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RECEIVED 31 January 2024

ACCEPTED 08 May 2024

PUBLISHED 28 May 2024

CITATION

Afridi M, Sadaaiappan B, Nassar AS,
Mahmoudi H, Khan MA and Mundra S (2024)
Mangrove sediments-associated bacterium
(*Bacillus* sp. SW7) with multiple plant growth-
promoting traits promotes the growth of
tomato (*Solanum Lycopersicum*).
Front. Mar. Sci. 11:1379439.
doi: 10.3389/fmars.2024.1379439

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Mangrove sediments-associated bacterium (*Bacillus* sp. SW7) with multiple plant growth-promoting traits promotes the growth of tomato (*Solanum Lycopersicum*)

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Global food production intensification presents a major hurdle to ensuring food security amidst a growing world population. Widespread use of chemical fertilizers in recent decades has risked soil fertility, compounded by the challenges posed by climate change, particularly in arid regions. To address these issues, adopting plant growth-promoting (PGP) bacteria stands out as a promising solution, offering multifaceted benefits to arid agroecosystems. We isolated a bacterial strain, SW7, from mangrove sediment, characterised the entire genome followed by phylogenetic analyses, and evaluated its *in-vitro* PGP activity. Subsequently, we examined its impact on tomato seed germination and plant growth. The strain SW7 exhibited growth on 11% NaCl, survival at 50°C, and possessed multiple PGP traits such as significant increase in seed germination rate (60.60 ± 38.85%), phosphate (83.3 g L⁻¹) and potassium (39.6 g L⁻¹) solubilization and produced indole acetic acid (3.60 ppm). Additionally, strain SW7 tested positive for ammonia, catalase, and oxidase enzyme production. The strain SW7 genome consists of 5.1 MB with 35.18% G +C content. Through genome-based phylogenetic and orthoANI analyses, the strain was identified as a novel *Bacillus* species, designated herein as *Bacillus* sp. SW7. In an eight-week shade-house experiment, inoculation of strain SW7 improved, leaf number, leaf density, leaf area index and mass water of tomatoes. Additional parameters, like chlorophyll a, chlorophyll b and carotenoids were not affected in SW7-inoculated tomatoes. In conclusion, *Bacillus* sp. SW7 exhibits multiple PGP traits and an adaptive capacity to high temperature and salinity, positioning it as a potential candidate for elevating the productivity of arid agroecosystems.

KEYWORDS

arid agroecosystem, *Bacillus* species, mangrove, plant growth-promoting bacteria, whole genome sequencing

1 Introduction

Global food production intensification is a major challenge in achieving food security (Devaux et al., 2020) amid a growing world population (Arndt et al., 2023). The increase in human population creates a hurdle in achieving food safety, this has led to use of large quantities of chemical fertilizer for the past decades, despite its harmful effects on the environment and humans (Yadav et al., 2018). Also, the climate changes escalate the situation, due to rise in temperature the percentage of arid land is increasing. The semi- to hyper-arid region of the world faces several issues that will possibly continue to endanger food security in the upcoming years (Ammar et al., 2023). To meet the growing demand for sustainable agriculture in arid regions, enhancing food security becomes attainable through the efficient utilization of limited water resources to boost domestic food production. However, intensive cropping needs sufficient water supply which is not adequate in arid regions (Goddek et al., 2023). At the moment, most of the countries in the arid regions are facing water shortages because their water supply is below the water poverty line of 1000 m³/capita/year, compared with the world average of 7240 m³/capita/year (Hejazi et al., 2023). Furthermore, cultivating crops in arid regions requires extensive usage of synthetic fertilizers to enhance productivity, which ultimately deteriorates the ecosystem (Liu et al., 2020). As a result, innovative alternate approaches are required in such regions to concurrently increase food production, water sustainability and reduce ecosystem deterioration (Abdullah et al., 2022).

Promoting sustainable agriculture involves gradually reducing reliance on chemical fertilizers and embracing bio-waste-derived substances, alongside leveraging the potential of microorganisms (Basu et al., 2021). Biofertilizers, microbial-based products, and alternatives like organic extracts and vermicompost teas have emerged to counteract the negative impacts of toxic agrochemicals (Yatoo et al., 2021). Among these microbial solutions, known for enhancing plant growth, disease control, mitigating plant stress and environmental friendliness, offer a viable path to improve soil fertility and mitigate agrochemical harm (Nosheen et al., 2021). Utilizing microbial formulations as fertilizers in agriculture holds promise for enhancing soil fertility while reducing the detrimental effects of agrochemicals (Sumbul et al., 2020). Employing effective PGP bacteria as biofertilizers and biological control agents presents a suitable alternative to lessen reliance on chemical fertilizers in crop production (Zainab et al., 2021).

In the Middle-Eastern and North African (MENA) region, PGP bacteria has a vital role in enhancing plant growth and crop yields by reducing the direct and indirect effects of biotic and abiotic stresses like high temperature, and salinity (Abdelaal et al., 2021; Reang et al., 2022). PGP bacteria mitigate the salinity damage in several crops through various mechanisms, including improved water uptake and osmolyte accumulation (glutamate, glycine betaine, proline and soluble sugars). Also, PGP bacteria increases antioxidant levels, synthesis of ACC deaminase, exopolysaccharides production and ionic homeostasis, which in turn enhances agricultural productivity even in saline-alkaline soil (Mishra et al., 2023). Currently, studies in various parts of the world are reporting improvements in the growth

and production of crops such as tomatoes (Fan et al., 2016; Astorga-Eló et al., 2021), maize (Ojuederie and Babalola, 2023), and wheat (Shankar and Prasad, 2023), attribute to the use of PGP bacteria. The utilization of PGP bacteria as inoculants has substantial potential for application in farming, and lowering the rate of chemical fertilizers (Bittencourt et al., 2023). PGP bacteria have drawn more interest recently, due to their potential usage as a biofertilizer to improve crop development and production. However, there is an increasing need to find novel PGP bacteria that adapt and grow in extreme conditions with new phyto-beneficial traits (Lutfullin et al., 2022).

