening of antibacterial activities of *Bacillus* spp. isolated from the Parangkusumo coastal sand dunes, Indonesia. *BIO Integration*, vol. 3, no. 3, pp. 132-137. <http://doi.org/10.15212/bioi-2022-0005>.
- HAMANE, S., ZERROUK, M.H., LYEMLAHI, A.E., AARAB, S., LAGLAOUI, A., BAKKALI, M. and ARAKRAK, A., 2020. Screening and characterization of phosphate-solubilizing rhizobia isolated from *Hedysarum pallidum* in the Northeast of Morocco. In: M. KUMAR, V. KUMAR and R. PRASAD, eds. *Phyto-microbiome in stress regulation*. Singapore: Springer, pp. 113124. http://doi.org/10.1007/978-981-15-2576-6_7.
- HOQUE, M.N., HANNAN, A., IMRAN, S., PAUL, N.C., MONDAL, M.F., SADHIN, M.M.R., BRISTI, J.M., DOLA, F.S., HANIF, M.A., YE, W., BRESTIC, M. and RHAMAN, M.S., 2023. Plant Growth-promoting rhizobacteria-mediated adaptive responses of plants under salinity stress. *Journal of Plant Growth Regulation*, vol. 42, no. 3, pp. 1307-1326. <http://doi.org/10.1007/s00344-022-10633-1>.
- HUSSAIN, S., SHAUKAT, M., ASHRAF, M., ZHU, C., JIN, Q. and ZHANG, J., 2019. Salinity stress in arid and semi-arid climates: effects and management in field crops. In: S. HUSSAIN, ed. *Climate change and agriculture*. London: IntechOpen. <http://doi.org/10.5772/intechopen.87982>.
- ISMAIL, A.B., SINGH, S., SARANGI, S.K., SRIVASTAVA, A.K. and BHOWMICK, M.K., 2022. Agricultural system transformation for food and income security in coastal zones. In: T.D. LAMA, U.K. MANDAL, S.K. SARANGI and H.S. SEN, eds. *Transforming coastal zone for sustainable food and income security*. Cham: Springer International Publishing, pp. 322.

- KHALIFA, A. and ALSOWAYEH, N., 2023. Whole-genome sequence insight into the plant-growth-promoting bacterium *priestia filamentosa* strain AZC66 obtained from zygothallium coccineum rhizosphere. *Plants*, vol. 12, no. 10, pp. 1944. <http://doi.org/10.3390/plants12101944>.
- KISHORE, N., PINDI, P.K. and RAM REDDY, S., 2015. Phosphate-solubilizing microorganisms: a critical review. In: B. BAHADUR, M. VENKAT RAJAM, L. SAHIJRAM and K. KRISHNAMURTHY, eds. *Plant biology and biotechnology*. New Delhi: Springer, vol. 1, pp. 307333. http://doi.org/10.1007/978-81-322-2286-6_12.
- LI, Y., YOU, X., TANG, Z., ZHU, T., LIU, B., CHEN, M.X., XU, Y. and LIU, T.Y., 2022. Isolation and identification of plant growth-promoting rhizobacteria from tall fescue rhizosphere and their functions under salt stress. *Physiologia Plantarum*, vol. 174, no. 6, pp. e13817. <http://doi.org/10.1111/ppl.13817>. PMID:36344445.
- LI, Z., LIU, Z., WANG, Y., WANG, X., LIU, P., HAN, M. and ZHOU, W., 2023. Improving soil phosphorus availability in saline areas by marine bacterium *Bacillus paramycoides*. *Environmental Science and Pollution Research International*, vol. 30, no. 52, pp. 112385-112396. <http://doi.org/10.1007/s11356-023-30273-6>. PMID:37831236.
- MAHMUD, K., MAKAJU, S., IBRAHIM, R. and MISSAOUI, A., 2020. Current progress in nitrogen fixing plants and microbiome research. *Plants*, vol. 9, no. 1, pp. 1. <http://doi.org/10.3390/plants9010097>. PMID:33375032.
- MANETSBERGER, J., CABALLERO GÓMEZ, N., SORIA-RODRÍGUEZ, C., BENOMAR, N. and ABRIOUEL, H., 2023. Simply Versatile: the use of peribacillus simplex in sustainable agriculture. *Microorganisms*, vol. 11, no. 10, pp. 1-13. <http://doi.org/10.3390/microorganisms11102540>. PMID:37894197.
