

Whole-genome sequences of three halotolerant *Bacillus* strains isolated from the rhizosphere of halophyte plants in Tunisia

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Abstract

Three halotolerant *Bacillus* strains were isolated from the rhizosphere of halophyte plants growing in saline environments, specifically from Sebket Kelbia, located in the semi-arid region of Tunisia. These strains are of particular interest due to their potential role in enhancing plant growth and tolerance under salt stress, thereby contributing to sustainable saline agriculture and soil remediation. Previously, partial 16S rRNA gene sequencing indicated that the isolates belong to the genus *Bacillus*. Here, we report their whole-genome sequences obtained using Illumina 250 bp paired-end sequencing. Genome analysis assigned strain 1 to *Bacillus subtilis*, and strains 2 and 3 to *Bacillus cereus*. The reads were assembled into 45, 21, and 22 contigs, with average coverage of 85.3x, 84.8x, and 56.9x, and total genome lengths of 4,787,672 bp, 5,732,988 bp, and 5,732,039 bp for strains 1, 2, and 3, respectively. The GC contents were 44.13 % for strain 1 and 35 % for strains 2 and 3. The genomic data generated in this study provide a valuable resource for further studies on molecular mechanisms underlying plant growth promotion and the role of halotolerant PGPR in improving plant adaptation to extreme environmental conditions.

Introduction

The rapid population growth and increasing pressure on agricultural ecosystems, have led to declining productivity and the degradation of sustainable agroecosystems (Steensland and Zeigler, 2021). Global changes increase are further accelerating the degradation of agricultural lands and affect food security in the world. Furthermore, the current rate of agricultural production growth remains insufficient to meet the food needs of the world's population by

2050 (Steensland and Zeigler, 2021). For these reasons, improving agricultural productivity has become a major global challenge, yet it remains complex and is largely influenced by soil and climate conditions, farming systems, and management strategies (Nemecek and Gaillard, 2010). Tunisian agriculture is also facing environmental constraints such as climate change, soil degradation and salinization and water scarcity. Technological approaches developed to improve soil quality have often proven costly, unsustainable, and only partially effective. In contrast, biological strategies based on the natural tolerance of plants to abiotic stress and the use of beneficial microorganisms have emerged as promising and environmentally friendly alternatives (Etesami and Maheshwari, 2018). Among these microorganisms, plant growth-promoting rhizobacteria (PGPR) play a key role. These bacteria colonize the rhizosphere and exert beneficial effects on plant growth through various direct mechanisms such as nitrogen fixation, phosphate solubilization, siderophore and phytohormone production, as well as indirect mechanisms, including biological control of pathogens and induction of systemic resistance (Vacheron et al., 2013; Khoso et al., 2024; Liu et al., 2025). PGPR enhance plant growth, biomass, and yield by improving nutrient availability and hormonal regulation (Khoso et al., 2024; Sun and Shahrajabian, 2025). They also strengthen plant tolerance to abiotic stresses, particularly salinity, by stimulating production of antioxidants and sodium-binding exopolysaccharides (EPS), while modulating ion homeostasis and ethylene levels (Atouei et al., 2019; Fouda et al., 2019; Arora et al., 2024). Among the most studied PGPR genera, *Bacillus* stands out for its capacity to survive under severe environmental conditions such as salinity and drought and to exert multiple plant-beneficial effects. In addition, bacterial strains such as *Bacillus subtilis* and *Bacillus cereus* have been widely reported to enhance root growth, increase nutrient availability, and suppress plant pathogens (Zhang et al., 2023; Chen et al., 2024; Liu et al., 2025).

The use of native halotolerant rhizobacteria (HT-PGPR) isolated from the rhizosphere of halophytes plants in saline biotopes represents a new eco-friendly and low cost biological approach for farmers and stakeholders. This approach also contributes to the rehabilitation of marginal saline lands and in the remediation of salt-affected soils. Therefore, the exploration and utilization of native halotolerant rhizobacterial *Bacillus* from halophytic ecosystems represent a promising strategy to improve plant growth and tolerance in saline environments while contributing to the development of sustainable agricultural practices capable of addressing soil degradation and meeting the increasing global food demand.

Recently, advances in high-throughput sequencing technologies have enabled the exploration of numerous extremophilic microbial genomes, thereby improving our understanding of how

these organisms survive and function under extreme conditions. In this context, halophilic bacteria that promote plant growth represent excellent model systems for studying various biological mechanisms. Here, we report three halotolerant *Bacillus*, one *Bacillus subtilis* and two *Bacillus cereus* strains, isolated from the rhizosphere of native halophyte plants growing in a saline biotope (Sebkhet Kelbia) located in a semi-arid region of Tunisia. These three strains were previously identified based on partial 16S rRNA gene sequences and deposited in GenBank under the following accession numbers: strain TS20Lm as *Bacillus inaquosorum* (*Bacillus subtilis* subsp. *inaquosorum*; AN ON832055), strain TS13Ai as *Bacillus thuringiensis* (*Bacillus cereus* group; ON832056), and strain TS14Hs as *Bacillus proteolyticus* (*Bacillus cereus* group; ON832057) (Slatni et al., 2024). Whole-genome sequencing refined their taxonomic assignments, showing that strain 1 corresponds to *Bacillus subtilis*, while strains 2 and 3 both correspond to *Bacillus cereus*. The complete genome sequences have now been deposited in GenBank under accession run numbers SRR27969764 (strain 1), SRR27977373 (strain 2), and SRR27996658 (strain 3).

