



























to survive in acidic and alkaline soil, thereby promoting plant growth. The bacterial isolates examined in this study could resist environmental conditions that could impair plant growth. It is critical for determining the efficacy of the bacterial isolate, and the result obtained confirms prior findings [23,49-52].

## 5. Conclusions

This is the first preliminary study on the microbial diversity of rhizospheric rhizobacteria soil, and it shows the presence of a *Pseudomonas* sp previously uncharacterized in Kenyan tomato rhizospheric soil. The current research considers all ten isolates promising PGPR isolates from the tomato rhizosphere that can improve plant growth based on the *in vitro* characterization. However, further characterization of these isolates in planta in the greenhouse and the field is necessary to determine their appropriateness as effective bio inoculants on tomato plants.

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## Competing Interests

The authors declare no conflict of interest.

## REFERENCES

- [1] M. Y. A. Zuluaga, K. M. Lima Milani, L. S. Azeredo Gonçalves, and A. L. Martinez de Oliveira, "Diversity and plant growth-promoting functions of diazotrophic/N-scavenging bacteria isolated from the soils and rhizospheres of two species of Solanum," *PLOS ONE*, vol. 15, no. 1, p. e0227422, Jan. 2020, doi: 10.1371/journal.pone.0227422.
- [2] A. Baez-Rogelio, Y. E. Morales-García, V. Quintero-Hernández, and J. Muñoz-Rojas, "Next generation of microbial inoculants for agriculture and bioremediation," *Microb. Biotechnol.*, vol. 10, no. 1, pp. 19–21, Jan. 2017, doi: 10.1111/1751-7915.12448.
- [3] R. de Souza, A. Ambrosini, and L. M. P. Passaglia, "Plant growth-promoting bacteria as inoculants in agricultural soils," *Genet. Mol. Biol.*, vol. 38, no. 4, pp. 401–419, Nov. 2015, doi: 10.1590/S1415-475738420150053.
- [4] A. N. Yadav, "Plant Microbiomes and Its Beneficial Multifunctional Plant Growth Promoting Attributes," *Int. J. Environ. Sci. Nat. Resour.*, vol. 3, no. 1, Jun. 2017, doi: 10.19080/IJESNR.2017.03.555601.
- [5] P. K. Agrawal, R. Kundan, and S. Agrawal, "CHARACTERIZATION OF *PSEUDOMONAS* SP FROM RHIZOSPHERE OF TOMATO PLANTS (*LYCOPERSICON ESCULENTUM*) AND ITS EFFICACY ON PLANT GROWTH PROMOTION," *J. Biol. Sci. Opin.*, vol. 3, no. 3, pp. 114–121, Jul. 2015, doi: 10.7897/2321-6328.03325.
- [6] M. Ahemad and M. Kibret, "Mechanisms and applications of plant growth promoting rhizobacteria: Current perspective," *J. King Saud Univ. - Sci.*, vol. 26, no. 1, pp. 1–20, Jan. 2014, doi: 10.1016/j.jksus.2013.05.001.
- [7] R. Hayat, S. Ali, U. Amara, R. Khalid, and I. Ahmed, "Soil beneficial bacteria and their role in plant growth promotion: a review," *Ann. Microbiol.*, vol. 60, no. 4, pp. 579–598, Dec. 2010, doi: 10.1007/s13213-010-0117-1.
- [8] B. R. Glick, "Plant Growth-Promoting Bacteria: Mechanisms and Applications," *Scientifica*, vol. 2012, pp. 1–15, 2012, doi: 10.6064/2012/963401.
- [9] Y. Gu, J. Wang, Z. Xia, and H.-L. Wei, "Characterization of a Versatile Plant Growth-Promoting Rhizobacterium *Pseudomonas mediterranea* Strain S58," *Microorganisms*, vol. 8, no. 3, p. 334, Feb. 2020, doi: 10.3390/microorganisms8030334.
- [10] A. J. Spiers, A. Buckling, and P. B. Rainey, "The causes of *Pseudomonas* diversity," *Microbiology*, vol. 146, no. 10, pp. 2345–2350, Oct. 2000, doi: 10.1099/00221287-146-10-2345.
- [11] S. Sah and R. Singh, "Phylogenetical coherence of *Pseudomonas* in unexplored soils of Himalayan region," *3 Biotech*, vol. 6, no. 2, p. 170, Dec. 2016, doi: 10.1007/s13205-016-0493-8.
- [12] C. Jimtha John *et al.*, "*Pseudomonas fluorescens* R68 assisted enhancement in growth and fertilizer utilization of *Amaranthus tricolor* (L.)," *3 Biotech*, vol. 7, no. 4, p. 256, Aug. 2017, doi: 10.1007/s13205-017-0887-2.
- [13] O. Behn, "Influence of *Pseudomonas fluorescens* and arbuscular mycorrhiza on the growth, yield, quality and resistance of wheat infected with *Gaeumannomyces graminis*," *J. Plant Dis. Prot.*, vol. 115, no. 1, pp. 4–8, Feb. 2008, doi: 10.1007/BF03356232.
- [14] J. Vacheron *et al.*, "Plant growth-promoting rhizobacteria and root system functioning," *Front. Plant Sci.*, vol. 4, 2013, doi: 10.3389/fpls.2013.00356.
- [15] L. F. Andrade *et al.*, "Analysis of the abilities of endophytic bacteria associated with banana tree roots to promote plant growth," *J. Microbiol.*, vol. 52, no. 1, pp. 27–34, Jan. 2014, doi: 10.1007/s12275-014-3019-2.
- [16] U. Fayaz, S. Banday, E. Shahnaz, N. A. Khan, S. M. Zargar, and A. H. Bhat, "Morpho-cultural, physiological and