The mangroves are true extremophiles, which evolved to grow in extreme ecological conditions such as arid and hot intertidal environments (Friis et al., 2023). The mangrove tree is known to tolerate high salinity and plays an important role in the carbon sequestration, nutrient cycle, conservation and production of coastal ecosystems (Osland et al., 2018; Costa et al., 2022). Microbes associated with the mangrove are known to tolerate high salinity. Several studies have demonstrated that bacterial isolates from mangrove sediments and rhizosphere have PGP potentials, make them potential sources for isolating PGP strains (Mishra et al., 2023).

This study aimed to: (i) isolate bacteria from mangrove sediment collected from Khor Al Yeefrac, Umm Al Quwain, UAE; (ii) assess their PGP traits in *in-vitro* conditions; (iii) characterize the whole genome of strain SW7 to identify taxonomy, phylogeny, and gene clusters responsible for PGP traits in extreme conditions; and (iv) evaluate the effect of strain SW7 on seed germination, as well as the growth and productivity of tomatoes (*Solanum Lycopersicum*) in a shade-house field trial condition.

2 Materials and methods

2.1 Study site and sampling collection

The mangrove forest in Umm Al Quwain extends approximately 12 km along the coast with a homogeneous mangrove species *Avicennia marina*. The sediment samples were collected from Khor Al Yeefrac (25°32'07.8" N, 55°38'04.2" E) in the Umm Al Quwain mangrove forest (east coast, UAE). The sediment samples were promptly transported to the lab and processed for bacterial screening within 24 hr under cooling conditions. Various physicochemical properties of mangrove sediment samples were analysed and reported in Tounsi-Hammami et al., 2024.

2.2 Bacterial isolation, identification, NaCl and heat stress analyses

Out of total 41 bacterial isolates, obtained from mangrove sediments, which exhibited multiple PGP traits (Tounsi-Hammami et al., 2024), we selected one promising bacterial strain SW7 for further testing characterisation and *in-vitro* assays. Additionally, we subjected the strain to salt tolerance assays

(NaCl 2–11% w/v) and heat stress tests following the method described by Mahdi et al. (2020).

2.3 *In vitro* assessment of multiple PGP traits

2.3.1 Phosphate and potassium solubilization

Phosphate solubilization activity was assessed by spot-inoculating pure colonies on PVK agar medium and incubating them for 3 days at 30°C. The Phosphate Solubilization Index (PSI) was calculated as described by Teymouri et al. (2016). For potassium solubilization, the Aleksandrov medium (5.0 g/L glucose, 0.5 g/L MgSO₄ · 7H₂O, 0.1 g/L CaCO₃, 0.006 g/L FeCl₃, 2.0 g/L Na₂ HPO₄, and feldspath 2.0 g/L) was used as the sole K source (Rana et al., 2011). After 24 hr, the colony was taken with a sterile plastic pipette, inoculated on solid Aleksandrov medium, and incubated at room temperature for 5 days. The formation of a clear zone around the growing colony indicated potassium solubilizing ability, and the solubilization index was calculated using the formula by Sadiq et al. (2013).

2.3.2 Ammonia and indole acetic acid production

Ammonia production was determined by growing strain in peptone water at 30°C for 4 days. Nessler's reagent was used to assess ammonia production, with a faint yellow colour indicating a small quantity and a deep yellow to brownish colour signifying maximum ammonia production (Sharma et al., 2021). Indole acetic acid (IAA) production was assessed by inoculating strain into Jensen's broth containing 2 mg mL⁻¹ tryptophan. The culture was incubated at 28°C for 48 hr with continuous shaking at 125 rpm. After centrifugation, a 1 mL aliquot of the supernatant was mixed with Salkowski's reagent and incubated at room temperature for 20 minutes in darkness, as described by Islam et al. (2016). The concentration of IAA was determined by measuring the absorbance at 530 nm and using a standard curve from pure IAA solutions.

2.3.3 Hydrogen cyanide production

Hydrogen cyanide (HCN) production was performed using a nutrient agar medium with 0.44% glycine. Filter paper strips were dipped in 2% sodium carbonate solution and 0.5% picric acid and stored at 30°C for 72 hr. Colour change from orange to red to brown on the filter paper indicated the level of HCN production by the isolate, and quantitative analysis was done by examining by dipping the altered filter paper strips in double distilled water and observing at 625 nm, following Lotfi et al. (2022).

2.3.4 Siderophore assay

Siderophore assay was conducted using Chrome azurol S agar medium as described by Petrillo et al. (2021). The presence of a yellow-orange halo surrounding bacterial growth indicated siderophore production. The siderophore production index (SPI) was calculated using the formula.

$$\text{SPI} = (\text{colored zone} + \text{colony}) / \text{colony diameters} - (1)$$

2.3.5 Oxidase and catalase enzyme

Oxidase and catalase enzyme activities were assessed using Kovacks's reagent and a 6% H₂O₂ solution, respectively. Positive results for oxidase were indicated by a lavender colour, while catalase activity was confirmed by the formation of air bubbles during the test (Prajakta et al., 2019).

2.4 Seed preparation, surface coating, *in-vitro/in-vivo* germination assay under shade-house conditions

We collected seeds of tomato (*Solanum Lycopersicum*, F1 hybrid; 96 seeds) and coated them following the method described earlier (Mahdi et al., 2020). In brief, we surface sterilized seeds with 5% sodium hypochlorite solution for 1 min, shaken in 70% ethanol for 1 min and washed 5 times in sterilized distilled water followed by air-drying under a laminar hood. The culture pellets (OD₆₀₀ nm = 0.8) were taken from fresh cultures via centrifugation at 10,000 rpm for 5 min. The bacterial pellets were resuspended in 10 mL of sterile distilled water, vortexed for 10 s, and used for seed treatment. The culture suspensions were used as seed dry-out with a ratio of 10 mL per (120, 96, 56) seeds for 1 hr. Next, seeds were air-dried and placed on petri plates filled with sterile filter paper. Thirty seeds were kept on each plate. The triplicates of each treatment were sustained, and seeds treated with sterile distilled water were used as a negative control. The petri plates were kept at 25°C in the dark and germination rates were observed after the 14 days of post-incubation. The total length and fresh and dry weight were measured. The germination rate and vigor index were calculated using Equations 1, 2 (Islam et al., 2016):

$$\text{Germination rate (\%)} = (\text{Number of germinated seeds} / \text{total seeds}) \times 100 \quad (1)$$

$$\text{Vigor index} = \text{Germination (\%)} \times \text{Total seedling length (cm)} \quad (2)$$