In addition, these strains exhibit high tolerance to salinity and represent promising biological tools for the remediation of saline soils and the enhancement of crop productivity under environmental constraints, particularly in salt-affected areas (Slatni et al., 2024). Therefore, the field application of these halotolerant plant growth-promoting rhizobacteria (HT-PGPR) represents a promising strategy for the development of sustainable saline agriculture. In this study, whole-genome sequencing was performed to generate genomic assemblies and facilitate research on the physiological, biochemical, and genetic characteristics of these halophilic strains. The publication of these genomic datasets will provide valuable insights into the molecular mechanisms of adaptation to extreme environments, as well as their metabolic pathways, and potential uses in biotechnology. Furthermore, the genomic data generated in this study constitute a valuable resource for future research aimed at elucidating the role of halotolerant PGPR in enhancing plant adaptation to challenging environmental conditions.

Material and Methods

Bacterial isolation

The three bacterial strains analyzed in this study were isolated from the rhizosphere of halophyte plants growing in saline environments in the semi-arid region of Tunisia, specifically from Sebkhet Kelbia. Strain 1 was isolated from the rhizosphere of the halophyte *Limoniastrum monopetalum*, strain 2 from *Arthrocnemum indicum* and strain 3 from *Holocnemum strobilaceum* (Slatni et al., 2024).

Whole Genome Sequencing, assembly and annotation

Bacterial culturing, DNA extraction, genome sequencing and assembly were conducted by microbesNG according to their protocols <https://microbesng.com/>. Genomic DNA libraries were prepared using the Nextera XT Library Prep Kit (Illumina, San Diego, USA) following the manufacturer's instructions with the following modifications: input DNA was increased 2-fold, and PCR elongation time increased to 45 seconds. Libraries were sequenced using the Illumina NovaSeq 6000 (Illumina, San Diego, USA) using a 250 bp paired-end protocol. Reads were trimmed using Trimmomatic version 0.30 (Bolger et al., 2014) with a sliding window quality cutoff of Q15. *De novo* assembly was performed using SPAdes version 3.7 (Bankevich et al., 2012), and contigs annotated using Prokka 1.11 (Seemann, 2014).

Results

Genome properties

Trimmed sequence read data and genome assembly data of these three Halotolerant *Bacillus* strains has been deposited in NCBI/GenBank under the BioProject PRJNA1076592. A summary of the genomic features of these three bacteria strains is listed in table 1. In fact, the results indicated that strain 1 belongs to *Bacillus subtilis* with NCBI GenBank accession run number SRR27969764, while strains 2 and 3 were identified as *Bacillus cereus* with NCBI GenBank accession run numbers respectively SRR27977373 and SRR27996658. In addition, the sequencing reads were assembled into 45, with an average coverage of 85.3X, and total genome lengths of 4,787,672 bp for *Bacillus subtilis*. For the two *Bacillus cereus* (strains 2 and 3), the sequencing reads were assembled into 21 and 22 contigs, with an average coverage of 84.8X and 56.9X and total genome lengths of 5,732,988 bp and 5,732,039 bp respectively. The genomic GC contents are 44.13 % for strain 1 and 34.88 % for strains 2 and 3.

Data availability

This whole-genome shotgun has been deposited at DDBJ/ENA/GenBank under accession numbers JBAHZR000000000, JBAJAF000000000 and JBAKBC000000000. Raw sequence reads have been deposited in the NCBI Sequence Read Archive under BioProject number PRJNA1076592 and run numbers SRR27969764, SRR27977373 and SRR27996658.

Statement on continuing work

The datasets are being shared prior to formal publication and should be considered preliminary. For any feedback from the community, please get in touch with TS (tarek.slatni@fst.utm.tn) for any information needed.

Table 1. Summary statistics of whole genome sequencing of the three *Bacillus* strains isolated from the rhizosphere of halophyte plants in Tunisia.

Bacterial strains	<i>Bacillus subtilis</i>	<i>Bacillus cereus</i>	<i>Bacillus cereus</i>
Source	Rhizosphere of <i>Limoniastrum monopetalum</i>	Rhizosphere of <i>Arthrocnemum indicum</i>	Rhizosphere of <i>Holocnemum strobilaceum</i>
Biosample	SAMN39946909	SAMN39953809	SAMN39971232
#contigs(>= 1000 bp)	45	21	22
Largest contig	1190034	1190034	2372662
Genome size (bp)	4787672	5732988	5732039
GC content (%)	44,13	34,88	34,88
Mean coverage	85,2513	84,7486	56,9299
N50	423087	869890	1134513
Number of reads	888201	1006814	668436
tmRNA	1	1	1
GenBank Accession (Assembly)	JBAHZR000000000	JBAJAF000000000	JBAKBC000000000
GenBank Accession (Raw reads)	SRR27969764	SRR27977373	SRR27996658

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