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Original Article

Isolation and Characterization of *Bacillus* spp. Producing Indole-3-Acetic Acid (IAA) and Solubilizing Inorganic Phosphate from the Rhizosphere of Medicinal Sage (*Salvia officinalis*)

Murat Güler [1] ✉:

[1] Ministry of National Education, Kırsehir, Türkiye

[1] https://orcid.org/0000-0002-3074-6458
✉: volvox2015@gmail.com

ABSTRACT

The production of indole-3-acetic acid (IAA) is a key characteristic of plant growth-promoting bacteria (PGPR). This hormone, synthesized by PGPR in the rhizosphere, is responsible for the division, elongation, and differentiation of plant cells and plays a crucial role in various physiological mechanisms in plants. 21 bacterial isolates were obtained from the rhizosphere of medicinal sage (*Salvia officinalis* L.) in this study. This study aimed to characterize the indigenous bacterial community in the rhizosphere of *S. officinalis* and evaluate their potential as microbial fertilizers, focusing on their IAA production and phosphate solubilization capabilities. Among these isolates, 9 were identified as *Bacillus* sp. through morphological and biochemical tests as well as the MALDI-TOF MS method. Furthermore,9 isolates, 5 (BCM-3, BTM-1, BN-5, BCM-4, and BGM-13) demonstrated the ability to dissolve inorganic phosphate, while 6 (BCM-3, BCM-4, BSM-1, BSM-2, BTM-1, and BN-5) produced indole-3-acetic acid (IAA) in varying percentages. IAA production was assessed in nutrient broth (NB) medium supplemented with 0.2% L-tryptophan and measured at different incubation times. The results revealed that maximum IAA production by *Bacillus cereus* BCM-3 and BCM-4 was achieved after 3 days of incubation, with the highest production observed in BCM-3 (129.8 µg ml^{-1}). Furthermore, *B. cereus* BCM-3 and BCM-4 exhibited the highest inorganic phosphate solubilization performance among all tested *Bacillus* isolates. Our findings demonstrated that BCM-3 and BCM-4, isolated from the rhizosphere of medicinal sage, have significant potential for use as microbial fertilizer applications due to their high IAA production and inorganic phosphate solubilization abilities.

Key words: Medicinal sage, *Bacillus* spp., PGPR , microbial fertilizers

Tıbbi Adaçayı (*Salvia officinalis* L.) Rizosferinden İndol-3-Asetik Asit (IAA) Üreten ve İnorganik Fosfatı Çözen Bacillus Türlerinin İzolasyonu ve Karakterizasyonu

ÖZ

İndol-3-asetik asit (IAA) üretimi, bitki büyümesini teşvik eden bakterilerin (PGPR) temel bir özelliğidir. Rizosferde bitki gelişimini teşvik eden rizobakteri (PGPR) tarafından sentezlenen bu hormon, bitki hücrelerinin bölünmesinden, uzamasından ve farklılaşmasından sorumludur ve bitkilerde çeşitli fizyolojik mekanizmalarda önemli bir rol oynar. Bu çalışmada, tıbbi adaçayı (*Salvia officinalis* L.) rizosferinden 21 bakteri izolatı elde edilmiştir. Bu izolatlardan dokuz tanesinin morfolojik, biyokimyasal testler ve MALDI TOF MS yöntemi ile *Bacillus* sp. olduğu belirlenmiştir. Dokuz izolat arasından beş tanesinin (BCM-3, BTM-1, BN-5, BCM-4 ve BGM-13) inorganik fosfat çözdüğü, beş tanesinin (BCM-3, BCM-4, BSM-1, BSM-2, BTM-1 ve BN-5) farklı oranlarda IAA ürettiği belirlenmiştir. IAA üretimi, % 0,2 L-triptofan eklenmiş nutrient broth (NB) ortamında farklı inkübasyon sürelerinde gerçekleşmiştir. Sonuçlar, *Bacillus cereus* BCM-3 ve BCM-4'ün maksimum IAA üretiminin 3 günlük inkübasyon süresinde gerçekleştiğini ortaya koymuştur. En iyi IAA üretimi, 3 günlük inkübasyon süresinde *B. cereus* BCM-3'ten (129.8 µg ml^{-1}) elde edilmiştir. Ayrıca, *B. cereus* BCM-3 ve BCM-4 izolatlarının test edilen tüm Basillus izolatları arasında en iyi inorganik fosfat çözme performansını sergilemiştir. Bulgularımız, tıbbi adaçayı topraklarından izole edilen BCM-3 ve BCM-4 izolatlarının yüksek IAA üretimi ve inorganik fosfat çözme

yetenekleri nedeniyle mikrobiyal gübre uygulamaları için önemli bir potansiyele sahip olduğunu göstermiştir. Bu çalışma, *Salvia officinalis* (adaçayı) rizosferindeki yerel bakteriyel topluluğu karakterize etmeyi, IAA üretme ve inorganik fosfat çözme yetenekleri ile mikrobiyal gübre potansiyellerini değerlendirmeyi amaçlamıştır.