- MEHMOOD, S., KHAN, A.A., SHI, F., TAHIR, M., SULTAN, T., MUNIS, M.F.H., KAUSHIK, P., ALYEMENI, M.N. and CHAUDHARY, H.J., 2021. Alleviation of salt stress in wheat seedlings via multifunctional bacillus aryabhattai pm34: an in-vitro study. *Sustainability (Basel)*, vol. 13, no. 14, pp. 8030. <http://doi.org/10.3390/su13148030>.
- NAFISAH, W., PRABANINGTYAS, S., WITJORO, A., SAPTAWATI, R.T. and RODIANSYAH, A., 2022. Exploration non-symbiotic nitrogen-fixing bacteria from several lakes in East Java, Indonesia. *Biodiversitas (Surakarta)*, vol. 23, no. 4, pp. 1752-1758. <http://doi.org/10.13057/biodiv/d230405>.
- NAG, P., SHRITI, S. and DAS, S., 2020. Microbiological strategies for enhancing biological nitrogen fixation in nonlegumes. *Journal of Applied Microbiology*, vol. 129, no. 2, pp. 186-198. <http://doi.org/10.1111/jam.14557>. PMID:31858682.
- NI'MATUZAHROR, MOCH, A., SUPRIYANTO, A., RUSTANTINA, B., JAIYAH, L. A., RAHMAWATI, A., NURHAYATI, H., SARI, S.K., KHIPTIYAH, A.M. and HURI, D., 2024. Biodiversity of hydrolytic enzymes-producing soil bacteria from a Durian Park, Jombang, Indonesia: beneficial prospect for sustainable agriculture. *Biodiversitas Journal of Biological Diversity*, vol. 25, no. 1, pp. 392403. <http://doi.org/10.13057/biodiv/d250146>.
- NICOLLA, A.C., IRSYAD, A.N., FIRDASIA, W., SARIFAH, Z., NILAMSARI, E.I., UMAH, N., DARADWINTA, R. and SUKIRNO, S., 2021. Comparison of Damselfly (Odonata: Zygoptera) diversity in wet dune slack habitat with canopied and non-canopied areas of Gumuk Pasir Parangkusumo, Yogyakarta, Indonesia. *IOP Conference Series. Earth and Environmental Science*, vol. 736, no. 1, pp. 012046. <http://doi.org/10.1088/1755-1315/736/1/012046>.
- OLIVEIRA, T.S., OLIVEIRA, B.F.R., ANDRADE, F.C.C., GUIMARÃES, C.R., GODOY, M.G. and LAPORT, M.S., 2022. Homoscleromorphaderived *Bacillus* spp. as potential sources of biotechnologically-relevant hydrolases and biosurfactants. *World Journal of Microbiology & Biotechnology*, vol. 38, no. 10, pp. 169. <http://doi.org/10.1007/s11274-022-03358-6>. PMID:35882683.
- PANDIARAJAN, J. and REVATHY, K., 2020. Cellulolytic potential of gut bacterial biomass in silkworm *Bombyx mori*. *Ecological Genetics and Genomics*, vol. 14, pp. 100045. <http://doi.org/10.1016/j.egg.2019.100045>.
- PARK, H.-J., SEO, B.-S., JEONG, Y.-J., YANG, H.I., PARK, S., BAEK, N., KWAK, J.-H. and CHOI, W.-J., 2022. Soil salinity, fertility and carbon content, and rice yield of salt-affected paddy with different cultivation period in southwestern coastal area of South Korea. *Soil Science and Plant Nutrition*, vol. 68, no. 1, pp. 53-63. <http://doi.org/10.1080/00380768.2021.1967082>.
- PATIL, K.S., LEE, S.-J., LE, V.V., JEON, S.H., CHAE, J.-C., 2017. Complete genome sequence of *Bacillus aryabhattai* K13 isolated from compost. *Korean Journal of Microbiology*, vol. 53, no. 4, pp. 332-333. <http://doi.org/10.7845/kjm.2017.7078>.
- PAZ, A., CARBALLO, J., PÉREZ, M.J. and DOMÍNGUEZ, J.M., 2016. *Bacillus aryabhattai* BA03: a novel approach to the production of natural value-added compounds. *World Journal of Microbiology & Biotechnology*, vol. 32, no. 10, pp. 159. <http://doi.org/10.1007/s11274-016-2113-5>.
