



Screening of Plant Growth-Promoting Halotolerant Bacteria Isolated from Weeds Rhizosphere Grown in Saline Soil

Luqman Qurata Aini^{1*)}, Nurul Aini²⁾, Wiwin Sumiya Dwi Yamika²⁾ and Adi Setiawan²⁾

¹⁾ Department of Plant Pest and Disease, Faculty of Agriculture, Universitas Brawijaya, Malang, East Java, Indonesia

²⁾ Department of Agronomy, Faculty of Agriculture, Universitas Brawijaya, Malang, East Java, Indonesia

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*) Corresponding author:

E-mail: luqman.fp@ub.ac.id

ABSTRACT

This study aimed to screen and characterize halotolerant bacterial isolates, which could enhance plant growth performance in saline soil. Halotolerant bacteria was isolated from weeds rhizosphere grown in saline soil of coastal agricultural land located at Brondong District, Lamongan Regency, East Java Province, Indonesia. This research was conducted from June to September 2018. Seven bacterial isolates can grow in a Nutrient Agar medium containing 10% of NaCl, suggesting that these bacteria were halotolerant. Furthermore, all bacterial isolates were shown to produce indol acetic acid (IAA) and do not induce a hypersensitive response when infiltrated into tobacco leaves. These results explain that these bacteria had potency as plant growth-promoting rhizobacteria (PGPR) and were not tend to be the plant pathogen. The growth of seedlings when inoculated in cucumber seed grown in saline media were higher than those in control. This result suggests that the halotolerant bacteria can enhance the development of cucumber seedlings in saline stress conditions. Three potential halotolerant bacteria i.e., SN22, SN23, SN26 were selected and molecularly identified as *Bacillus megaterium*, *Bacillus* sp., and *B. megaterium*, respectively.

INTRODUCTION

Indonesia has 106,000 km of coastline with more than one million hectares of land potential for agriculture (Arifin, Herawati, Mujiyo, & Widijanto, 2021). However, agricultural land near the coastline is potentially exposed to salinity caused by tidal waves and seawater intrusion (Gopalakrishnan, Hasan, Sanaul Haque, Jayasinghe, & Kumar, 2019). One of the efforts to overcome soil high salinity is by using tolerant crops to high salinity conditions. However, several studies report that few cultivars of tomato, cucumber, corn, and rice were shown susceptible to salinity stress (Baghbani, Forghani, & Kadkhodaie, 2013; Hoffmann, Berni, Hausman, & Guerriero, 2020; Reddy, Kim, Yoon, Kim, & Kwon, 2017; Shtereva, Vassilevska-Ivanova, & Karceva, 2015). Efforts to address the high salinity problem in soil are not sufficient only with tolerant plant cultivars since limited plant cultivars were easy

against salinity stress (Shrivastava & Kumar, 2015). Therefore, improving soil fertility in saline soils through the seasonal planting arrangements and using microbial technology to improve plant growth in a high saline environment were promised.

Microbial technology has been widely used to enhance soil fertility and reduce synthetic chemicals by using microbes to function as biofertilizers (Nosheen, Ajmal, & Song, 2021; Ortiz & Sansinenea, 2022). Microbial technology to manage salinity in soil can be done by utilizing microbes, especially soil inhabitant bacteria that tolerate growing in soil with high salinity levels called halotolerant bacteria (Shrivastava & Kumar, 2015). Several studies have reported the finding of several types of halotolerant bacteria which can help plants to overcome salinity stress and grow better on saline (Egamberdieva, Shurigin, Gopalakrishnan, & Sharma, 2014; Iqbal, Khalid, Zahir, & Ahmad, 2016;

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Zafar-ul-Hye, Farooq, Zahir, Hussain, & Hussain, 2014). Some of these bacteria were even capable of producing plant growth regulators, defined as plant growth-promoting bacteria (PGPB) (Islam *et al.*, 2016; Paul & Lade, 2014). PGPB can improve nutrient acquisition and soil fertility in saline soil and enhance crop productivity (Shilev, 2020; Teo *et al.*, 2022). These bacteria used to live and grow naturally (indigenous) by colonizing the rhizosphere of plants on soil that contains high salinity (Shrivastava & Kumar, 2015). Isolation and utilization of such indigenous bacteria improve crop resistance against salinity stress and enhance microbial biodiversity in saline soils (Etesami & Beattie, 2018).

Information on the utilization of indigenous halotolerant rhizobacteria of saline soil, particularly those isolated from weeds inhabited in saline soil, is still limited. Hence more studies are needed to obtain high potential halotolerant bacteria. Therefore, this study aimed to obtain halotolerant bacteria recovered from the rhizosphere of weeds grown in saline soil of farmland located in the coastal area in Lamongan District, East Java which has the potency of plant growth-promoting bacteria (PGPB).

MATERIALS AND METHODS

Halotolerant Bacteria Sampling and Isolation

The sampling location was conducted in the coastal farmland in Brondong District, Lamongan Regency, East Java, from June to September 2018. Isolation of halotolerant bacteria originating from the rhizosphere of weeds grown in saline soil was performed by the method described by Munif, Hallmann, & Sikora (2013) with minor modification. The samples used in this study were the rhizosphere, i.e., roots and surrounding soil roots of weeds grown in saline soil of agricultural land. A total of 10 g of the sample were suspended in 90 ml of sterile aqua dest and then subjected to serial dilution. The suspension in each dilution series (100 µl) was spread over nutrient agar (NA) plates, and then the culture was incubated for 24 hours at 27-28°C. The growing bacterial colonies were then purified on a fresh NA plate and then subjected to further screening by growing them in NA plates containing NaCl with a concentration of 10%. The purified bacteria recovered from colonies grown on the agar plates containing 10% NaCl were determined as halotolerant bacteria and stored for the next experimental stage.