2.5 Preparation of coated tomato seeds for greenhouse assay

The tomato seeds were first put through the float test to determine their viability (Degwale et al., 2022). Seeds were placed in sterilized water and observed for their floating characteristics. Floating seeds were discarded as it may indicate their inability to germinate, however, seeds that sunk were considered viable and continued to the next stage of the sowing. Next, 5 centrifuge tubes (5 ml) were prepared for strain SW7, they were then labelled accordingly and filled with 1ml of the SW7 strain inoculum (10⁸ cfu/ml) using a micropipette, followed by adding 10 seeds to each tube using sterilized forceps. Afterwards, the centrifuge tubes containing the inoculum and seeds were placed in the shaker at

37°C to incubate for 24 hours. In the final step, coated seeds were removed from the incubation and the inoculum was removed using the pipette which left the 50 coated seeds ready for sowing.

Once the seeds were coated, the sowing preparations began. The seedling potting trays with 50 pits each were prepared with peat moss substrate. Each tray was labelled according to treatment and placed inside the greenhouse at the International Centre of Biosaline Agriculture (ICBA, Dubai, UAE) to start the sowing process. The conditions of the greenhouse were similar to those described by (Rath and Ghosal, 2020). Small dents were made in the soil and sterilized forceps were then used to pick up each seed and plant one in each pit to cover it with soil. The trays were then irrigated and left to start the germination process in greenhouse conditions. The trays were watered 3 times daily, just enough to always keep the soil moist for seeds to germinate under ideal conditions. Once the seedlings started growing the amount of irrigation was slightly increased to accommodate the growth.

After 4 weeks of monitoring the seedling growth and collecting data under the greenhouse conditions, the well-grown seedlings were selected and transplanted from the potting trays straight to the ground inside the shade house to receive near-constant irrigation to allow continuous growth for another 4 weeks. Shade house conditions were like described by (Quamruzzaman et al., 2021). Shade house conditions were not strictly controlled, it was based on the weather, temperature, and humidity of the day. A saganizer garden hand was used to make small holes in the soil then filled with a mixture of NPK fertilizer and natural humic substance (organic soil conditioner) with a ratio of 1:3 to further help seedling growth and help transplanted seedlings grow well under the shade house field trial conditions. The seedlings were irrigated twice a day until eight weeks as described earlier (Savva and Frenken, 2002). The shoot height was measured once in a week upto eight weeks to compare the influence of strain on the growth of tomato plants (Supplementary Figure S1).

2.6 *In vitro* plant growth promotion attributes

The soil plant analysis development (SPAD) analyses were conducted to assess the nutritional status of the fully matured leaves (three replicates per plant and 5 plants per group) using a 502P chlorophyllometer (Konica Minolta, Inc., Tokyo, Japan). We also calculated the leaf area index (LAI) by measuring the leaf length (L) and width (W) using the following formula ($L \times W \times 0.75$; where 0.75 represents the leaf compensating factor. We collected three leaves per plant and measured the water content by calculating fresh (F_w) and dry (D_w) weight recorded after incubating at 104°C for 2 and 80°C for 72 hr and following this formula: leaf water content (%) = $(F_w - D_w) / F_w * 100$. Leaf density was measured using the gravimetric method, where leaf mass is divided by leaf area, typically expressed in grams per square meter (g/m^2). For chlorophyll contents we collected three leaves per plant. Briefly, we mixed 0.2 g of frozen leaf samples with 4 mL dimethyl

sulphoxide for 24 hr followed by the absorbance measurement at wavelengths of 663 nm, 645 nm, and 470 nm. Chl a, Chl b, Chl a + b and carotenoid contents were determined by using the following equations described by Batool et al. (2020). The statistical analyses of control vs inoculated samples were carried out using student's t-test and the results were considered significant at $P < 0.05$.

2.7 DNA extraction and whole genome characterization

Genomic DNA was extracted from the overnight grown culture of strain SW7 using E.Z.N.A soil DNA isolation kit, with minor modifications. Further to perform whole genome sequencing we constructed the library using Illumina Nextera XT sample prep and index kits, as well as the MiSeq Reagent Micro Kit v2 (Illumina, San Diego, CA, USA). We performed 150 bp x 2 paired-end sequencing using MiSeq Illumina sequencer. The quality of raw reads was assessed with the FastQC V 0.12.1 quality control tool, and then using sickle the sequences were “Filter by quality” tool to remove low-quality reads (with a Phred score below 20) as well as reads with a 90% high-quality score distribution. We used SPAdes in two different ways for assembling the contigs 1. The trimmed reads were assembled using the default parameter for isolates (-isolates) and 2. We also used SPAdes from the Partic server (<http://www.bv-brc.org>) (Davis et al., 2019) to join forward and reverse reads and then assemble them into larger contigs using a 60-bp overlap and a 300-bp length threshold. Then the assembled contigs were polished using RACON (Vaser et al., 2017) and Pilon (Walker et al., 2014) with 2 and 4 iterations respectively. Based on the Quast 5.2.0 (Gurevich et al., 2013) assembly statistics, the assembly using Patric SPAdes had better quality with less number of contigs and mismatches. Future ragtag program (Alonge et al., 2019) was used to reorder the assembled contigs into a complete genome by filling the gaps in the sequences using a closed genome as a reference. Annotation was performed with Prokka by using the assembled genome (Seemann, 2014) and Rapid Annotation using Subsystem Technology (RAST) (Overbeek et al., 2014).