- PUTRO, S.T. and PRASETIYOWATI, S.H., 2019. Challenges in collecting primary data for environmental research purposes: a case study in Parangtritis sand dune, Yogyakarta. *IOP Conference Series. Earth and Environmental Science*, vol. 243, no. 1, pp. 012004. <http://doi.org/10.1088/1755-1315/243/1/012004>.
- RAWAT, P., DAS, S., SHANKHDHAR, D. and SHANKHDHAR, S.C., 2021. Phosphate-Solubilizing Microorganisms: Mechanism and Their Role in Phosphate Solubilization and Uptake. *Journal of Soil Science and Plant Nutrition*, vol. 21, no. 1, pp. 49-68. <http://doi.org/10.1007/s42729-020-00342-7>.
- REN, W., LI, P., WANG, X., CHE, Y., LONG, H., ZHANG, X., CAI, X., HUANG, A., ZENG, Y. and XIE, Z., 2022. Cross-habitat distribution pattern of *Bacillus* communities and their capacities of producing industrial hydrolytic enzymes in Paracel Islands: habitat-dependent differential contributions of the environment. *Journal of Environmental Management*, vol. 323, pp. 116252. <http://doi.org/10.1016/j.jenvman.2022.116252>. PMID:36126600.
- RUMANTI, I.A., HAIRMANIS, A., NUGRAHA, Y., NAFISAH, SUSANTO, U., WARDANA, P., SUBANDIONO, R.E., ZAINI, Z., SEMBIRING, H., KHAN, N.I., SINGH, R.K., JOHNSON, D.E., STUART, A.M. and KATO, Y., 2018. Development of tolerant rice varieties for stress-prone ecosystems in the coastal deltas of Indonesia. *Field Crops Research*, vol. 223, pp. 75-82. <http://doi.org/10.1016/j.fcr.2018.04.006>.
- SANTOS, P.C., FANG, Z., MASON, S.W., SETUBAL, J.C. and DIXON, R., 2012. Distribution of nitrogen fixation and nitrogenase-like sequences amongst microbial genomes. *BMC Genomics*, vol. 13, no. 1, pp. 1-12. <http://doi.org/10.1186/1471-2164-13-162>.
- SARMAH, R. and SARMA, A.K., 2023. Phosphate solubilizing microorganisms: a review. *Communications in Soil Science and Plant Analysis*, vol. 54, no. 10, pp. 1306-1315. <http://doi.org/10.1080/00103624.2022.2142238>.
- SATYAPAL, G.K., MISHRA, S.K., SRIVASTAVA, A., RANJAN, R.K., PRAKASH, K., HAQUE, R. and KUMAR, N., 2018. Possible bioremediation of arsenic toxicity by isolating indigenous bacteria from the middle Gangetic plain of Bihar, India. *Biotechnology Reports (Amsterdam, Netherlands)*, vol. 17, pp. 117-125. <http://doi.org/10.1016/j.btre.2018.02.002>.
- SHAHID, I., HAN, J., HANOOQ, S., MALIK, K.A., BORCHERS, C.H. and MEHNAZ, S., 2021. Profiling of metabolites of *Bacillus* spp. and their application in sustainable plant growth promotion and

- biocontrol. *Frontiers in Sustainable Food Systems*, vol. 5, pp. 605195. <http://doi.org/10.3389/fsufs.2021.605195>.
- SHAHID, M., ZEYAD, M.T., SYED, A., SINGH, U.B., MOHAMED, A., BAHKALI, A.H., ELGORBAN, A.M. and PICHTEL, J., 2022. Stress-tolerant endophytic isolate *priestia aryabhatai* BPR-9 modulates physio-biochemical mechanisms in wheat (*Triticum aestivum* L.) for enhanced salt tolerance. *International Journal of Environmental Research and Public Health*, vol. 19, no. 17, pp. 10883. <http://doi.org/10.3390/ijerph191710883>.
- SHULTANA, R., KEE ZUAN, A.T., YUSOP, M.R. and SAUD, H.M., 2020. Characterization of salt-tolerant plant growth-promoting rhizobacteria and the effect on growth and yield of saline-affected rice. *PLoS One*, vol. 15, no. 9, pp. e0238537. <http://doi.org/10.1371/journal.pone.0238537>. PMID:32886707.
- SUI, L., WANG, J., YANG, X. and WANG, Z., 2020. Spatial-temporal characteristics of coastline changes in Indonesia from 1990 to 2018. *Sustainability (Basel)*, vol. 12, no. 8, pp. 1-28. <http://doi.org/10.3390/su12083242>.