Anahtar kelimeler: Tıbbi adaçayı*, Bacillus* spp., PGPR , mikrobiyal gübre

INTRODUCTION

Auxins are a diverse group of carboxylic acid signaling molecules that regulate various physiological processes in plants. These are synthesized primarily in the aerial parts, such as the shoot apex, auxins are transported to the sub-aerial regions of the plant (Park et al., 2017). Indole-3-acetic acid (IAA), a key auxin, plays a crucial role in plant-microbe interactions and directly enhances plant growth. IAA produced by plant growth-promoting rhizobacteria (PGPR), including Bacillus species, is synthesized through five tryptophan-dependent metabolic pathways: indole-3-acetamide (IAM), indole-3-acetonitrile (IAN), tryptophan side-chain oxidase (TSO), tryptamine (TAM), and indole-3-pyruvate (IPyA) (Lin et al., 2015). Bacterial auxins, particularly indole-3-acetic acid (IAA), promote root growth by supporting the development of lateral roots, thereby increasing root surface area (Kong et al., 2020). This enhanced root architecture improves nutrient uptake, ultimately contributing to better plant growth and yield. Auxins also influence overall plant biology and activate additional mechanisms employed by plant growth-promoting bacteria (PGPB) to support plant growth and development. Members of the genus *Bacillus*, which have the ability to synthesize IAA and solubilize phosphate, constitute an important group of plant growth promoting rhizobacteria (PGPR), which are abundant in the rhizosphere (Etesami and Glick, 2024). Numerous studies have been reported on indigenous IAA-producing *Bacillus* strains (Lim and Kim, 2009; Prashanth and Mathivanan, 2010).

Medicinal plants such as sage have been used for thousands of years to provide nutrients, treat health disorders, and prevent diseases. Sage (*Salvia officinalis* L.-family Lamiacea) is an important medicinal and aromatic plant that grows in various parts of the world is widely used for its essential oils in the food, medicine and perfumery (Sharma et al., 2019). The harmful effects of chemical fertilizers on the environment and human health, coupled with excessive price increases, have necessitated the development of sustainable approaches in the cultivation of medicinal and aromatic plants such as sage. This highlights the importance of eco-friendly alternative fertilizers that can enhance plant growth (Marcelino et al., 2023). Currently, the application of plant growth-promoting rhizobacteria (PGPR), one of the environmentally friendly and sustainable agricultural approaches, holds significant potential for enhancing plant growth and nutrient uptake (Uçar et al., 2023). Anbi et al. (2020) stated that the application of plant growth-promoting rhizobacteria (PGPR) led to significant increases in plant height, leaf area, number of leaves, number of flowering shoots, chlorophyll a, and chlorophyll b contents in *Salvia officinalis*. Yolci et al. (2022) reported that the application of plant growth-promoting rhizobacteria (PGPR), including *Azospirillum lipoferum*, *Bacillus megaterium*, and *Frateuria aurentia*, mitigated the toxic effects of increasing boron doses in medicinal sage. Furthermore, PGPR treatments led to significant increases in total anthocyanin, total flavonoid, chlorophyll b, and total chlorophyll contents compared to the untreated control. Additionally, it has been reported that the application of indole-3-acetic acid (IAA)-producing and phosphate-solubilizing bacteria (*Pseudomonas fluorescens* RC512, *Bacillus megaterium* RC16, and *Bacillus subtilis* RC17), either individually or in combination, significantly increased plant height, seed weight, and number of branches in *Nigella damascena* (Akçura and Çakmakçı, 2023).

Although research on IAA-producing bacteria in the rhizosphere has increased recently, studies on the medicinal plant rhizosphere are still limited. The latest research underscores the importance of advancing the development of microbial formulations using local isolates that demonstrate activity across diverse rhizospheric ecosystems and plant species (Compant et al., 2019). Therefore, the development of microbial fertilizers that align with sustainable agricultural practices that contribute to environmental protection has the potential to substantially reduce the reliance on chemical fertilizers. This study aims to identify *Bacillus* spp. in the rhizosphere of the medicinal plant *S. officinalis*, widely used in medicine and pharmacy, and to determine their abilities to produce IAA and solubilize inorganic phosphate.