The functional annotation of predicted genes was carried out based on COGclassifier (<https://pypi.org/project/cogclassifier>), the amino acid sequences obtained from rast annotations was used to predict the functional genes using Kyoto Encyclopaedia of Genes and Genomes database (KEGG; <http://www.genome.jp/kegg/>; <https://www.kegg.jp/blastkoala/>; https://www.genome.jp/eg-bin/find_pathway_object), and the Gene Ontology database (GO; <http://www.geneontology.org/>). As a final step, DNA sequences, genes, and predicted non-coding RNA sequences were integrated into the GenBank file format and the circle map of the genome was created using the Circos version 0.69-6 (Krzywinski et al., 2009). The raw sequencing reads and assembly were deposited in the National Centre of Biotechnology Information (NCBI) database under the accession number PRJNA1068844 and the assembled genome was deposited under the accession number CP150440.

2.8 Multilocus sequence typing and phylogenomic analysis

We performed MLST analysis to identify the strain SW7 using seven house-keeping genes such as *glpF* (glycerol uptake facilitator protein), *gmk* (guanylate kinase, putative), *ilvD* (dihydroxy-acid dehydratase), *pta* (phosphate acetyltransferase), *pur* (phosphoribosylaminoimidazolecarboxamide), *pycA* (pyruvate carboxylase) and *tpi* (triosephosphate isomerase) (Priest et al., 2004). To confirm the taxonomic identification, we conducted a phylogenetic analysis using the Type (Strain) Genome server (TYGS) (<https://tygs.dsmz.de>). The genome sequences were pairwise compared with closely related genomes using Genome BLAST Distance Phylogeny (GBDP) (Camacho et al., 2009) along with *B. anthracis* Ames (three other strains), *B. cereus* ATCC 4342 (11 other *Bacillus* strains), 11 *Bacillus* sp. isolated from the international space station (ISS) (Venkateswaran et al., 2017b) and with two PGP reported *Bacillus* species (*Bacillus* sp. RZAMS9 (Bonatelli et al., 2020), and *Bacillus* sp. D5 (PGP) (Magotha et al., 2021) and one plasmidless *B. megaterium* QM B1515 (Eppinger et al., 2011). The distance from the genome blast was employed to determine the closest genome to the strain SW7. Subsequently, GGDC 3.0 was utilized to calculate the digital DNA-DNA hybridization (DDH) value and confidence intervals. These intergenomic distances were then used to infer a balanced minimum evolution tree with branch support via FASTME (V2.1.6.1), including SPR post-processing (Athmika et al., 2021), with 100 pseudo-bootstrap replicates. The rooted tree was constructed and visualized using PhyD3 (Kim et al., 2017). The OrthoANI value with the closest strain was calculated using the FastANI software (Lee et al., 2016). Also, we pairwise compare the *Bacillus* sp. SW7 using mauve aligner (Darling et al., 2010) with other PGP *Bacillus* sp. RZ2MS9 (PGPB), non-PGP *Bacillus cereus* (Q1 strain and S1-R2T1-FB) and *Bacillus anthracis* str Ames.

3 Results

3.1 Isolation, biochemical characterization and identification of bacterial strain

The strain SW7 is a gram-positive bacterium with a circular shape, cream colour, with raised elevation (7 nm diameter) on an agar plate. Biochemical tests showed that the strain was positive for oxidase, and catalase activity whereas negative for urease, decarboxylase, and ornithine decarboxylase (Supplementary Table S1). Similarly, the strain tested negative for lysine, citrate utilization, H₂S production, sorbitol, rhamnose, sucrose melibiose and amygdalin. The strain utilized various carbon sources such as ortho-Nitrophenyl- β -galactoside (ONPG), arginine, tryptophan, gelatinase, glucose, mannitol, inositol and arabinose. It displayed both phosphate (clear zone 8.00 \pm 0.00 mm; 83.3 g/L⁻¹) and potassium (23.00 \pm 0.57 mm; 39.6 g/L⁻¹) solubilization activity. The strain tested positive for ammonia (0.5 ppm) and IAA (3.60 ppm) production and no HCN production and siderophore activity were recorded. The salt and

heat stress assay showed the strain was able to tolerate 11% NaCl (Figure 1A) and can grow up to 50°C (Figure 1B).

3.2 Seed germination and shade house assay

The strain SW7 inoculated seeds (60.60 \pm 38.85%) had a significantly higher germination rate compared to the control (50.6 \pm 35.04%; p-value = 0.02). However, the seedling length (4.86 \pm 1.38 cm vs. 3.99 \pm 0.14 cm, p-value = 0.38) and vigor index (297.1 \pm 10.4 vs. 378.8 \pm 107.6, p-value = 0.31) didn't differ significantly between control and inoculated seeds.

The SW7 exhibits similar values for most plant growth parameters, including Chlorophyll a and b, total chlorophyll, carotenoids, and chlorophyll-carotenoid ratio. For example, the SPAD value varied only 0.6% between the SW7-inoculated and control plant groups. However, SW7-inoculated tomato plants exhibited significantly higher (p > 0.05) leaf numbers, density, and mass water (Figure 1C). Also, increased leaf area index and leaf water content were observed in the SW7-inoculated plants, suggesting broader leaves. These findings strongly suggest a positive impact of the inoculation treatment on the overall growth and physiological aspects of the tomato plants.

3.3 Whole-genome characterization of strain SW7

The assembled genome of strain SW7 comprises a circular genome of 5,155,809 bp with G+C content of 35.17% (Figure 2). In total, the genome consists of 5,424 predicted protein-coding sequences (CDSs), 02 rRNA, and 69 tRNA genes. The assembled genome has a completeness of 98.56% with zero percent contamination. Approximately, 4,104 protein-coding genes were predicted to be functional, and 1,320 hypothetical proteins. Among the predicted CDSs, around 3,736 CDSs (68.88%) were assigned to the Clusters of Orthologous Groups (COG) database. For more details on COG categories, KEGG pathways, Enzymatic (Reactions) Commission numbers (EC) and GO terms assigned to the strain SW7 (refer Supplementary Results S1, Supplementary Figure S2).

