

Whole genome sequence *Ensifer* sp. VA4 a nitrogen fixing bacterium associated with *Vigna anguiculata* L. walp. from Timimoun (Algeria)

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Abstract

This study reports the whole-genome sequence (WGS) of an endophytic bacterial strain isolated from efficient root nodules of *Vigna unguiculata* collected from Ksar Ben Aissa (Tmimoun, Algeria). The strain, identified as *Ensifer* sp., exhibits nitrogen-fixing activity and associates with the host plant in a symbiotic manner, contributing to nitrogen nutrition in low-fertility soils. Whole-genome sequencing will provide valuable insights into the genetic basis of nodule-associated endophytic behaviour and nitrogen fixation, facilitating the development and application of this strain in sustainable agriculture in desert environments.

Key words: *Vigna unguiculata*, nodules, WGS, Desert, *Ensifer*, nitrogen fixation

Introduction

Leguminous plants play a central role in soil fertility and sustainable agriculture due to their unique ability to acquire atmospheric nitrogen through symbiotic associations with nodule-forming bacteria such as *Rhizobium*, *Bradyrhizobium*, and *Ensifer* (Kearsley et al., 2024; Srivastava et al., 2025). These microorganisms infect the roots of legumes, induce the formation of specialized structures called root nodules, and within these nodules carry out biological nitrogen fixation (BNF), converting molecular nitrogen (N_2) into ammonia (NH_3), which is then assimilated by the host plant (Chiurazzi et al., 2025). This process significantly reduces the dependence on synthetic nitrogen fertilizers, mitigating environmental impacts such as nitrate leaching and greenhouse gas emissions (Bhatt et al., 2025).

The genus *Ensifer* (formerly included within *Sinorhizobium*) comprises a group of α -proteobacteria best known for their ability to form nitrogen-fixing root nodules on a wide range of leguminous plants, including crops such as *Vigna unguiculata* (cowpea), *Medicago*, *Trifolium*, and *Cicer* species (Fagorzi et al., 2020). Within these nodules, *Ensifer* strains differentiate into bacteroids that reduce atmospheric nitrogen (N_2)

into ammonia, a form readily assimilated by the host plant, thereby improving nitrogen nutrition without the need for synthetic fertilizers (Pacheco et al., 2023). This symbiotic nitrogen fixation not only boosts legume productivity but also enriches the soil with biologically fixed nitrogen, benefiting subsequent crops in rotation systems and contributing to more sustainable agricultural practices (Qiao et al., 2024).

In addition to their well-defined symbiotic role, *Ensifer* strains often exhibit traits that extend beyond nodule formation. Many isolates possess plant growth-promoting abilities such as indole-3-acetic acid (IAA) production, phosphate solubilization, siderophore secretion, and tolerance to abiotic stresses including salinity, drought, and heavy metals (Bhatt et al., 2026). These features enable *Ensifer* to persist in diverse soils and to support host plants under suboptimal or marginal agricultural conditions (Cambeis et al., 2025).

Given these capacities, efficient symbiotic nitrogen fixation and broad plant growth-promoting functions, *Ensifer* represents a highly promising candidate for the development of targeted microbial inoculants in agriculture. Integrating *Ensifer* strains into microbial consortia that also include free-living diazotrophs and other beneficial rhizobacteria offers the potential to enhance nitrogen availability throughout the cropping season, stabilize yields under stress, and reduce reliance on chemical fertilizers. The present study focuses on an *Ensifer* sp. strain isolated from root nodules of *Vigna unguiculata*, aiming to characterize its genome, nitrogen fixation-related genes, and plant growth-promoting traits, with a view to its potential application in sustainable legume-based cropping systems.

Material and Methods

1. Sample collection and isolation

Root nodules of *Vigna unguiculata* were collected from plants growing at an agricultural parcel Ksar Ben Aissa (Timimoun), Algeria. Nodules were carefully detached from the root system,

placed in sterile polyethylene bags, and transported to the laboratory in an icebox. Samples were processed within 24 h of collection and the bacterium was isolated from surface-sterilized nodules using the direct nodule isolation method. An individual nodule was opened with a sterile scalpel and using a toothpick, the bacterium was streaked directly onto yeast extract–mannitol (YEM) medium. After incubation, at $28 \pm 2^\circ\text{C}$ for 7 days, single colonies were selected and further purified and named VA4.

3. Whole-genome sequencing of bacteria

The genomic DNA of strain VA4 was obtained by MicrobesNG. The concentration of DNA was quantified using the Quant iT dsDNA HS kit (Thermo Fisher Scientific), and libraries were constructed employing the Nextera XT kit (Illumina) on a Hamilton Microlab STAR system. Sequencing was conducted on an Illumina NovaSeq 6000 (Illumina, San Diego, USA) utilising a 250 bp paired-end technique. Reads underwent adapter trimming utilising Trimmomatic v0.30 (Bolger et al., 2014) with a sliding window quality threshold of Q15. De novo assembly was conducted with SPAdes v3.7 (Bankevich et al., 2012), and contigs were annotated with Prokka v1.11 (Seemann, 2014).

Results

Genomic features

The sequence data is available from NCBI/GenBank under the BioProject: [PRJNA1429578](#).

The whole genome sequencing of the strain VA4 (BioSample SAMN56136948) was conducted. The sequence reads were assembled using QUAST, yielding 44 contigs (≥ 1000 bp). The largest contig measured 632,243 base pairs (bp), while the total genome assembly length for strain VA4 was 6,335,590 bp. The genomic G + C content of strain VA4 was determined to be 62.39%, with a mean coverage of $26.31\times$ and an $N50$ of 358,937 bp. The total number of

genes, including 5,994 protein-coding genes (CDS), 54 tRNA genes, and 8 rRNA genes, is presented in [Table 1](#).

Data availability

The raw reads are available under GenBank accession SRR38007193, and the genome assembly under JBVQPO000000000.1. Based on Average Nucleotide Identity (ANI) analysis and current taxonomic classification, the genome sequence of strain VA4 will be further characterized for species assignment within the *Ensifer* genus.

Statement on continuing work

The datasets are being shared prior to formal publication and should be considered preliminary. We encourage and welcome feedback from the scientific community. Please get in touch with **Nadjette Djemouai** (djemouai.nadjette@univ-ghardaia.dz) for more information.

105 **Table 1.** Summary statistics for the genomes studied

Bcterial strain	Biosample	Number of contigs (>=1000 bp)	Largest contig	Total length	GC (%)	Mean cover age	N50	CDS	tRNA	rRNA	GenBank accession (Assembly)	GenBank accession (reads)
VA4	SAMN56136948	44	632,243	6,335,590	62.39	26.31	358,937	5,994	54	8	JBVQPO000000000.1	SRR38007193

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Acknowledgements

The sequencing of the bacterial strain was supported by GetGenome and The Sainsbury Laboratory, Norwich, UK, with support from the Gatsby Charitable Foundation and the Biotechnology and Biological Sciences Research Council (BBSRC). We gratefully acknowledge Prof. Sophien Kamoun and Dr. James Canham as well as all the people involved in GetGenome initiative.

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