

Complete genomes of *Priestia megaterium* and *Enterobacter ludwigii*: Non-symbiotic endophytes from algerian cowpea nodules

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Abstract

This study reports the isolation and whole-genome sequencing of three non-symbiotic, nitrogen-fixing bacterial strains recovered from root nodules of cowpea (*Vigna unguiculata*) grown in a Saharan environment. Although associated with legume nodules, these strains are non-symbiotic but still capable of nitrogen fixation. Whole-genome sequence analysis assigned the isolates to *Priestia megaterium* (2 strains) and *Enterobacter* (1 strain) species, revealing the presence of genes related to nitrogen metabolism and plant growth promotion. The identification of free-living or non-symbiotic diazotrophs within cowpea nodules highlights the complexity of the nodule microbiome and suggests a potential role of these bacteria in enhancing nitrogen availability and plant performance under Saharan conditions.

Key words: *Vigna anguiculata*, nodules, WGS, Desert, *Priestia*, *Enterobacter*

Introduction

Legume root nodules are classically regarded as specialized organs hosting symbiotic nitrogen-fixing rhizobia, such as *Bradyrhizobium*, *Rhizobium*, and *Ensifer*, which reduce atmospheric nitrogen into ammonia in exchange for plant-derived carbon (Coba de la Peña et al., 2018). However, recent studies have shown that nodules also harbor a diverse microbiome, including non-symbiotic or endophytic bacteria that are not essential for nodule formation but may contribute to nitrogen acquisition and plant growth (Santoyo, 2022). In particular, several free-living and nodule-associated diazotrophs have been reported to fix nitrogen independently of the canonical symbiotic system, adding a supplementary layer of nitrogen input in legume-based cropping systems (Bellenger et al., 2014; Ladha et al., 2022).

Cowpea (*Vigna unguiculata*) is a drought-tolerant legume widely cultivated in arid and semi-arid regions, including the Sahara, where low soil fertility and water scarcity limit crop productivity (Kim et al., 2025). In such environments, biological nitrogen fixation becomes a key strategy to sustain production without relying heavily on chemical fertilizers (Soumare et al., 2020). While symbiotic rhizobia are well-documented in cowpea nodules, less is known about the diversity and functional role of non-symbiotic nitrogen-fixing bacteria inhabiting these structures (Klepa et al., 2022; Mbah et al., 2022; Yohannes et al., 2024). Evidence suggests that members of genera such as *Priestia* and *Enterobacter* can colonize root nodules as endophytes or opportunistic associates, where they may contribute to nitrogen fixation and other plant growth-promoting traits (Zhu et al., 2025).

In this context, we report the isolation of four non-symbiotic nitrogen-fixing bacterial strains from root nodules of cowpea plants grown in a Saharan environment. Whole-genome sequencing assigned these isolates to *Priestia megaterium* and *Enterobacter* species, revealing genomic features associated with nitrogen metabolism and adaptation to arid conditions. This study aims to characterize these nodule-associated diazotrophs and to explore their potential contribution to nitrogen nutrition and plant growth in dryland agricultural systems, highlighting the importance of non-symbiotic bacteria within the legume nodule microbiome.

Material and Methods

1. Sample collection and isolation

Nodules of *Vigna unguiculata* were collected from plants growing at three different localities within El Golea province, Algeria. Cowpea plants grown at the Université des Sciences et de la Technologie Houari Boumediene (USTHB) experimental field station, Hassi Ghanem (El-Golea) and Hofra region in El-Golea oasis were collected. First, 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.

The bacteria were isolated from surface-sterilized nodules using the suspension dilution method. A 10^{-3} dilution was prepared in sterile saline solution, and 100 μ L of this dilution was plated onto yeast extract–mannitol (YEM) medium supplemented with the antifungal agent cycloheximide at 50 μ g/mL. Plates were incubated at 28 ± 2 °C for 10 days. Distinct bacterial colonies were selected, purified by successive streaking, and designated as isolates VA1, VA7, and VA9. Glycerol stocks of each purified isolate were prepared and stored at -20 °C for long-term conservation.

3. Whole-genome sequencing of bacteria

The genomic DNA of the three strains was processed by MicrobesNG. The libraries were constructed employing the Nextera XT kit (Illumina) on a Hamilton Microlab STAR system. Sequencing was ran on an Illumina NovaSeq 6000 (Illumina, San Diego, USA) utilising a 250 bp paired-end technique. Reads underwent adapter trimming using 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

Genome sequencing and assembly quality

Three bacterial endophyte strains (VA1, VA7, VA9) isolated from healthy cowpea root nodules underwent whole-genome sequencing, yielding high-quality assemblies (Table 1). VA1 (SAMN57186234) and VA7 (SAMN56134205), both identified as *Priestia megaterium*, produced near-complete genomes of 5.86 Mb and 5.71 Mb, respectively, with identical 37.5% GC content, 38-49 contigs (≥ 1 kb), N_{50} values of 969.5 kb, and excellent coverage ($62.6\times$ - $86.6\times$). On the other hand, VA9 (SAMN56001665), identified as *Enterobacter ludwigii*, generated a smaller, high-GC (54.5%) genome (4.75 Mb, 19 contigs, 780.5 kb N_{50} , $56.2\times$ coverage).

All assemblies met stringent quality standards for comparative genomics ($N_{50} > 780$ kb, < 50 contigs, $> 50\times$ coverage), enabling comprehensive functional annotation. Complete assemblies were deposited in GenBank: GCA_056786235.1 (VA1), GCA_055710015.1 (VA7), GCA_055759495.1 (VA9), with raw reads SRR38049304, SRR38067822, and SRR38067821. These strains establish a foundation for defined consortium formulation, stability testing, and comparative transcriptomics during *Vigna* colonization, critical steps toward commercial microbial inoculant development for sustainable legume production. Additionally, mining these

99 genomes will elucidate the molecular interactions enabling non-symbiotic bacterial nodule
100 colonization in cowpea, expanding understanding of beneficial plant-microbe associations
101 beyond rhizobial symbiosis.

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

Bcterial strain	Biosample	Number of contigs (≥ 1000 bp)	Largest contig	Total length	GC (%)	Mean coverage	N_{50}	CDS	tRNA	rRNA	GenBank accession (Assembly)	GenBank accession (reads)
VA1	SAMN57186234	49	1251898	5864871	37.5	62.5635x	969.5 kb	6,066	128	58	GCA_056786235.1	SRR38049304
VA7	SAMN56134205	38	1253776	5713152	37.5	86.5636x	969.5 kb	5,898	111	55	GCA_055710015.1	SRR38067822
VA9	SAMN56001665	19	830146	4748790	54.5	56.2218x	780.5 kb	4,602	77	29	GCA_055759495.1	SRR38067821

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