3.4 Molecular evidence of PGP potential of strain *Bacillus* sp. SW7

The functional genes responsible for plant growth-promoting traits were identified in the genome sequence of *Bacillus* sp. SW7. *Bacillus* sp. SW7 is identified as a non-pathogenic strain since it lacks plasmids pXO1 and pXO2, which carry the *capBCAD* operon for capsule protein and the gene *lef*, *cya*, and *pagA* responsible for the lethal factor (LF) or edema factor (EF), respectively. The genome of strain *Bacillus* sp. SW7 includes genes responsible for phosphate solubilization (*pstA*, *pstB*, *pstC*), potassium solubilization (*ackA* and *mdh*), siderophore production (*dhbACBEF*), indole acetic acid (*ipdC*), nitrogen fixation (*nifS*), hydrogen cyanide

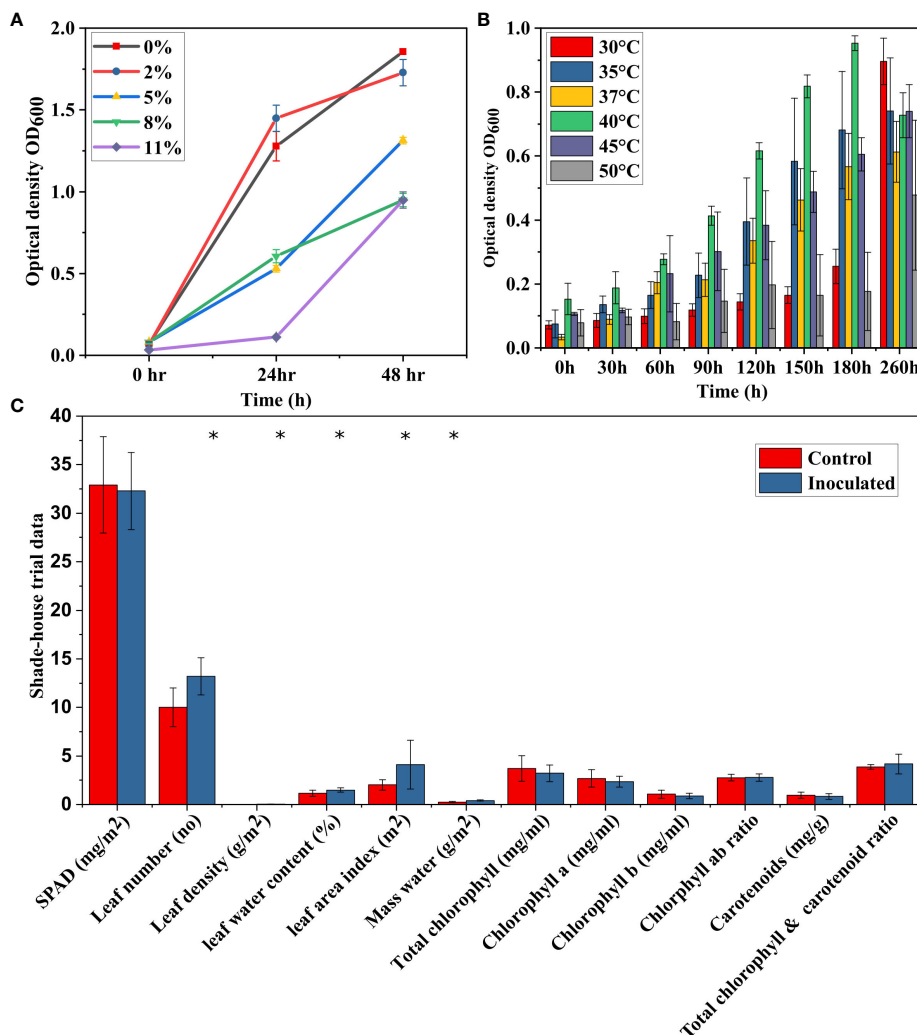


FIGURE 1

The ability of *Bacillus* sp. SW7 strains to tolerate salinity ranging from 0–11% NaCl (A) and their heat stress tolerance, showing growth up to 50°C (B). (C) Effect of *Bacillus* sp. SW7 inoculation on tomato plant growth parameters. The experiment consisted of 5 trials, each performed in triplicate, and data were analyzed using Student's t-test. Shade-house evaluation of *Bacillus* sp. SW7 inoculation on various growth parameters in tomato plants, with * indicating significant differences.

production (*hcnA*, *hcnB*, *hcnC*), catalase (*katE*), oxidase (*qoxC*), arginine decarboxylase (*speA_1*), beta-galactosidase (*lacA*), inositol (*iolA* & C) and sorbitol (*sdhA*, *sdhB*).

3.5 Genes conferring for PGP fitness

Bacillus sp. SW7 genome comprised of genes responsible for heat shock proteins (*dnaJ*, *K*, *GrpE* and *groS*, *L*), cold shock proteins (*cspA*, and *D*), and osmoregulated proline transporter gene (*OpuE*, *opeU*). Stress response protein SCP2 (*yceC*), general stress protein (*yceD_1* and 2) are part of the genome. The genome also has some antioxidants genes (*sodA1*, *sodA2*, *katE* and *qoxC*), reflecting the need for survival under the extreme environments from where they have been isolated. We also found the *xerC* gene that is responsible for recombinase, which is critical for the PGP bacteria to be an effective rhizosphere colonizer.

3.6 Phylogenetic analysis

The whole genome-based taxonomic analysis using Type Strain Genome Server (TYGS) revealed that strain SW7 is closely related to the *Bacillus anthracis* str. Ames (Figure 3), with dDDH (*d0*) of 83.4%, and relatively close to other *Bacillus cereus* and *Bacillus* species isolated from the international space station with high ($\geq 82\%$) dDDH value (Supplementary Table S2).