Characterization of Halotolerant Bacteria

Bacterial morphological characters such as shape, color, and edges of the colony were obtained from observations of single colonies grown on the NA plate. In addition, gram reaction, cell shape, and spore production were observed microscopically after Gram and spore staining by a method according to Schaad, Jones, & Chun (2001). The halotolerant bacterial isolates were also tested on a 1-month-old tobacco plant by infiltrating the bacterial suspension on tobacco leaves to determine hypersensitivity reactions (HR).

Selection of Halotolerant Bacteria in Production of Indole-3-Acetic Acid (IAA)

Halotolerant bacterial isolates were quantitatively tested for their capability to excrete IAA. The assay was performed using selective media containing L-tryptophan (Dawwam, Elbeltagy, Emara, Abbas, & Hassan, 2013). The bacterial isolates were cultivated in a liquid Nutrient Broth (NB) containing tryptophan (0.1 g/l) for three days at a temperature of 28°C in the dark. The bacterial culture was then centrifuged (10,000 rpm for 10 minutes). Next, two ml of supernatant were incorporated with 4 ml of Salkowski reagent (7.5 ml FeCl₃·7H₂O 0.5 M; 150 ml concentrated H₂SO₄; 250 ml sterile aqua dest), homogenized, and then left for 15 minutes. When the solution changed its color, the absorbance was measured using a spectrophotometer (wavelength of 530 nm). Finally, the absorbance was calculated into the standard curve equation to determine the IAA concentration.

The Effect of Halotolerant Bacteria Inoculation on the Growth of Cucumber Seedling

Cucumber seeds were surface sterilized by soaking with either 95% alcohol for 5 minutes and then 5% NaClO solution for 1 minute. The seeds were then rinsed three times using sterile distilled water. They were then dipped in 48 hours of halotolerant bacterial culture suspension for 15 minutes. About 25 cucumber seeds were put into seed paper and placed on a Petri dish. The seed paper was moistened with NaCl solution (EC value of 5 dS/m). The cucumber seeds were grown for two weeks and subjected to the observation of growth parameters, i.e., the percentage of germination, root length, and hypocotyl length.

Molecular Identification of Halotolerant Bacteria

The molecular identification of the halotolerant bacteria selected as PGPR was performed by sequencing the 16S rRNA gene. Genomic DNA from halotolerant bacteria was isolated using Presto™ Mini gDNA Bacteria Kit (Gene aid). The 16S rRNA gene in genomic DNA was amplified by PCR reaction using KOD FX Neo (Toyobo, Japan). DNA amplification was performed on a PCR machine with the following reaction stages: heating at 95°C for 1 minute, annealing at 55°C for 1 minute, and elongating at 72°C for 5 minutes. The PCR results were then subjected to gel electrophoresis with 1% agarose in the TBE buffer (Green and Sambrook, 2012). The amplicon of the PCR result was then

purified and sequenced. The sequence of the 16S rRNA gene was used for the search for a homologous series of 16S rRNA sequences in the DNA database (GenBank) using the BLASTN software available on the NCBI website. The phylogenetic tree was constructed using MEGA version 7 (Kumar, Stecher, & Tamura, 2016).

Data Analysis

The effect of halotolerant bacteria inoculation on cucumber seedlings was analyzed with ANOVA. If the results were significantly different among the treatments, a posthoc test was used with the Duncan Multiple Range Test (DMRT) at an error level of 5%. Data were analyzed using SPSS 22.0, and visualized by ggplot2 in RStudio.