- molecular characterisation of *Sphaceloma ampelinum* causing anthracnose of grapes in temperate region of India and its management," *Indian Phytopathol.*, vol. 74, no. 4, pp. 949–957, Dec. 2021, doi: 10.1007/s42360-021-00408-1.
- [17] N. Tripathi and A. Sapra, "Gram Staining," in *StatPearls*, Treasure Island (FL): StatPearls Publishing, 2022. Accessed: May 12, 2022. [Online]. Available: <http://www.ncbi.nlm.nih.gov/books/NBK562156/>
- [18] C. Abiola and V. O. Oyetayo, "Isolation and Biochemical Characterization of Microorganisms Associated with the Fermentation of Kersting's Groundnut (*Macrotyloma geocarpum*)," *Res. J. Microbiol.*, vol. 11, no. 2, pp. 47–55, Feb. 2016, doi: 10.3923/jm.2016.47.55.
- [19] Y. Jiang *et al.*, "Identification and Characterization of *Arthrobacter nicotinovorans* JI39, a Novel Plant Growth-Promoting Rhizobacteria Strain From Panax ginseng," *Front. Plant Sci.*, vol. 13, p. 873621, May 2022, doi: 10.3389/fpls.2022.873621.
- [20] Q. Chen and S. Liu, "Identification and Characterization of the Phosphate-Solubilizing Bacterium *Pantoea* sp. S32 in Reclamation Soil in Shanxi, China," *Front. Microbiol.*, vol. 10, p. 2171, Sep. 2019, doi: 10.3389/fmicb.2019.02171.
- [21] A. Verma, Y. Patidar, and A. Vaishampayan, "Isolation and purification of potassium solubilizing bacteria from different regions of India and its effect on crop's yield," *Indian J Microbiol Res*, vol. 3, no. 4, p. 483, 2016, doi: 10.18231/2394-5478.
- [22] N. Bechtaoui *et al.*, "Characterization of plant growth promoting rhizobacteria and their benefits on growth and phosphate nutrition of faba bean and wheat," *Biol. Open*, vol. 8, no. 7, 2019, doi: 10.1242/bio.043968.
- [23] F. Ghadamgahi *et al.*, "Plant Growth-Promoting Activity of *Pseudomonas aeruginosa* FG106 and Its Ability to Act as a Biocontrol Agent against Potato, Tomato and Taro Pathogens," *Biology*, vol. 11, no. 1, p. 140, Jan. 2022, doi: 10.3390/biology11010140.
- [24] A. Y. Z. Khalifa, A.-M. Alsyeeh, M. A. Almalki, and F. A. Saleh, "Characterization of the plant growth promoting bacterium, *Enterobacter cloacae* MSR1, isolated from roots of non-nodulating *Medicago sativa*," *Saudi J. Biol. Sci.*, vol. 23, no. 1, pp. 79–86, Jan. 2016, doi: 10.1016/j.sjbs.2015.06.008.
- [25] M. R. Green, J. Sambrook, and J. Sambrook, *Molecular cloning: a laboratory manual*, 4th ed. Cold Spring Harbor, N.Y: Cold Spring Harbor Laboratory Press, 2012.
- [26] S. Altschul, "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs," *Nucleic Acids Res.*, vol. 25, no. 17, pp. 3389–3402, Sep. 1997, doi: 10.1093/nar/25.17.3389.
- [27] D. S. Treves, "Review of three DNA analysis applications for use in the microbiology or genetics classroom," *J. Microbiol. Biol. Educ.*, vol. 11, no. 2, pp. 186–187, 2010, doi: 10.1128/jmbe.v11i2.205.
- [28] R. C. Edgar, "MUSCLE: multiple sequence alignment with high accuracy and high throughput," *Nucleic Acids Res.*, vol. 32, no. 5, pp. 1792–1797, Mar. 2004, doi: 10.1093/nar/gkh340.