3.7 MLST analysis and ANI

Although the phylogenetic analyses suggested that the strain is closely related to *Bacillus anthracis* str Ames, due to the absence of pXO1 and pXO2 biomarkers, we would like to perform MLST analysis using seven housekeeping genes. This analysis reveals that the strain SW7 is identified as *Bacillus cereus*, with the following

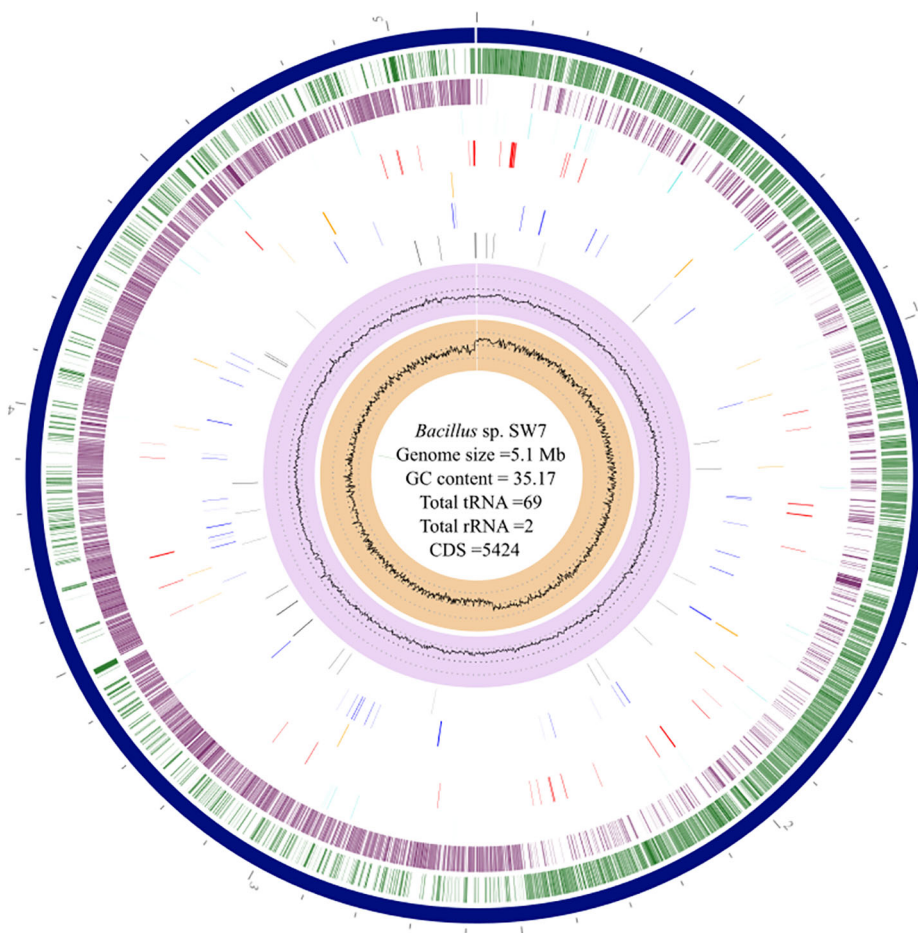


FIGURE 2

Circos plot representing the genome of *Bacillus* sp. SW7. The innermost circle is shown in sandy brown for GC skew, while the second innermost circle, in light violet represents for GC content. Drug target and transporter regions are indicated by black and blue lines respectively. Orange and red lines highlight virulence factor and AMR genes. The second (green) and third (purple) circles from the outside represent the CDS in the forward and reverse direction, followed by non-coding sequences represented in light cyan.

housekeeping genes: *glp*(3), *gmk* (23), *ilv* (132), *pta* (152), *pur* (38), *pyc* (43), and *tpi* (99). To assess the consistency of evolutionary distance, the SW7 genome was compared using average nucleotide identity (ANI), which shows an ANI value of 97.32% against *Bacillus*-sp.S1-R2T1-FB (*Bacillus* isolated from international space station) (Supplementary Table S3). Moreover, other *Bacillus* species isolated from the ISS and non-virulent *Bacillus anthracis* exhibit greater than 97% average nucleotide identity (ANI) to *Bacillus* sp. SW7 (Supplementary Table S3). Utilizing the mauve aligner, we identified that *Bacillus* sp. SW7 shares more homologous regions with *Bacillus*-sp.S1-R2T1-FB (ISS), followed by *Bacillus anthracis* str. Ames. Additionally, significant homology was observed with the PGP *Bacillus* sp. RZ2MS9 (Supplementary Figure S3).