MATERIALS AND METHODS

Isolation and purification of rhizobacterial isolates

Rhizospheric soil samples were collected in May 2024 from medicinal sage in the Medicinal Plants Garden of the Department of Field Crops, Faculty of Agriculture (39°57'44.2"N, 32°51'36.7"E) Ankara University. The samples were taken from the plant rhizosphere at 3 different locations at a depth of 10 cm. Each soil sample was labeled and brought to the laboratory to study them under aseptic conditions. Rhizospheric bacteria were

isolated from 1 g of dried soil samples using the serial dilution technique. The soil samples were diluted in a series from 10^{-1} to 10^{-6}, and aliquots of each dilution were spread on to nutrient agar (NA) solidified Petri dishes. The plates were incubated at 28 °C, and distinct bacterial colonies were subsequently selected and subcultured many times until pure colonies were obtained (Figure 1).

Identification of rhizobacterial isolates

The physiological and biochemical characteristics of the bacterial isolates, along with Gram staining and endospore formation tests, were analyzed following the protocols described by Tsegaye et al. (2019). Rhizobacterial isolates, presumed to belong to the *Bacillus* genus and characterized as Gram-positive with spore-forming ability, were identified using the MALDI-TOF MS (Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry) technique. Microorganisms were identified based on their distinct molecular fingerprints using the MALDI Biotyper CA System. The protein profiles of microbial biomolecules, including proteins, peptides, sugars, and polymers, were ionized and subjected to an electric and/or magnetic field. The resulting spectral profiles were compared graphically to reference data in the system's database, allowing for precise identification at the genus and species level (Sivri and Öksüz, 2019). Figure 1 shows the flowchart of the process obtained from the isolation of *Bacillus* spp. from *S. officinalis* rhizospheric soil sample to determine their IAA-producing and phosphate-solubilizing properties.