- SULTANA, S., PAUL, S.C., PARVEEN, S., ALAM, S., RAHMAN, N., JANNAT, B., HOQUE, S., RAHMAN, M.T. and KARIM, M.M., 2020. Isolation and identification of salt-tolerant plant-growth-promoting rhizobacteria and their application for rice cultivation under salt stress. *Canadian Journal of Microbiology*, vol. 66, no. 2, pp. 144-160. <http://doi.org/10.1139/cjm-2019-0323>. PMID:31714812.
- TANG, A., HARUNA, A.O., MAJID, N.M.A. and JALLOH, M.B., 2020. Potential PGPR properties of cellulolytic, nitrogen-fixing, phosphate-solubilizing bacteria in rehabilitated tropical forest soil. *Microorganisms*, vol. 8, no. 3, pp. 1-22. <http://doi.org/10.3390/microorganisms8030442>.
- TSOTETSI, T., NEPHALI, L., MALEBE, M. and TUGIZIMANA, F., 2022. *Bacillus* for plant growth promotion and stress resilience: what have we learned? *Plants*, vol. 11, no. 19, pp. 2482. <http://doi.org/10.3390/plants11192482>.
- ULLAH, S., BANO, A., ULLAH, A., SHAHID, M.A. and KHAN, N., 2022. A comparative study of plant growth promoting rhizobacteria (PGPR) and sowing methods on nutrient availability in wheat and rhizosphere soil under salinity stress. *Rhizosphere*, vol. 23, pp. 100571. <http://doi.org/10.1016/j.rhisph.2022.100571>.
- VU, M.T., GERALDI, A., DO, H.D.K., LUQMAN, A., NGUYEN, H.D., FAUZIA, F.N., AMALLUDIN, F.I., SADILA, A.Y., WIJAYA, N.H., SANTOSO, H., MANUHARA, Y.S.W., BUI, L.M., HARIYANTO, S. and WIBOWO, A.T., 2022. Soil mineral composition and salinity are the main factors regulating the bacterial community associated with the roots of coastal sand dune halophytes. *Biology (Basel)*, vol. 11, no. 5, pp. 695. <http://doi.org/10.3390/biology11050695>.
- WEN, J., REN, C., HUANG, N., LIU, Y. and ZENG, R., 2015. Draft genome of bagasse-degrading bacteria *Bacillus aryabhatai* GZ03 from deep sea water. *Marine Genomics*, vol. 19, pp. 13-14. <http://doi.org/10.1016/j.margen.2014.11.004>.
- YANG, S., NING, Y., LI, H. and ZHU, Y., 2024. Effects of *Priestia aryabhatai* on phosphorus fraction and implications for coremediating Cd-contaminated farmland with plant-microbe technology. *Plants*, vol. 13, no. 2, pp. 268. <http://doi.org/10.3390/plants13020268>.
- YOON, S.H., HA, S.M., KWON, S., LIM, J., KIM, Y., SEO, H. and CHUN, J., 2017. Introducing EzBioCloud: A taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. *International Journal of Systematic and Evolutionary Microbiology*, vol. 67, no. 5, pp. 1613-1617. <http://doi.org/10.1099/ijsem.0.001755>. PMID:28005526.
- ZELAYA-MOLINA, L.X., GUERRA-CAMACHO, J.E., ORTIZ-ALVAREZ, J.M., VIGUERAS-CORTÉS, J.M., VILLA-TANACA, L. and HERNÁNDEZ-RODRÍGUEZ, C., 2023. Plant growth-promoting and heavy metal-resistant *Priestia* and *Bacillus* strains associated with pioneer plants from mine tailings. *Archives of Microbiology*, vol. 205, no. 9, pp. 1-23. <http://doi.org/10.1007/s00203-023-03650-5>.
- ZHANG, G. and DONG, Y., 2022. Design and application of an efficient cellulose-degrading microbial consortium and carboxymethyl cellulase production optimization. *Frontiers in Microbiology*, vol. 13, pp. 1-17. <http://doi.org/10.3389/fmicb.2022.957444>.
- ZHU, J., LI, M. and WHELAN, M., 2018. Phosphorus activators contribute to legacy phosphorus availability in agricultural soils: a review. *The Science of the Total Environment*, vol. 612, pp. 522-537. <http://doi.org/10.1016/j.scitotenv.2017.08.095>. PMID:28865270.