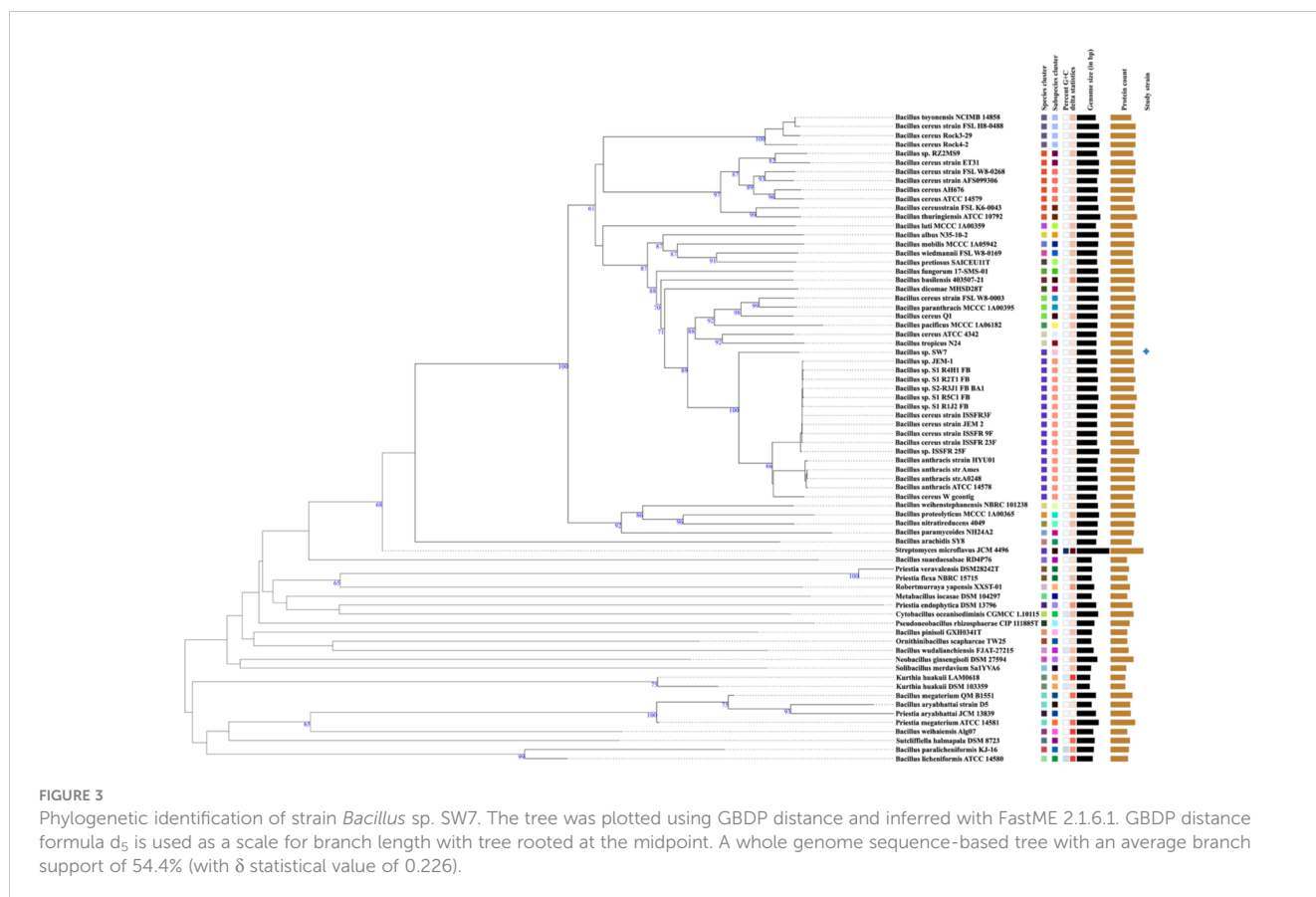
4 Discussion

The study shows a positive effect of strain SW7 inoculation on tomato seed germination, plant leaf number, density and mass water. Moreover, the strain displays multiple PGP traits such as the

ability to produce ammonia, and IAA, solubilize potassium and phosphate. It can withstand 11% NaCl salinity and 50°C of temperature. Whole-genome analysis confirms the presence of multiple PGP and stress-alleviating genes, making the strain a potential candidate as a PGP bacterium to mitigate stress in marginal environments. Furthermore, the phylogenetic analysis reveals that the strain belongs to a novel *Bacillus* sp.

4.1 *Bacillus* sp. SW7 inoculation enhanced the tomato seed germination and plant growth

We observed a significantly higher seed germination rate and higher vigor index in tomatoes after the inoculation of *Bacillus* sp. SW7. This is in agreement with a previous study showing that inoculation of *Bacillus* species such as *Bacillus pumilus* and *Bacillus firmus* enhances seed germination of tomatoes under both saline and non-saline conditions (Patani et al., 2023). Fan et al. (2016) demonstrated that the inoculation of *B. megaterium* increased the



seed germination and vigor index in salt-affected tomato plants. Notably, a consortium involving other PGP bacteria (*Paenibacillus polymyxa* and *Brevibacillus*), *B. subtilis*, and *B. alitudinis* also increases the seed germination rate in tomatoes (Astorga-Eló et al., 2021).

Furthermore, the inoculation of *Bacillus* sp. SW7 proved to enhance various growth parameters of tomato plants after 8 weeks in a shade-house experiment. Leaf number, density, and mass water in the *Bacillus* sp. SW7 inoculated tomatoes significantly increased compared to the control group. These findings align with previous studies that demonstrated the inoculation of *Bacillus* species enhancing various physiological parameters in tomatoes grown in high salt conditions (Patani et al., 2023). Similarly, *Bacillus cereus* was found to improve plant growth parameters in soybean plants (Khan et al., 2020) and increase leaf number, length, and area index in peanuts (*Arachis hypogaea*) (Gohil et al., 2022), which is consistent with the findings of this study, suggesting that *Bacillus* sp. SW7 is a promising candidate for PGP, capable of mitigating arid conditions and enhancing plant growth.

4.2 Genomic analysis for PGP traits in *Bacillus* sp. SW7 and *in-vitro* confirmation through shade-house experiment

The genome analysis of strain SW7 revealed a size of 5.15 Mb with a GC content of 35.17%, encompassing 5,424 CDSs, consistent

with previously reported genomes of related isolates in the NCBI database (Leontidou et al., 2020). A comparable genome size, GC content, and number of Coding DNA Sequences (CDS) were observed in *Bacillus* sp. strain RZ2MS9, known for its multiple PGP traits (Bonatelli et al., 2020).

PGP bacterial strains, in general, play a crucial role in enhancing plant development, contributing to activities like phosphate and potassium solubilization, nitrogen fixation, HCN production, and the secretion of essential plant hormones such as IAA, abscisic acid, and cytokinin etc (Hakim et al., 2021). *Bacillus* sp. SW7 was found to produce plant growth promoting factor NH_3 , demonstrated phosphate and potassium solubilization activity and produce the hormone IAA. Genome analysis uncovered the presence of genes associated with multiple PGP traits, encompassing phosphate (operon *pstABC*), and potassium solubilization (*ackA* and *mdh*), nitrogen fixation (only *nifS*), as well as the production of IAA (*ipdC*). Various studies have highlighted the diverse PGP potentials of *Bacillus* species. For example, *Bacillus* sp. PG-8, isolated from Panchagavya, significantly improved plant growth by solubilizing insoluble phosphate and producing IAA (Gohil et al., 2022). Furthermore, *Bacillus* sp. SW7 possesses a two-component signal transduction system for phosphate uptake (*phoBPR* genes, and the *phnXW*) genes crucial for the solubilization of organophosphorus phosphonate compounds, making them bioavailable for the plant. Similarly, complete traits of phosphonate solubilization gene clusters were reported in PGP *Bacillus firmus* I-1582, which promotes tomato growth and decreases nematode counts in the

rhizosphere (Susič et al., 2020). Notably, various *Bacillus* species are known to have phosphate solubilization genes that promote plant growth, such as *Bacillus* sp. strain D5 (Bonatelli et al., 2020), *Bacillus* sp. Strain RZ2MS9 (Magotra et al., 2021) and *B. subtilis* EA-CB0575 (Franco-Sierra et al., 2020).