- [29] K. Tamura, G. Stecher, and S. Kumar, "MEGA11: Molecular Evolutionary Genetics Analysis Version 11," *Mol. Biol. Evol.*, vol. 38, no. 7, pp. 3022–3027, Jun. 2021, doi: 10.1093/molbev/msab120.
- [30] S. Sah, S. Krishnani, and R. Singh, "*Pseudomonas* mediated nutritional and growth promotional activities for sustainable food security," *Curr. Res. Microb. Sci.*, vol. 2, p. 100084, Dec. 2021, doi: 10.1016/j.crmicr.2021.100084.
- [31] A. S. Kashyap *et al.*, "Unraveling Microbial Volatile Elicitors Using a Transparent Methodology for Induction of Systemic Resistance and Regulation of Antioxidant Genes at Expression Levels in Chili against Bacterial Wilt Disease," *Antioxidants*, vol. 11, no. 2, p. 404, Feb. 2022, doi: 10.3390/antiox11020404.
- [32] N. P. Nordstedt, L. J. Chapin, C. G. Taylor, and M. L. Jones, "Identification of *Pseudomonas* Spp. That Increase Ornamental Crop Quality During Abiotic Stress," *Front. Plant Sci.*, vol. 10, p. 1754, Jan. 2020, doi: 10.3389/fpls.2019.01754.
- [33] D. Comeau, C. Balthazar, A. Novinscak, N. Bouhamdani, D. L. Joly, and M. Filion, "Interactions Between *Bacillus* Spp., *Pseudomonas* Spp. and *Cannabis sativa* Promote Plant Growth," *Front. Microbiol.*, vol. 12, p. 715758, Sep. 2021, doi: 10.3389/fmicb.2021.715758.
- [34] S. Kalam, A. Basu, and A. R. Podile, "Functional and molecular characterization of plant growth promoting *Bacillus* isolates from tomato rhizosphere," *Heliyon*, vol. 6, no. 8, p. e04734, Aug. 2020, doi: 10.1016/j.heliyon.2020.e04734.
- [35] Ghent University, Belgium and M. Höfte, "The use of *Pseudomonas* spp. as bacterial biocontrol agents to control plant diseases," in *Burleigh Dodds Series in Agricultural Science*, Wageningen University & Research, The Netherlands and J. Köhl, Eds. Burleigh Dodds Science Publishing, 2021, pp. 301–374. doi: 10.19103/AS.2021.0093.11.
- [36] A. F. Abd El-Rahman, H. A. Shaheen, R. M. Abd El-Aziz, and D. S. S. Ibrahim, "Influence of hydrogen cyanide-producing rhizobacteria in controlling the crown gall and root-knot nematode, *Meloidogyne incognita*," *Egypt. J. Biol. Pest Control*, vol. 29, no. 1, p. 41, Dec. 2019, doi: 10.1186/s41938-019-0143-7.
- [37] M. M. Paulin, A. Novinscak, C. Lanteigne, V. J. Gadkar, and M. Filion, "Interaction between 2,4-Diacetylphloroglucinol- and Hydrogen Cyanide-Producing *Pseudomonas brassicacearum* LBUM300 and *Clavibacter michiganensis* subsp. *michiganensis* in the Tomato Rhizosphere," *Appl. Environ. Microbiol.*, vol. 83, no. 13, pp. e00073-17, Jul. 2017, doi: 10.1128/AEM.00073-17.
- [38] H.-B. Li *et al.*, "Genetic Diversity of Nitrogen-Fixing and Plant Growth Promoting *Pseudomonas* Species Isolated from Sugarcane Rhizosphere," *Front. Microbiol.*, vol. 8, p. 1268, Jul. 2017, doi: 10.3389/fmicb.2017.01268.
- [39] B. Sivasankari and M. Anandharaj, "Isolation and Molecular Characterization of Potential Plant Growth Promoting *Bacillus cereus* GGBSTD1 and *Pseudomonas* spp. GGBSTD3 from Vermisources," *Adv. Agric.*, vol. 2014, pp. 1–13, 2014, doi: 10.1155/2014/248591.