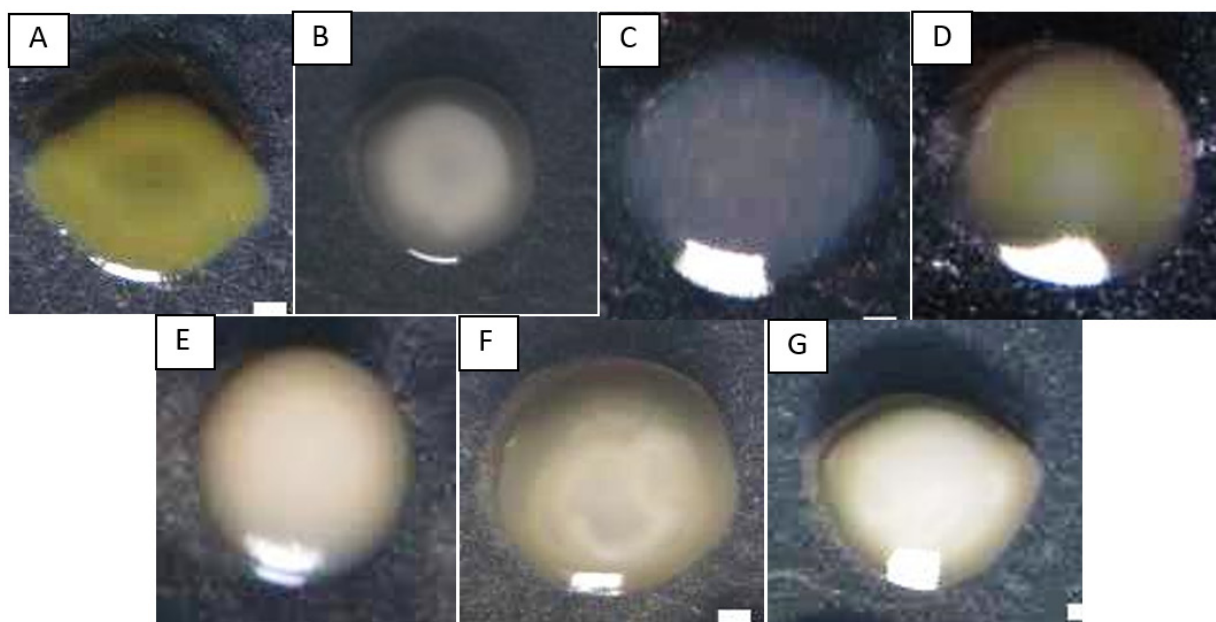


Fig. 1. Colony morphology of halotolerant bacterial isolates: A. SN1, B. SN2, C. SN6, D. SN15, E. SN22, F. SN23, and G. SN26

Table 1. Morphology, cell morphology, and the hypersensitive reaction of halotolerant bacterial isolates

Isolates	Colony Morphology			Cell Morphology			Hypersensitive Reaction
	Form	Color	Margin	Gram	Shape	Spore Production	
SN1	Round	White	-	+	bacilli	-	-
SN2	Round	White, translucent	-	+	bacilli	-	-
SN6	Round	White, opaque	-	+	bacilli	+	-
SN15	Round	Yellow	-	+	bacilli	-	-
SN22	Round	White, opaque	-	+	bacilli	+	-
SN23	Round	White	-	+	bacilli	+	-
SN26	Round	White, opaque	-	+	bacilli	+	-

RESULTS AND DISCUSSION

Characterization of Halotolerant Bacteria

Twenty-three bacteria have been isolated from the rhizosphere of weeds inhabited in saline farmland. Seven bacterial isolates could be grown on an NA medium containing 10% NaCl. Hence it could be determined that the seven bacterial isolates were halotolerant bacteria. Seven isolates have different colony morphology (Fig. 1). They can not produce a hypersensitive reaction when the bacterial cells were infiltrated on tobacco leaves, indicating that they tend to had no potential as plant pathogens. All isolates were Gram-positive bacilli but differ in spore-forming ability (Table 1). Only four halotolerant bacterial isolates, i.e., SN6, SN22, SN23, and SN26, produce spores that are the main character of the genus of *Bacillus*. Several bacterial species that have been found as halotolerant are reported to be Gram-positive *Bacillus* (Obeidat, 2017; Ruginescu *et al.*, 2020). Berrada *et al.* (2012) also reveal that 102 of 124 bacteria isolated from marsh and saltern ecosystem in Morocco are Gram-positive halotolerant bacteria dominated by the genus of *Bacillus*, *Jeotgalibacillus*, *Planococcus*, *Staphylococcus*, and *Thalobacillus*.

IAA Producing Halotolerant Bacteria

The screening for IAA-producing bacteria shows that all bacterial isolates can excrete IAA with a concentration of 1.15 to 10.08 ppm (Table 2). These results indicate that all bacterial isolates were potential PGPR since IAA functions as a plant hormone to promote plant growth. It has been widely described that certain types of bacteria that originated from the plant rhizosphere were capable of producing IAA. For example, Chaiarn & Lumyong (2011) found that 18.05% of 216 bacteria isolated from rice plants in Thailand can produce IAA. Mohite (2013) reports that 10 bacteria isolated from banana, wheat, and maize rhizosphere were IAA producers and identified as *Lactobacillus acidophilus*, *L. casei*, *B. megaterium*, *B. subtilis*, and *B. cereus*. Mike-Anosike, Braide, & Adeleye (2018) also reported several bacteria isolated from *Panicum maximum* rhizosphere identified as *Staphylococcus* sp., *Micrococcus* sp., *Bacillus* sp., and *Pseudomonas* were able to produce IAA ranging 4.0 mg/l to 10.0 mg/l.

Table 2. Production of IAA by halotolerant bacteria

Isolate	IAA concentration (ppm)
SN1	3.10
SN2	2.50
SN6	1.15
SN15	5.24
SN22	10.08
SN23	2.10
SN26	1.35

The Effect of Halotolerant Bacteria Inoculation on Cucumber Seedlings in Salinity Stress Condition

Inoculation of halotolerant bacterial isolates affects the cucumber seedlings' growth during the germination stage under the salinity stress conditions. The seed germination percentage in the inoculation of five halotolerant bacterial isolates, i.e., SN6, SN15, SN22, SN23, and SN26, were higher than control (Fig. 2A). SN26 and SN23 show the highest percentage of seed germination. However, the percentage of seed germination in the inoculation of two halotolerant bacterial isolates, i.e., SN1 and SN2, did not differ compared to that of control. These results suggested that the injection of five halotolerant bacterial isolates, i.e., SN6, SN15, SN22, SN23, and SN26, enhanced the seed germination in cucumber seedlings under the salinity stress condition. Ramadoss, Lakkineni, Bose, Ali, & Annapurna (2013) also reported that wheat seed germination was enhanced by more than 50% through inoculation with halotolerant bacteria strain compared to an uninoculated seed. A higher percentage of germination due to inoculation of halotolerant bacteria was also reported on alfalfa and sugar beet (Ansari, Shekari, Mohammadi, Biró, & Végvári, 2017; Zhou, Zhao, & Tian, 2017).