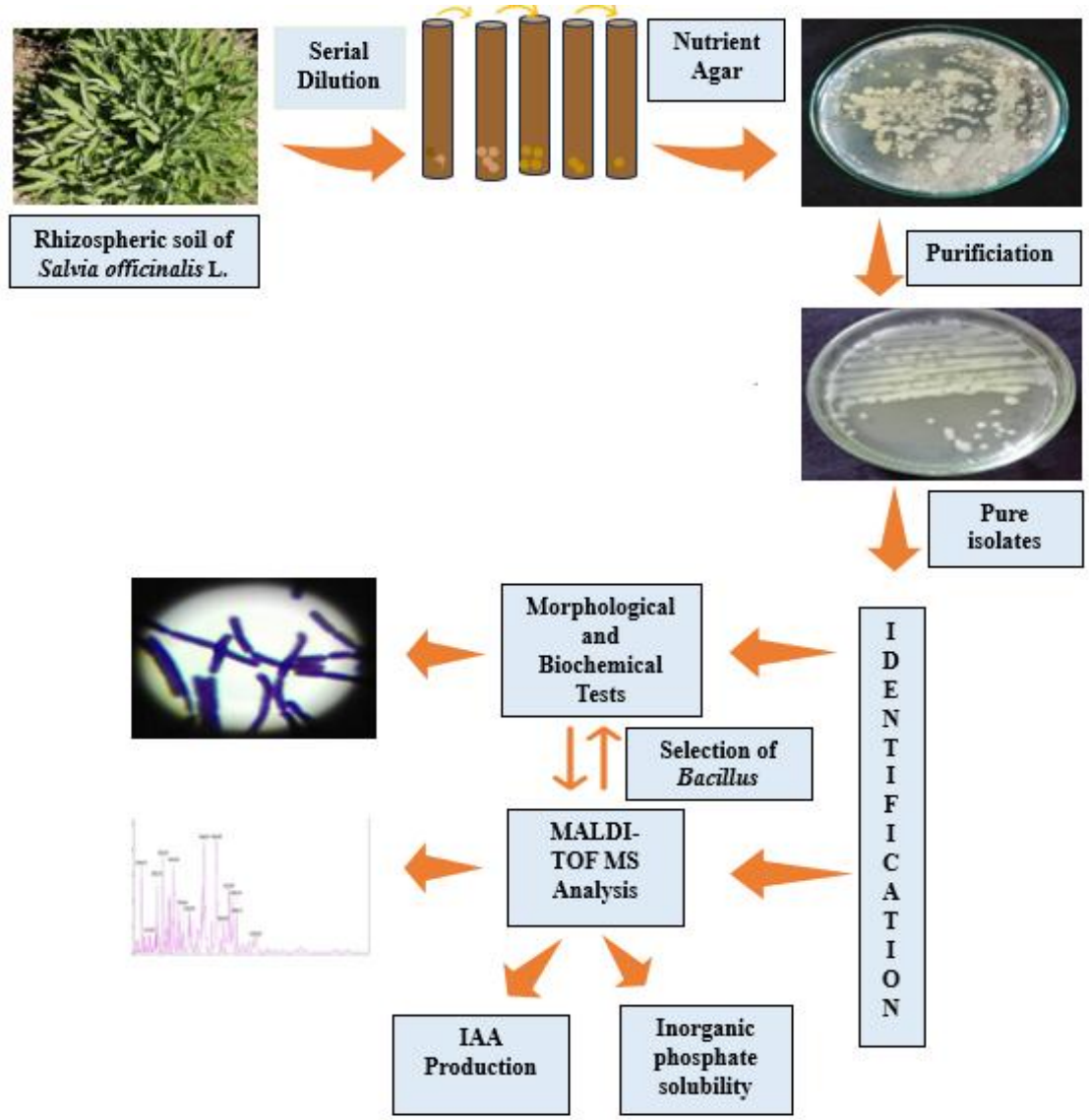

Figure 1. Process flowchart for identification and characterization of *Bacillus* spp. isolates from *S. officinalis* rhizospheric soil samples

Determination of inorganic phosphate dissolving isolates

The bacterial isolates were tested for their ability to solubilize phosphate according to the methods of Pikovskaya (1948) using Pikovskaya agar (PKA) medium (0.2 g L^{-1} NaCl, 10 g L^{-1} glucose, 0.2 g L^{-1} KCl, 5 g L^{-1} $Ca_3(PO_4)_2$, 0.5 g L^{-1} $(NH_4)_2SO_4$, 0.1 g L^{-1} $MgSO_4 \cdot 7H_2O$, 0.002 g L^{-1} $FeSO_4 \cdot 7H_2O$, 0.5 g L^{-1} yeast extract, 0.002 g L^{-1} $MnSO_4 \cdot H_2O$, and 1000 ml distilled water). The plates were inoculated with bacteria and incubated at 28±2 °C for 7 days. Colony, forming a clear halo zone around them, indicating phosphate solubilization. The experiments were performed in triplicate. The Phosphate solubilization index (PSI) was determined using measurements taken after seven days of growth from a point inoculation on a PKA medium at 28±2 °C (Meena et al., 2015).

Solubilization index = (Colony diameter + Halo zone diameter)/Colony diameter

Determination of Indole-3-Acetic Acid (IAA) producing isolates

The ability of the isolates to produce Indole-3-Acetic Acid (IAA) was evaluated following the protocol by Sarwar and Kremer (1995). The production of IAA was assessed in cultures grown in nutrient broth (NB) medium supplemented with 0.2 % L-tryptophan, incubated at 30 °C with shaking at 200 rpm for 4 days. One milliliter of culture was sampled every 24 hours and stored at -20 °C for analysis. Following the method outlined by Gang et al. (2019), 100 µL of Salkowaski reagent ($FeCl_3$, 0.5 M; $HClO_4$, 35%) was combined with an equal volume (100 µL) of the culture supernatant (obtained by centrifuging at 10,000 rpm for 5 min) in a 96-well microplate. The mixture was incubated in the dark at room temperature for 30 minutes. After the color developed, the absorbance of the mixture was measured spectrophotometrically (SHIMADZU UV mini-1240 Spectrophotometer) at 530 nm using both a standard curve created from known IAA concentrations and an uninoculated NB medium as controls.

Statistical analysis

Phosphate solubilization index (SI) and IAA production data were analyzed in triplicate using JMP Pro 17.0 statistical software. Dependent variables with a normal distribution were presented as mean ± standard deviation (SD) (Genç and Soysal, 2018).

RESULTS AND DISCUSSION

Evaluation of morphological and biochemical properties of isolates

A total number of 21 isolates were obtained from the rhizospheric soil samples of *S. officinalis*. After screening, 9 isolates were tentatively identified as *Bacillus* sp. based on standard methods, including Gram staining (positive), catalase test (positive), endospore formation (positive), as outlined in Bergey's Manual of Systematic Bacteriology (Krieg and Holt, 1984). Thereafter, isolates were confirmed as *Bacillus* sp. using the MALDI-TOF MS method. According to the MALDI-TOF MS analysis results, this genus included *B. simplex* (BSM-1 and BSM-2), *B. cereus* (BCM-3 and BCM-4), *B. thuringiensis* (BTM-1), *B. niacini* (BN-5), *Bacillus* spp. (BLM-7), *B. mycoides* (BMM-9), *B. megaterium* (BGM-13). While the catalase test of all isolates was positive, the oxidase test was negative. Moreover, all isolates were capable of sporulation. Additionally, all other isolates tested positive for motility. The morphological and biochemical results of the isolates are presented in Table 1.