Phytohormones, particularly IAA, are vital for regulating the growth, division, differentiation, and response of plant cells to light and gravity (Asim et al., 2023) further promoting root expansion and nutrient uptake. Earlier studies have demonstrated the positive effects of *Bacillus* species on plant growth. For instance the inoculation of *Bacillus* sp. PG-8 (Gohil et al., 2022), *Bacillus thuringiensis* (Batista et al., 2021), and *Bacillus siamensis* (Saleem et al., 2021), has been demonstrated to enhance the growth of peanuts, tomatoes and cotton respectively. These *Bacillus* strains were found to alleviate stress by producing IAA. The genome of *B. megaterium* RmBm31 (Dahmani et al., 2020), encodes genes for multiples PGP traits including IAA, HCN, phosphate/potassium solubilization, aligning with the findings of the present study.

The *Bacillus* sp. SW7 harbours other PGP genes, including HCN (*hcnA*, *hcnB*, *hcnC*) and siderophore genes (*dhbACBEF*). However, both HCN production and siderophore activity was not observed in our *in-vitro* assessment, possibly due to the specific condition provided during the experiment. Typically, siderophores are produced under unfavourable or low-iron conditions to capture iron from the surrounding environment (Sultana et al., 2021). Studies have shown that the addition of siderophores can more effectively improve plant growth than the addition of Fe(III) (De Serrano, 2017). Genome analysis of various *Bacillus* strains with PGP potential, including *B. subtilis* EA-CB0575 (Franco-Sierra et al., 2020), *B. megaterium* RmBm31 (Dahmani et al., 2020), has revealed the presence of siderophore genes. Additionally, the application of *Bacillus amyloliquefaciens* Ba13 increased beneficial bacteria in the rhizosphere and rhizoplane of tomatoes by producing siderophores, which compete for soluble Fe(III). This competition leads to a decrease in soil fungi, and genome analysis revealed that *B. amyloliquefaciens* has two genes encoding siderophores (Bacillibactin) (Ji et al., 2021).

4.3 Strain SW7 phylogenetically different from *B. anthracis* and *B. cereus* and represent novel species

The whole-genome blast analysis revealed that the strain SW7 is closely related to *Bacillus anthracis* str. Ames. However, the strain SW7 lacks the pOX1 and pOX2 plasmids. To confirm the taxonomy, we performed MLST, which indicated that SW7 belongs to the *Bacillus cereus* group based on the housekeeping genes. Further, the phylogenetic analysis revealed that the strain SW7 is placed in a different clade from *Bacillus anthracis* str. Ames and other *Bacillus cereus* species, including isolates from the International Space Station (Venkateswaran et al., 2017b). About the recent phylogenetic investigation of the family Bacillaceae which are polyphyletic, it is difficult to trace the evolutionary lineages (Patel and Gupta, 2020). Herein we place the strain SW7 as a novel *Bacillus* species based on phylogenetics and MLST, which is

phylogenetically different from *B. anthracis* and *B. cereus*. Although *B. anthracis* is a common soil bacterium, which are obligate and opportunistic animal pathogen belonging to *Bacillus cereus* sensu lato, and *B. thuringiensis*, some *Bacillus anthracis* strains lack the virulent plasmids pOX1 as well as pOX2 contributed positively to plant growth (Azeem et al., 2023).

5 Conclusion

Our study reveals that the newly identified PGP bacteria *Bacillus* sp. SW7 from mangrove sediments significantly improves tomato seed germination rates and increases the number of leaves and density, enhancing leaf area index compared to the untreated control group. The *in-vitro* and genome analysis confirm the presence of multiple PGP functions in *Bacillus* sp. SW7, including IAA, and NH₃ production, phosphate and potassium solubilization, which contribute to tomato plant growth promotion. Furthermore, the genome encompasses stress-tolerant genes related to heat and salinity, suggesting the suitability of the strain to arid environments. These experimental evidences and genomic traits data positioned the *Bacillus* sp. SW7, as a promising candidate for arid agroecosystems, offers potential benefits for crop growth in marginal environments using a sustainable and environmentally friendly alternative. This study recommends the utilization of the *Bacillus* sp. SW7 to enhance crop growth and productivity, particularly in challenging arid agricultural settings. However, before considering the SW7 strain for larger-scale field trials, it is crucial to conduct further preliminary studies under greenhouse conditions. This should involve selecting different tomato seed varieties, assessing seed viability, and optimizing SW7 inoculation rates. Furthermore, it is recommended to evaluate the effectiveness of the SW7 strain on tomato seedling growth under varying arid agroecosystem marginal environmental conditions, such as salinity, water availability, and reduced synthetic fertilization rates. Addressing these aspects will provide a more comprehensive understanding of the SW7 strain's potential and inform its practical implementation in agriculture.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/[Supplementary Material](#).

Author contributions

MA: Writing – original draft, Data curation, Methodology, Visualization. BS: Methodology, Visualization, Writing – original draft, Formal analysis, Software, Validation, Writing – review & editing. AN: Formal analysis, Methodology, Writing – review & editing, Validation. HM: Validation, Writing – review & editing, Visualization, Data curation. MK: Methodology, Writing – review & editing, Conceptualization, Funding acquisition, Investigation, Project administration. SM: Conceptualization, Funding

acquisition, Investigation, Project administration, Writing – review & editing, Resources, Supervision, Writing – original draft.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. The authors thank UAE University for providing UAEU-ZU Joint Research project (R22024; 12R139) and the Zayed University for Research Incentive Fund (RIF) project-R21074.

Acknowledgments

We acknowledge the UAE University for providing a UAEU-ZU (12R139/R22024) research grant to SM and MK and Zayed University Research Incentive Fund (RIF) project-R21074 awarded to MK and HM. We thank Amna Almarri, Meera Ashkanani, Aroosa Zeb, and Aneesa Rasheed Anwar for helping with SW7 strain seed coating, salt and heat stress assay; AlSayeda Zahra Salman for preliminary characterization of PGP traits isolates and Qurban Ali for his help in preparing new figures.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2024.1379439/full#supplementary-material>.

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