Cucumber seedlings with halotolerant bacterial isolates show longer roots than uninoculated seedlings (Fig. 2B). The length of hypocotyl shows higher in the cucumber seedling inoculated with all halotolerant bacterial isolates than that of the uninoculated seedling (Fig. 2C). These results suggest that the inoculation of the halotolerant bacteria enhances the growth of root and hypocotyl of cucumber seedlings when grown in salinity stress conditions. Yañez-Yazlle, Romano-Armada, Acreche, Rajal, & Irazusta (2021) report

that halotolerant bacteria isolated under saline stress can induce root length of chia (*Salvia hispanica* L.) and quinoa (*Chenopodium quinoa* Willd.) from 60% to 92%. Albdaawi, Khyami-Horani, Ayad, Alananbeh, & Al-Sayaydeh (2019) also

report six halotolerant bacterial strains as PGPR increased survival on inoculated plants on high levels salinity conditions as indicated on seedling root and hypocotyl growth and when compared to uninoculated plants.

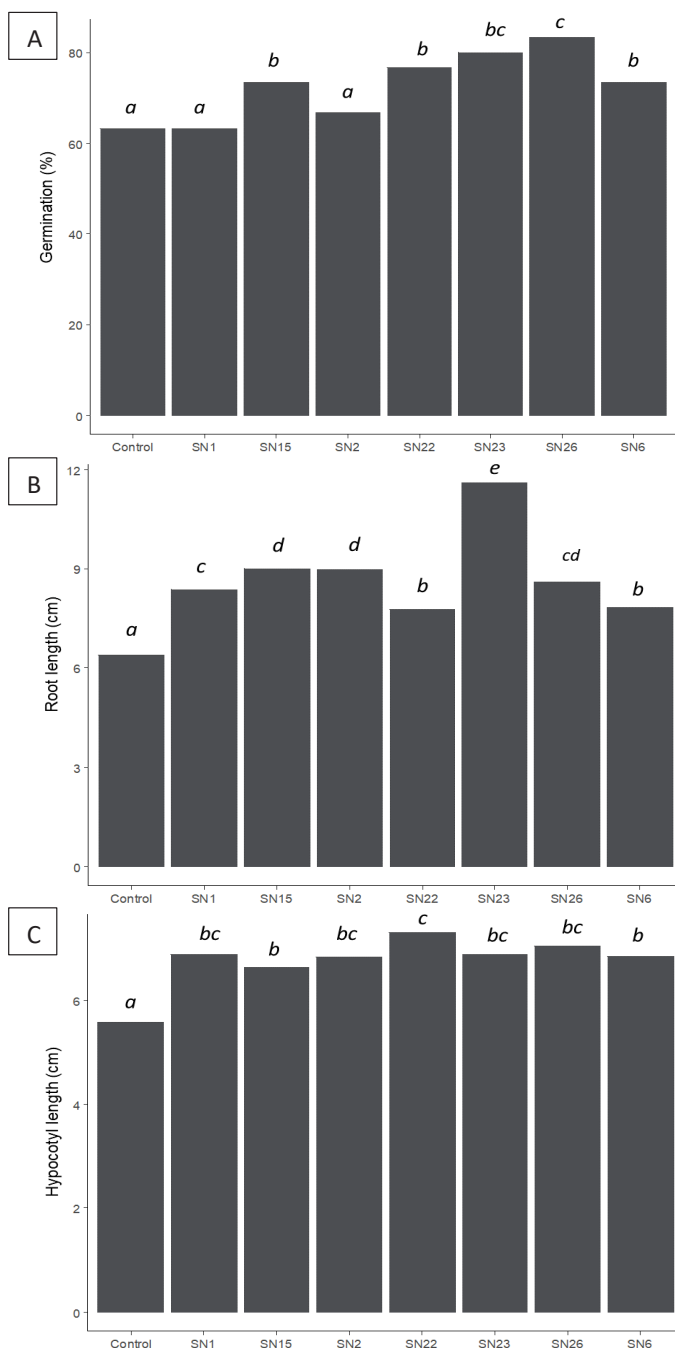


Fig. 2. The effect of halotolerant bacteria inoculation on cucumber seedlings based on (A) the percentage of seed germination, (B) root length, and (C) hypocotyl length. Bar with different letters is significantly different between isolates, at $p < 0.05$, according to DMRT.

Molecular Identification of Potential Halotolerant Bacteria as PGPR

In this study, three isolates of halotolerant bacteria had high potential as PGPR, i.e., SN 22, SN 23, and SN 26, based on their ability to produce IAA and enhance cucumber seedlings under the high salinity condition. Based on the results of BLAST-N using the DNA sequence of the gene encoding 16S rRNA, the halotolerant bacterial isolate of SN23 has 99.93% similarity to *Bacillus* sp. strain NE3RP2, SN26 had 99.58% similarity to *B. megaterium* strain MUGA128, and SN 22 had 99.93% similarity to *B. megaterium* strain WSH-002 (Table 3). Furthermore, the phylogenetic tree developed using maximum likelihood showed that each halotolerant bacterial isolate was clustered following the result of affinity. For example, the phylogenetic tree analysis using 16S rDNA showed SN23 and *B. megaterium*

NBRI161 in one clade, and the affinity of strains in trees showed 72% (Fig. 3).