Table 1. Morphological and biochemical properties of *Bacillus* spp. isolated from the rhizosphere of *S. officinalis*

Strain no	Gram reaction	Shape	Colony color	Endospore formation	Catalase test	Oxidase test	Motility	MALDI TOF MS result	NCBI Number
BSM-1	+	Rod	white	+	+	-	+	*B. simplex*	1478
BCM-3	+	Rod	white	+	+	-	+	*B. cereus*	1396
BTM-1	+	Rod	white	+	+	-	+	*B. thuringiensis*	1428
BN-5	+	Rod	cream	+	+	-	+	*B. niacini*	86668
BSM-2	+	Rod	white	+	+	-	+	*B. simplex*	1478
BLM-7	+	Rod	white	+	+	Not Detected	+	*Bacillus spp.*	1386
BCM-4	+	Rod	white	+	+	-	+	*B. cereus*	1396
BMM-9	+	Rod	white	+	+	-	+	*B. mycoides*	1405
BGM-13	+	Rod	white	+	+	-	+	*B. megaterium*	1404

Note: +, positive; –, negative *

The MALDI-TOF MS identification method relies on detecting intracellular molecules, particularly ribosomal proteins, to classify microorganisms at the genus, species, and strain levels. This technique is recognized as one of the most reliable tools for identifying microbial species across disciplines like agriculture, food, and medicine, owing to its high accuracy and rapid results (Solntceva et al., 2021). Çelikten and Bozkurt (2018) employed the MALDI-TOF method to identify 120 bacterial isolates obtained from the wheat rhizosphere in their search on plant growth-promoting bacteria. Similarly, Öksel et al. (2022) utilized MALDI-TOF MS to identify bacterial species from wheat rhizospheres. Ünlü et al. (2023) used the MALDI-TOF MS technique to identify bacterial strains isolated from the alfalfa rhizosphere, classifying them into genera like *Bacillus*, *Pseudomonas*, *Stenotrophomonas*, *Lysinibacillus*, *Acinetobacter*, and *Enterobacter* in another study. Similarly, Güler, (2024) used the MALDI-TOF MS method to identify phosphate solubilizing bacteria in the rhizosphere of *Thymus vulgaris.*

Evaluation of inorganic phosphate-solubilizing and IAA-producing *Bacillus*

The results of present study revealed that out of all 9 isolated *Bacillus* genera, 5 *Bacillus* species gave a halo zone which means their ability of phosphate solubilizing . In the current study*, B. cereus* BCM-3*, B. thuringiensis* BTM-1, *B. niacini* BN-5*, B. cereus* BCM-4, *and B. megaterium* BGM-13 could dissolve inorganic phosphate (Figure 2B). P solubilization index of bacterial isolates was defined in between 3.94 to 6.58 on PKA (Pikovskaya medium) agar. According to the Phosphate solubilization index of bacterial isolates in PKA agar medium, the maximum phosphate solubilization index (PSI) value was obtained from *B. cereus* BCM-3 . This was followed by *B. cereus* BCM-4 and *B. megaterium* BGM-13 with PSI value of with PSI value of 6.35 and 5.21 respectively. The phosphate solubilization index (SI) of the isolates are shown in Figure 2A.

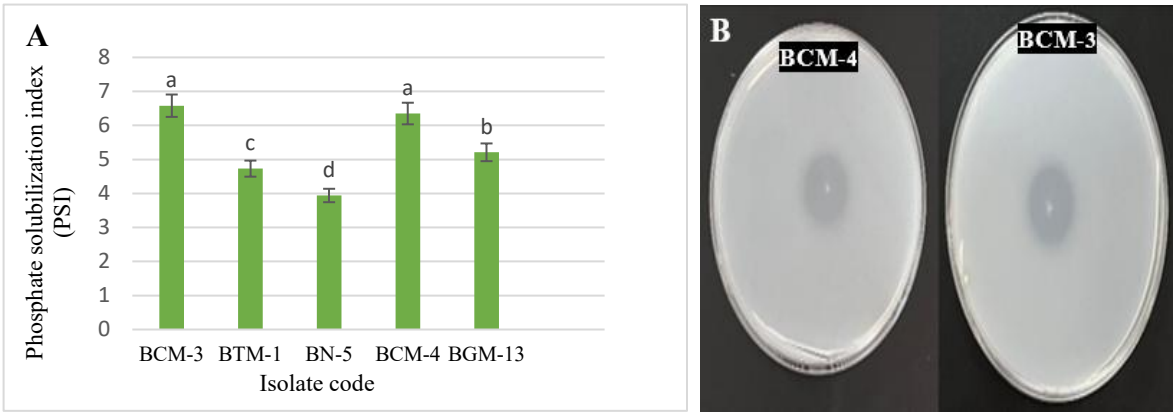

Figure 2. (A) Phosphate solubilization index (PSI) of five *Bacillus* sp. capable of dissolving inorganic phosphate in PKA medium over a 7-days period. (B) Phosphate-solubilizing bacteria formed transparent zones on PKA agar medium. All values are presented as the mean of three replicates. Bars labeled with different letters indicate statistically significant differences (p> 0.05). Error bars denote the standard deviation (SD) of the means.

