

Draft Genome Sequence of a Novel Species of *Halococcus* (Strain IIIV-5B), an Endophytic Archaeon Isolated from the Leaf Tissue of *Avicennia germinans*

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ABSTRACT Strain IIIV-5B was isolated from the leaf tissue of the black mangrove, *Avicennia germinans*. This microorganism belongs to the genus *Halococcus*. Here, we present the draft genome sequence of strain IIIV-5B, a novel species of this genus. The 3,869,808-bp genome has a G+C content of 63.9% and around 3,812 coding sequences.

Mangroves are woody plants often located in the intertidal areas of tropical and subtropical regions (1) that provide a suitable habitat for multiple species and contribute to the stabilization of coastlines by preventing erosion and protecting the shore from tidal waves (2–8). A novel strain (IIIV-5B) belonging to the genus *Halococcus*, first reported by Schoop in 1935 (9), was isolated and characterized during a prokaryotic biodiversity survey of halophilic and halotolerant endophytes in leaves of *Avicennia germinans* at the solar salterns in Cabo Rojo, Puerto Rico. Here, we present the draft genome sequence of this putative novel species, as reports of endophytic haloarchaea are scarce.

Strain IIIV-5B was isolated from surface-sterilized leaf tissue in Sehgal-Gibbons medium (10) containing 15% NaCl (wt/vol). The leaf tissue was placed onto the surface of solid medium and incubated at 30°C. Growing colonies were selected and purified by plate streaking until pure cultures were obtained. Genomic DNA extraction was performed using the Promega Wizard genomic DNA purification kit and sequenced at MicrobesNG in Birmingham, United Kingdom. Genomic DNA libraries were generated using a Nextera XT library prep kit (Illumina, San Diego, CA, USA) following the manufacturer's instructions, with the exception of the use of 2 ng of DNA instead of 1 ng as input and an increase of the PCR elongation time to 1 min. Pooled libraries were quantified using the Kapa Biosystems library quantification kit for Illumina. Libraries were sequenced using an Illumina HiSeq instrument (250-bp paired-end protocol). Adapters were trimmed with Trimmomatic 0.30 (11), *de novo* assembly was performed using SPAdes version 3.7 (12), and contigs were annotated with Prokka 1.11 (13). Genome sequences were then analyzed using the Rapid Annotations using Subsystems Technology (RAST) server (14–16). A draft genome sequence of 3,869,808 bp was assembled using 74 contigs (Table 1). Features in this genome include a G+C content of 63.96%, 2 CRISPR repeats, and around 3,812 coding sequences, 3 of which are suggested to encode rRNAs and 49 of which are suggested to encode tRNAs. Similar values were obtained with the NCBI Prokaryotic Genome Annotation Pipeline (3,877,752 bp, 63.9% G+C content, and 3,812 coding sequences).

Sequences corresponding to the 16S rRNA and *rpoB* genes were retrieved from the IIIV-5B annotated genome to perform taxonomical characterization. To determine the phylogenetic distance from other *Halococcus* spp., the 16S rRNA gene was obtained

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TABLE 1 Genome statistics for strain IIIV-5B

Statistic	Value
Sequencing	
No. of reads	279,550
Genome coverage (×)	30
Assembly	
Size (bp)	3,869,808
No. of contigs	74
G+C content (%)	63.96
N ₅₀ (bp)	99,920
Gene models	
Total no. of coding sequences	3,812
Total no. of RNAs	49

from the RAST server and uploaded to EZ-Taxon (17), where taxonomically close relatives were retrieved. 16S rRNA gene sequences were downloaded, aligned using ClustalW, and edited in Molecular Evolutionary Genetics Analysis X (MEGA X) software (18). The neighbor-joining tree method (19) was used to determine phylogenetic