These results suggest that the molecular identification was consistent with the results of morphological and physiological characterizations. All the three halotolerant bacteria were from the genus of *Bacillus*, indicating that the genus of *Bacillus* used to had the capacity to grow under salinity stress. Several species of *Bacillus* were reported can improve the crop growth under salinity stress, such as *B. megaterium* on cucumber (Nadeem *et al.*, 2016), *B. subtilis* on rice (Jha & Subramanian, 2014; Jha, Subramanian, & Patel, 2011), *B. safensis* and *B. cereus* on wheat (Saxena, Kumar, Chakdar, Anuroopa, & Bagyaraj, 2020), *B. subtilis* on maize (Ferreira *et al.*, 2018), *B. subtilis* on radish plant (Sedki & El-Mohamedy, 2012), and *B. subtilis* on Indian bassia (Abeer *et al.*, 2015).

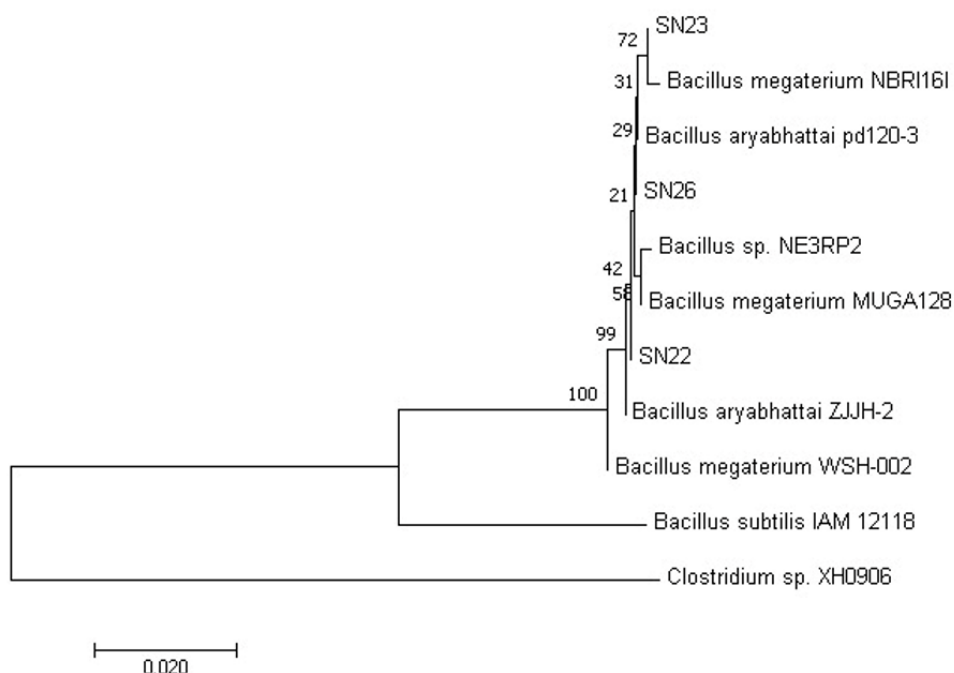


Fig. 3. Maximum likelihood tree, numbers above branches indicate branch lengths. The accession numbers on the right are identified from sequences from GenBank, and other sequences are obtained from the BOLD public database.

Table 3. The results of halotolerant bacteria identification potential as PGPR by similarity index of 16S rRNA gene sequence

Isolate	Max. Score	E. Value	Ident	Accession Code	Species Name
SN 22	2531	0.0	99.93%	CP003017.1	<i>Bacillus megaterium</i>
SN 23	2509	0.0	99.93%	KT900606.1	<i>Bacillus</i> sp.
SN 26	2498	0.0	99.58%	KT719649.1	<i>Bacillus megaterium</i>

The identification of potential endophytic bacteria as biostimulants showed the dominance of *Bacillus* sp. Several *Bacillus* species can survive in high temperatures (thermotolerant) and high salinity (halotolerant) (Farace *et al.*, 2015; Ji *et al.*, 2022).

Based on the results, it has been suggested that the halotolerant bacteria enhanced the growth of cucumber seedlings higher than that in control without bacteria. Bacteria isolated from saline farmland have been reported to be a biostimulant in enhancing plant growth. One of the mechanisms of soil microorganisms in helping plant growth is their ability to produce phytohormones (de Souza, Ambrosini, & Passaglia, 2015). Phytohormones can be produced by certain microorganisms or plants that affect plants' physiological processes (Egamberdieva, Wirth, Alqarawi, Abd_Allah, & Hashem, 2017). Bacteria that can grow in extreme conditions will enhance the production of plant hormones. This hormone accelerates growth so that the yield of plants obtained enhances. Some soil microorganisms are known to have the ability to produce IAA phytohormones in halophilic plant areas (Orhan, 2016). The finding of plant growth-promoting halotolerant bacteria recovered from weeds rhizosphere is relatively new. It can be used as a novel approach to obtain the halotolerant bacteria that also play a role as PGPR.