Deficiency of phosphorus, is very crucial after nitrogen, and limit plant growth. Numerous microorganisms, including bacteria, fungi, actinomycetes, and algae, demonstrate the capacity to solubilize and mineralize phosphorus. Plant growth-promoting rhizobacteria (PGPR) facilitate enhanced phosphate uptake by plants by converting it from insoluble to soluble forms (Fahsi et al. 2021). Massucato et al. (2022) has mentioned that these bacteria provide phosphorus to plants through different mechanisms, with some relying on enzymatic processes (e.g., phytases and/or phosphatases) and others facilitating phosphorus availability via the extrusion of H^+ ions and the release of organic acids derived from microbial metabolisms. According to Rawat et al. (2021), the most prevalent phosphate-solubilizing *Bacillus* spp., *Enterobacter* spp., and *Pseudomonas* spp. available in the rhizosphere. Moreover, earlier studies have revealed *Bacillus* spp. as an effective phosphate solubilizer (Saeid et al., 2018; Gomez-Ramirez and Uribe-Velez, 2021). Abbas et al. (2019) has indicated that rhizospheric *Bacillus cereus* isolated from Iraqi soil dissolved phosphate by forming halo zones at high levels (100%). Kumar et al. (2020) determined that *B. cereus* LPR2 isolated from spinach rhizosphere is a very effective phosphate solubilizing bacteria, forming a large halo region (20 mm) in PKA medium. Soares et al. (2023) investigated phosphate solubilization index at different pH levels and determined that the rhizospheric *B. cereus* UFT42 exhibited a phosphate solubilization index (PSI) exceeding 3 across all tested pH ranges (7.0, 6.0, and 5.5). The variation in PSI may be linked to differences in the population of rhizospheric bacteria across diverse ecological

conditions, which could be influenced by various soil factors such as nutrient status, acidity, moisture content, organic matter, and soil enzyme activity. In the current study, the phosphate solubilization index of *B. cereus* BCM-3 and BCM-4 was determined as 6.58 and 6.35, respectively. Similar findings for *B. cereus* were reported by Arif et al. (2017) and Zhou et al. (2021).

Previous research has revealed that rhizospheric soil hosts a significantly larger population of phosphate-solubilizing bacteria when compared to non-rhizospheric soil (Linu et al., 2019; Ibáñez et al., 2021). Qureshi et al. (2012) has reported that *Bacillus megaterium* isolated from cotton rhizosphere had a solubilization Index of 3.8 using PKA medium. Fahsi et al. (2021) determined that the Inorganic P-solubilizing activity of *B. megaterium* J11 isolated from jujube rhizosphere in National Botanical Research Institute's (NBRIP) liquid medium was very low (20.5 mg L^{-1}) . Djuuna et al. (2022) reported that phosphate-solubilizing bacteria (PSB) comprise 50 % of the total microbial community in soil. Lin et al. (2023) reported that more than half of the *Bacillus* isolates obtained from soybean rhizosphere indicated phosphate-solubilizing activity. Among these isolates, *B. megaterium* P68 was found to solubilize phosphate at a rate of 461.86 mg L^{-1} after 7 days of incubation in NBRIP medium. On the other hand, the phosphate solubilization index of *B. megaterium* BGM-13 was determined as 5.21. Similar findings were noted for *B. megaterium* by Zhang et al. (2019) and Kang et al. (2021). Many studies have shown that rhizospheric *Bacillus thuringiensis* is an effective phosphate solubilizer (Delfim et al., 2018; Liu et al., 2021). AL Kahtani et al. (2020) documented that *Bacillus thuringiensis* exhibited phosphate solubilization efficiencies (PSE) ranging from 7.6 to 9.6 among 13 isolates derived from the rhizosphere of the medicinal plants *Fagonia mollis* and *Achillea fragrantissima*. Gaikwad et al. (2021) determined the phosphate solubilizing efficiency of soil-based *B. thuringiensis* in PKA medium as 5.6. The phosphate solubilization index of *B. thuringiensis* MMS-3 was determined as 3.1 in the present study.