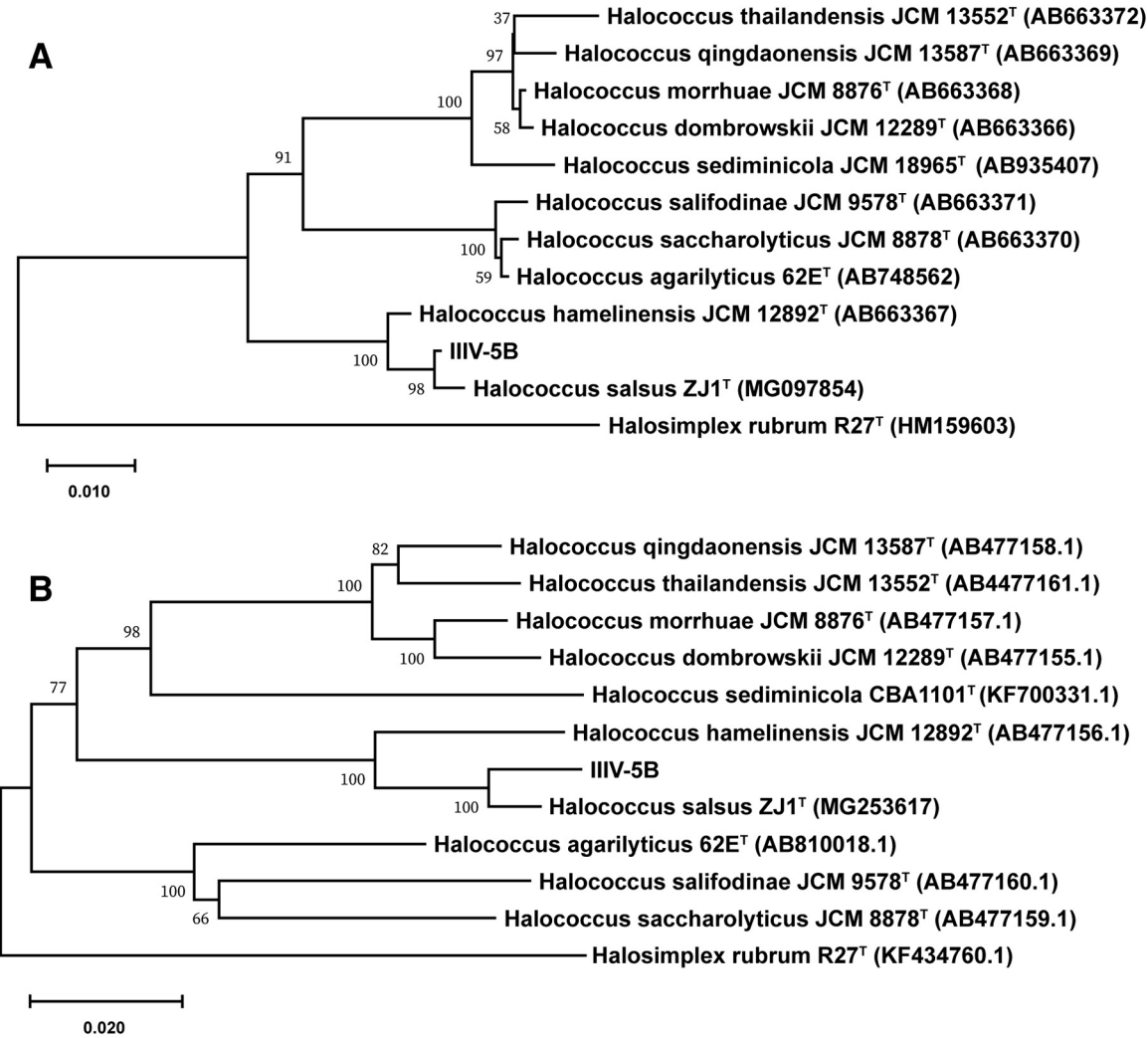


FIG 1 Neighbor-joining phylogenetic tree of the 16S rRNA (A) and *rpoB'* (B) gene sequences of *Halococcus* spp. and strain IIIV-5B using the p-distance model generated with MEGA X. Bootstrap values as percentages of 1,000 are shown. The bar represents 1 substitution per every 10 nucleotides. *Halosimplex rubrum* R27^T was used as the outgroup.

distances of 16S rRNA and *rpoB*' gene sequences between IIIV-5B and closely related strains (Fig. 1A and B). Default parameters were used for all software. The 16S rRNA gene of IIIV-5B was found to be 99.5% identical to that of *Halococcus salsus* ZJ1. Phylogenetic analysis of the *rpoB*' gene suggests that strain IIIV-5B might represent a new species of *Halococcus*.

Data availability. The draft genome sequence of strain IIIV-5B has been deposited in GenBank under the accession number [ZQWE00000000](https://www.ncbi.nlm.nih.gov/nuclseq/ZQWE00000000). The raw data have been deposited in the SRA under the accession number [PRJNA490534](https://www.ncbi.nlm.nih.gov/sra/PRJNA490534).

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