CONCLUSION

Seven bacterial isolates were halotolerant bacteria. All isolates were Gram-positive bacilli but differed in spore-forming ability (SN 1, SN 2, SN6, SN 15, SN 22, SN23, SN 26). Bacterial isolates that produce the highest IAA were SN22, and SN23 and SN26 isolates can enhance the germination of cucumber plants. The molecular identification has shown that SN22, SN23, and SN26 isolates were identified as *B. megaterium*, *Bacillus* sp., and *B. megaterium*, respectively.

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REFERENCES

- Abeer, H., Abd_Allah, E. F., Alqarawi, A. A., Al-Huqail, A. A., Alshalawi, S. R. M., Wirth, S., & Dilfuza, E. (2015). Impact of plant growth promoting *Bacillus subtilis* on growth and physiological parameters of *Bassia indica* (Indian bassia) grown under salt stress. *Pakistan Journal of Botany*, 47(5), 1735–1741. Retrieved from [https://www.pakbs.org/pjbot/PDFs/47\(5\)/18.pdf](https://www.pakbs.org/pjbot/PDFs/47(5)/18.pdf)
- Albdaiwi, R. N., Khyami-Horani, H., Ayad, J. Y., Alananbeh, K. M., & Al-Sayaydeh, R. (2019). Isolation and characterization of halotolerant plant growth promoting rhizobacteria from durum wheat (*Triticum turgidum* subsp. *durum*) cultivated in saline areas of the dead sea region. *Frontiers in Microbiology*, 10, 01639. <https://doi.org/10.3389/fmicb.2019.01639>
- Ansari, M., Shekari, F., Mohammadi, M. H., Biró, B., & Végvári, G. (2017). Improving germination indices of alfalfa cultivars under saline stress by inoculation with beneficial bacteria. *Seed Science and Technology*, 45(2), 475–484. <https://doi.org/10.15258/sst.2017.45.2.02>
- Arifin, I., Herawati, A., Mujiyo, S., & Widijanto, H. (2021). *Semar Sandy-app*: monitoring system for sandy soil irrigation based on android application. *IOP Conference Series: Earth and Environmental Science*, 637, 012002. <https://doi.org/10.1088/1755-1315/637/1/012002>
- Baghbani, A., Forghani, A. H., & Kadkhodaie, A. (2013). Study of salinity stress on germination and seedling growth in greenhouse cucumber cultivars. *Journal of Basic and Applied Scientific Research*, 3(3), 1137–1140. Retrieved from https://www.researchgate.net/profile/Amir-Hossein-Forghani/publication/280092542_Study_of_salinity_stress_on_germination_and_seedling_growth_in_greenhouse_cucumber_cultivars/links/57c5140f08aeb04914358439/Study-of-salinity-stress-on-germination-and-seedling-growth-in-greenhouse-cucumber-cultivars.pdf
- Berrada, I., Willems, A., De Vos, P., El fahime, E. M., Swings, J., Bendaou, N., ... Amar, M. (2012). Diversity of culturable moderately halophilic and halotolerant bacteria in a marsh and two salterns a protected ecosystem of Lower Loukkos (Morocco). *African Journal of Microbiology Research*, 6(10), 2419–2434. <https://doi.org/10.5897/AJMR11.1490>
- Chaihar, M., & Lumyong, S. (2011). Screening and optimization of indole-3-acetic acid production and phosphate solubilization from rhizobacteria aimed at improving plant growth. *Current Microbiology*, 62(1), 173–181. <https://doi.org/10.1007/s00284-010-9674-6>
- Dawwam, G. E., Elbeltagy, A., Emara, H. M., Abbas, I. H., & Hassan, M. M. (2013). Beneficial effect of plant growth promoting bacteria isolated from