IAA is a plant hormone that regulate root growth by promoting the division and elongation of root cells with root cap cuticles which protects the root meristem, induction of longer roots having more lateral hairy roots, which play a vital role in absorbing nutrients by the plants (Lau et al., 2020). The tested strains produced different levels of IAA depending on their growth in the NB medium supplemented with L-tryptophan. According to the results of the current study, 6 out of 9 *Bacillus* sp. were able to produce IAA at different rates (Figure 3). According to spectrophotometric analysis, the highest IAA production was indicated after 3 days using *B. cereus* BCM-3 with phosphate solubilization efficiency (PSE) of 129.8 µg ml^{-1}. This was followed by *B. cereus* BCM-4 with PSE of 114.5 µg ml^{-1} and *B. simplex* BSM-1 with PSE of 93.1 µg ml^{-1}, respectively. The lowest IAA production was determined in *B. thuringiensis* BTM-1 with PSE of 13.9 µg ml^{-1} on day 1. In comparison, *B. niacini* BN-5 and *B. simplex* BSM-2 yielded PSE of 18.1 µg ml^{-1} and 27.3 µg ml^{-1} of produced IAA, respectively, during the same incubation period. Figure 3 show experimental data for OD 530 nm and its equivalence in IAA (µg ml^{-1}) measured during different incubation periods.

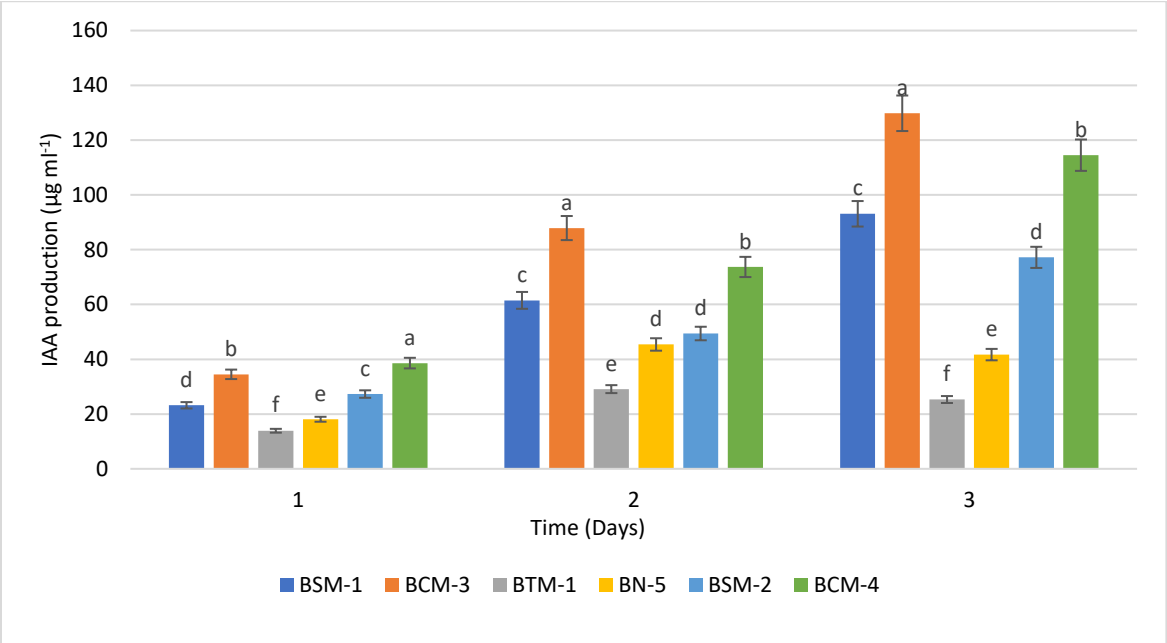

Figure 3. IAA production by the six *Bacillus* sp. cultured in nutrient broth supplemented with 0.2% L-tryptophan over a period of three days. All values are presented as the mean of three replicates. Bars labeled with different

letters indicate statistically significant differences (p> 0.05). Error bars denote the standard deviation (SD) of the means.

Several researchers have reported *Bacillus* spp. species from various plant roots to be good IAA producers (Miljaković et al., 2020; Ali et al., 2023). Sindhu et al. (2017) determined that *B. subtilis*, *B. flexus*, *B. cereus*, *B. megaterium*, and *B. endophyticus* produced IAA ranging from 4.0 to 24.3 µg ml^{-1}. Wagi and Ahmed (2019) determined that the rhizospheric isolate *B. cereus* (So3II) produced very high IAA (241.6 mg ml^{-1}) after 24 h of incubation. Hyder et al. (2020) reported that *B. cereus* KSL-24 isolated from chili pepper produced 56.2 µg ml^{-1} of IAA, while *B. cereus* KSL-8T produced 29.7 µg ml^{-1} of IAA. According to Widawati (2020), bacterial IAA production may not occur in the absence of L-tryptophan. In a recent study, Pakar et al. (2024) reported that rhizospheric *B. cereus* strain PM38 synthesized Indole 3-acetic acid (IAA) at a very high rate (166 µg ml^{-1}). In the current study, *B. cereus* BCM-3 and *B. cereus* BCM-4 isolates produced 129.8 and 114.5 µg ml^{-1} IAA after 3 days, respectively. The findings of our study are similar to Saboor et al. (2024), who determined IAA produced by *B. cereus* isolated from the *Vigna radiata* root.