- [40] Y. Yan *et al.*, "Nitrogen fixation island and rhizosphere competence traits in the genome of root-associated *Pseudomonas stutzeri* A1501," *Proc. Natl. Acad. Sci.*, vol. 105, no. 21, pp. 7564–7569, May 2008, doi: 10.1073/pnas.0801093105.
- [41] J. M. Chai and A. Adnan, "Effect of different nitrogen source combinations on microbial cellulose production by *Pseudomonas aeruginosa* in batch fermentation," *IOP Conf. Ser. Mater. Sci. Eng.*, vol. 440, p. 012044, Oct. 2018, doi: 10.1088/1757-899X/440/1/012044.
- [42] T. C. Bang, S. Husted, K. H. Laursen, D. P. Persson, and J. K. Schjoerring, "The molecular–physiological functions of mineral macronutrients and their consequences for deficiency symptoms in plants," *New Phytol.*, vol. 229, no. 5, pp. 2446–2469, Mar. 2021, doi: 10.1111/nph.17074.
- [43] M. Astriani, Siti Zubaidah, Abdul Latief Abadi, and Endang Suarsini, "*Pseudomonas plecoglossicida* as a novel bacterium for phosphate solubilizing and indole-3-acetic acid-producing from soybean rhizospheric soils of East Java, Indonesia," *Biodiversitas J. Biol. Divers.*, vol. 21, no. 2, Jan. 2020, doi: 10.13057/biodiv/d210220.
- [44] B. K. Jha, M. Gandhi Pragash, J. Cletus, G. Raman, and N. Sakthivel, "Simultaneous phosphate solubilization potential and antifungal activity of new fluorescent *pseudomonad* strains, *Pseudomonas aeruginosa*, *P. plecoglossicida* and *P. mosselii*," *World J. Microbiol. Biotechnol.*, vol. 25, no. 4, pp. 573–581, Apr. 2009, doi: 10.1007/s11274-008-9925-x.
- [45] T. Widowati, Nuriyanah, and H. Sukiman, "Production of indole acetic acid by *Enterobacter cloacae* H3 isolated from Mungbean (*Vigna radiata*) and its potential supporting the growth of soybean seedling," *IOP Conf. Ser. Earth Environ. Sci.*, vol. 308, no. 1, p. 012040, Aug. 2019, doi: 10.1088/1755-1315/308/1/012040.
- [46] F. Vásquez-Ponce, S. Higuera-Llantén, M. S. Pavlov, S. H. Marshall, and J. Olivares-Pacheco, "Phylogenetic MLSA and phenotypic analysis identification of three probable novel *Pseudomonas* species isolated on King George Island, South Shetland, Antarctica," *Braz. J. Microbiol.*, vol. 49, no. 4, pp. 695–702, Oct. 2018, doi: 10.1016/j.bjm.2018.02.005.
- [47] D. Chiniquy *et al.*, "Microbial Community Field Surveys Reveal Abundant *Pseudomonas* Population in Sorghum Rhizosphere Composed of Many Closely Related Phylotypes," *Front. Microbiol.*, vol. 12, p. 598180, Mar. 2021, doi: 10.3389/fmicb.2021.598180.
- [48] J. I. Rilling, J. J. Acuña, P. Nannipieri, F. Cassan, F. Maruyama, and M. A. Jorquera, "Current opinion and perspectives on the methods for tracking and monitoring plant growth–promoting bacteria," *Soil Biol. Biochem.*, vol. 130, pp. 205–219, Mar. 2019, doi: 10.1016/j.soilbio.2018.12.012.
- [49] T. Gashaw, B. Sitotaw, and S. Yilma, "Evaluation of Rhizosphere Bacterial Antagonists against *Ralstonia solanacearum* Causing Tomato (*Lycopersicon esculentum*) Wilt in Central Ethiopia," *Int. J. Agron.*, vol. 2022, pp. 1–9, Apr. 2022, doi: 10.1155/2022/6341555.
- [50] M. Yaghoubi Khangahi, S. Strafella, I. Allegretta, and C. Crecchio, "Isolation of Bacteria with Potential Plant-Promoting Traits and Optimization of Their Growth Conditions," *Curr. Microbiol.*, vol. 78, no. 2, pp. 464–478, Feb. 2021, doi: 10.1007/s00284-020-02303-w.
- [51] O. A. Fasusi, A. E. Amoo, and O. O. Babalola, "Characterization of plant growth-promoting rhizobacterial isolates associated with food plants in South Africa," *Antonie Van Leeuwenhoek*, vol. 114, no. 10, pp. 1683–1708, Oct. 2021, doi: 10.1007/s10482-021-01633-4.
- [52] A. Getahun, D. Muleta, F. Assefa, and S. Kiros, "Plant Growth-Promoting Rhizobacteria Isolated from Degraded Habitat Enhance Drought Tolerance of *Acacia* (*Acacia abyssinica* Hochst. ex Benth.) Seedlings," *Int. J. Microbiol.*, vol. 2020, pp. 1–13, Oct. 2020, doi: 10.1155/2020/8897998.