- the roots of potato plant. *Annals of Agricultural Sciences*, 58(2), 195–201. <https://doi.org/10.1016/j.aas.2013.07.007>
- de Souza, R., Ambrosini, A., & Passaglia, L. M. P. (2015). Plant growth-promoting bacteria as inoculants in agricultural soils. *Genetics and Molecular Biology*, 38(4), 401–419. <https://doi.org/10.1590/S1415-475738420150053>
- Dogan, M., Tipirdamaz, R., & Demir, Y. (2010). Salt resistance of tomato species grown in sand culture. *Plant, Soil and Environment*, 56(11), 499–507. <https://doi.org/10.17221/24/2010-pse>
- Egamberdieva, D., Shurigin, V., Gopalakrishnan, S., & Sharma, R. (2014). Growth and symbiotic performance of chickpea (*Cicer arietinum*) cultivars under saline soil conditions. *Journal of Biological and Chemical Research*, 31(1), 333–341. Retrieved from <https://repo.mel.cgiar.org/handle/20.500.11766/5357>
- Egamberdieva, D., Wirth, S. J., Alqarawi, A. A., Abd Allah, E. F., & Hashem, A. (2017). Phytohormones and beneficial microbes: essential components for plants to balance stress and fitness. *Frontiers in Microbiology*, 8, 2104. <https://doi.org/10.3389/fmicb.2017.02104>
- Etesami, H., & Beattie, G. A. (2018). Mining halophytes for plant growth-promoting halotolerant bacteria to enhance the salinity tolerance of non-halophytic crops. *Frontiers in Microbiology*, 9, 148. <https://doi.org/10.3389/fmicb.2018.00148>
- Farace, G., Fernandez, O., Jacquens, L., Coutte, F., Krier, F., Jacques, P., ... Dorey, S. (2015). Cyclic lipopeptides from *Bacillus subtilis* activate distinct patterns of defence responses in grapevine. *Molecular Plant Pathology*, 16(2), 177–187. <https://doi.org/10.1111/mpp.12170>
- Ferreira, N. C., de Cassia Lima Mazzuchelli, R., Pacheco, A. C., de Araujo, F. F., Antunes, J. E. L., & de Araujo, A. S. F. (2018). *Bacillus subtilis* improves maize tolerance to salinity. *Ciencia Rural*, 48(8), 6–9. <https://doi.org/10.1590/0103-8478cr20170910>
- Gopalakrishnan, T., Hasan, Md K., Sanaul Haque, A. T. M., Jayasinghe, S. L., & Kumar, L. (2019). Sustainability of coastal agriculture under climate change. *Sustainability*, 11(24), 7200. <https://doi.org/10.3390/su11247200>
- Hahm, M.-S., Son, J.-S., Hwang, Y.-J., Kwon, D.-K., & Ghim, S.-Y. (2017). Alleviation of salt stress in pepper (*Capsicum annum* L.) plants by plant growth-promoting rhizobacteria. *Journal of Microbiology and Biotechnology*, 27(10), 1790–1797. <https://doi.org/10.4014/jmb.1609.09042>
- Hoffmann, J., Berni, R., Hausman, J.-F., & Guerriero, G. (2020). A review on the beneficial role of silicon against salinity in non-accumulator crops: Tomato as a model. *Biomolecules*, 10(9), 1284. <https://doi.org/10.3390/biom10091284>
- Hussain, S., Zhang, J.-H., Zhong, C., Zhu, L.-F., Cao, X.-C., Yu, S.-M., ... Jin, Q.-Y. (2017). Effects of salt stress on rice growth, development characteristics, and the regulating ways: A review. *Journal of Integrative Agriculture*, 16(11), 2357–2374. [https://doi.org/https://doi.org/10.1016/S2095-3119\(16\)61608-8](https://doi.org/https://doi.org/10.1016/S2095-3119(16)61608-8)
- Iqbal, M. A., Khalid, M., Zahir, Z. A., & Ahmad, R. (2016). Auxin producing plant growth promoting rhizobacteria improve growth, physiology and yield of maize under saline field conditions. *International Journal of Agriculture and Biology*, 18(1), 37–45. <https://doi.org/10.17957/IJAB/15.0059>
- Islam, F., Yasmeen, T., Arif, M. S., Ali, S., Ali, B., Hameed, S., & Zhou, W. (2016). Plant growth promoting bacteria confer salt tolerance in *Vigna radiata* by up-regulating antioxidant defense and biological soil fertility. *Plant Growth Regulation*, 80(1), 23–36. <https://doi.org/10.1007/s10725-015-0142-y>
- Jha, Y., & Subramanian, R. B. (2014). PGPR regulate caspase-like activity, programmed cell death, and antioxidant enzyme activity in paddy under salinity. *Physiology and Molecular Biology of Plants*, 20(2), 201–207. <https://doi.org/10.1007/s12298-014-0224-8>
- Jha, Y., Subramanian, R. B., & Patel, S. (2011). Combination of endophytic and rhizospheric plant growth promoting rhizobacteria in *Oryza sativa* shows higher accumulation of osmoprotectant against saline stress. *Acta Physiologiae Plantarum*, 33(3), 797–802. <https://doi.org/10.1007/s11738-010-0604-9>
- Ji, C., Tian, H., Wang, X., Song, X., Ju, R., Li, H., ... Liu, X. (2022). *Bacillus subtilis* HG-15, a halotolerant rhizoplane bacterium, promotes growth and salinity tolerance in wheat (*Triticum aestivum*). *BioMed Research International*, 2022, 9506227. <https://doi.org/10.1155/2022/9506227>
- Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*, 33(7), 1870–1874. <https://doi.org/10.1093/molbev/msw054>