Similar to other *Bacillus* species, *Bacillus simplex* has been indicated as an efficient producer of IAA. For instance, Cochard et al. (2022) reported that endophytic *B. simplex* B26 and B33 isolated from *Solanum lycopersicum* root produced 35.3 and 61 µg m^{-1} IAA, respectively, after 48 h. Çakmakçı et al. (2023) determined that among fifteen bacterial strains isolated from various acidic rhizospheric soils, *B. simplex* RC236 and *B. simplex* TE142 produced 41.3 and 63.8 µg m^{-1} IAA, respectively. In a recent study, Thakur et al. (2024) determined that *B. simplex* isolated from the rhizosphere of *Crocus sativus* produced 74.8 µg ml^{-1} IAA. Similarly, *B. simplex* BSM-1 and *B. simplex* BSM-2 isolates produced 93.1 and 77.2 µg ml^{-1} IAA after 3 days, respectively . Although there are studies on the plant growth promoting properties of *Bacillus niacini*, studies on its IAA production are limited. Cedeño-García et al. (2018) reported that *B. niacini* GN8 isolated from *Medicago sativa* rhizosphere produced IAA at low value (14.03 µg ml^{-1}). In a similar study, Alemneh et al. (2021) determined that rhizospheric *B. niacini* with phosphate solubilizing ability produced low value (<10 µg ml^{-1}) IAA. On the other hand, Abdullahi et al. (2022) reported that rhizospheric *B. niacini* SA3 produced high value (96.56 µg ml^{-1}) of IAA. *B. niacini* BN-5 produced 41.7 µg ml^{-1} IAA after 3 days in the current study. Our findings are consistent with the results of Widawati et al. (2024), who observed IAA producing *B. niacini* from *Santalum album*. There are many studies reporting that *Bacillus thuringiensis*, is particularly well known for its insecticidal activities. It also supports plant growth by producing IAA. For instance, Raheem et al. (2018) determined that *B. thuringiensis* D-2, isolated from the rhizosphere of drought-tolerant *Acacia arabica*, produced 5.6 µg ml^{-1} of IAA, as confirmed by HPLC analysis. Batista et al. (2021), investigated the effect of tryptophan (Trp) concentration on *B. thuringiensis* RZ2MS9 IAA production, determined that the application of 1 g L^{-1} Trp to the medium increased IAA secretion by the bacteria 5-fold. Figueredo et al. (2023) reported that the rhizospheric strain *B. thuringiensis* RZ2MS9 synthesized 4.59 µg ml^{-1} of IAA in a tryptophan-free medium, while the addition of 5 mM tryptophan to the medium increased IAA production to 7.67 µg ml^{-1}. Their findings are consistent with this study, in which 0.2 % tryptophan was added to the growth medium. *B. thuringiensis* BTM-1 produced 41.7 µg ml^{-1} IAA after 3 days in the current study. The results are in line with Esertaş et al. (2024), who reported that *B. thuringiensis* 5O9 isolated from the roots of *Phalaenopsis* spp. produced 11.66 µg ml^{-1} IAA.

CONCLUSION

Plant growth-promoting bacteria are biofertilizers that enhance plant growth, improve resistance to diseases, and increase soil fertility. Their use in agriculture offers an environmentally friendly and sustainable alternative by reducing the need for chemical fertilizers. This is first report about a bacterium isolated from *S. officinalis* rhizosphere in Turkiye. Studies on IAA-producing *Bacillus* species isolated from the rhizosphere of the medicinal plants are limited. *Bacillus cereus* BCM-3 and BCM-4 isolated from the rhizosphere of medicinal sage have significant potential as plant growth-promoting (PGP) agents in terms of their IAA production and inorganic phosphate solubilization abilities. To achieve a broader ecological assessment, it is necessary to compare isolates from different plant rhizospheres and investigate a wider range of bacterial species. Further research is needed to elucidate the molecular and functional characteristics of these two isolates in detail and to support their effective use in field conditions.

Declaration of interests

The author declares that there is no conflict of interest.

Author Contributions

Murat Güler: Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; software; writing— original draft; writing—review and editing.

ORCID

Murat GÜLER https://orcid.org/0000-0002-3074-6458

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