- Mike-Anosike, E. E., Braide, W., & Adeleye, S. A. (2018). Studies on Indole Acetic Acid (IAA) production by rhizobacteria and growth promoting potentials. *International Journal of Advanced Research in Biological Sciences*, 5(2), 133–140. Retrieved from <https://ijarbs.com/pdfcopy/feb2018/ijarbs14.pdf>
- Mohite, B. (2013). Isolation and characterization of indole acetic acid (IAA) producing bacteria from rhizospheric soil and its effect on plant growth. *Journal of Soil Science and Plant Nutrition*, 13(3), 638–649. <https://doi.org/10.4067/S0718-95162013005000051>
- Munif, A., Hallmann, J., & Sikora, R. A. (2013). The influence of endophytic bacteria on *Meloidogyne incognita* infection and tomato plant growth. *Journal of the International Society for Southeast Asian Agricultural Sciences*, 19(2), 68–74. Retrieved from <http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.663.2040&rep=rep1&type=pdf>
- Nadeem, S. M., Ahmad, M., Naveed, M., Imran, M., Zahir, Z. A., & Crowley, D. E. (2016). Relationship between in vitro characterization and comparative efficacy of plant growth-promoting rhizobacteria for improving cucumber salt tolerance. *Archives of Microbiology*, 198(4), 379–387. <https://doi.org/10.1007/s00203-016-1197-5>
- Nosheen, S., Ajmal, I., & Song, Y. (2021). Microbes as biofertilizers, a potential approach for sustainable crop production. *Sustainability*, 13(4), 1868. <https://doi.org/10.3390/su13041868>
- Obeidat, M. (2017). Isolation and characterization of extremely halotolerant *Bacillus* species from Dead Sea black mud and determination of their antimicrobial and hydrolytic activities. *African Journal of Microbiology Research*, 11(32), 1303–1314. <https://doi.org/10.5897/AJMR2017.8608>
- Orhan, F. (2016). Alleviation of salt stress by halotolerant and halophilic plant growth-promoting bacteria in wheat (*Triticum aestivum*). *Brazilian Journal of Microbiology*, 47(3), 621–627. <https://doi.org/10.1016/j.bjm.2016.04.001>
- Ortiz, A., & Sansinenea, E. (2022). The role of beneficial microorganisms in soil quality and plant health. *Sustainability*, 14(9), 5358. <https://doi.org/10.3390/su14095358>
- Paul, D., & Lade, H. (2014). Plant-growth-promoting rhizobacteria to improve crop growth in saline soils: a review. *Agronomy for Sustainable Development*, 34(4), 737–752. <https://doi.org/10.1007/s13593-014-0233-6>
- Ramadoss, D., Lakkineni, V. K., Bose, P., Ali, S., & Annapurna, K. (2013). Mitigation of salt stress in wheat seedlings by halotolerant bacteria isolated from saline habitats. *SpringerPlus*, 2(1), 6. <https://doi.org/10.1186/2193-1801-2-6>
- Reddy, I. N. B. L., Kim, B.-K., Yoon, I.-S., Kim, K.-H., & Kwon, T.-R. (2017). Salt tolerance in rice: Focus on mechanisms and approaches. *Rice Science*, 24(3), 123–144. <https://doi.org/10.1016/j.rsci.2016.09.004>
- Ruginescu, R., Gomoiu, I., Popescu, O., Cojoc, R., Neagu, S., Lucaci, I., ... Enache, M. (2020). Bioprospecting for novel halophilic and halotolerant sources of hydrolytic enzymes in brackish, saline and hypersaline lakes of Romania. *Microorganisms*, 8(12), 1903. <https://doi.org/10.3390/microorganisms8121903>
- Saxena, A. K., Kumar, M., Chakdar, H., Anuroopa, N., & Bagyaraj, D. J. (2020). *Bacillus* species in soil as a natural resource for plant health and nutrition. *Journal of Applied Microbiology*, 128(6), 1583–1594. <https://doi.org/https://doi.org/10.1111/jam.14506>
- Schaad, N. W., Jones, J., & Chun, W. (2001). *Laboratory guide for identification of plant pathogenic bacteria*. St Paul, MN: American Phytopathological Society. Retrieved from <https://www.cabdirect.org/cabdirect/abstract/20013064240>
- Sedki, R., & El-Mohamedy, R. (2012). Biological control of *Pythium* root rot of broccoli plants under greenhouse conditions. *Journal of Agricultural Technology*, 8(3), 1017–1028. Retrieved from https://www.researchgate.net/publication/268203858_Biological_control_of_Pythium_root_rot_of_Broccoli_plants_under_greenhouse_condition
- Shilev, S. (2020). Plant-growth-promoting bacteria mitigating soil salinity stress in plants. *Applied Sciences*, 10(20), 7326; <https://doi.org/10.3390/app10207326>
- Shrivastava, P., & Kumar, R. (2015). Soil salinity: A serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation. *Saudi Journal of Biological Sciences*, 22(2), 123–131. <https://doi.org/10.1016/j.sjbs.2014.12.001>
- Shtereva, L. A., Vassilevska-Ivanova, R. D., & Karceva, T. V. (2015). Effect of salt stress on some sweet corn (*Zea mays* L. var. *saccharata*) genotypes. *Archives of Biological Sciences*, 67(3), 993–1000. <https://doi.org/10.2298/ABS150121062S>

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- Teo, H. M., Aziz, A., Wahizatul, A. A., Bhubalan, K., Siti, N. M. S., Muhamad, S. C. I., & Ng, L. C. (2022). Setting a plausible route for saline soil-based crop cultivations by application of beneficial halophyte-associated bacteria: A review. *Microorganisms*, 10(3), 657. <https://doi.org/10.3390/microorganisms10030657>
- Yañez-Yazlle, M. F., Romano-Armada, N., Acreche, M. M., Rajal, V. B., & Irazusta, V. P. (2021). Halotolerant bacteria isolated from extreme environments induce seed germination and growth of chia (*Salvia hispanica* L.) and quinoa (*Chenopodium quinoa* Willd.) under saline stress. *Ecotoxicology and Environmental Safety*, 218, 112273. <https://doi.org/10.1016/j.ecoenv.2021.112273>
- Zafar-ul-Hye, M., Farooq, H. M., Zahir, Z. A., Hussain, M., & Hussain, A. (2014). Application of ACC-deaminase containing rhizobacteria with fertilizer improves maize production under drought and salinity stress. *International Journal of Agriculture and Biology*, 16(3), 591–596. Retrieved from http://www.fspublishers.org/published_papers/37071_..pdf
- Zhou, N., Zhao, S., & Tian, C.-Y. (2017). Effect of halotolerant rhizobacteria isolated from halophytes on the growth of sugar beet (*Beta vulgaris* L.) under salt stress. *FEMS Microbiology Letters*, 364(11), fnx091. <https://doi.org/10.1093/femsle/fnx091>