

# Genome Sequence of *Aeromicrobium erythreum* NRRL B-3381, an Erythromycin-Producing Bacterium of the *Nocardioideae*

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***Aeromicrobium erythreum* NRRL B-3381 has a 3,629,239-bp circular genome that has 72% G+C content. There are at least 3,121 coding sequences (CDSs), two rRNA gene operons, and 47 tRNAs. The genome and erythromycin (*ery*) biosynthetic gene sequences provide resources for metabolic and combinatorial engineering of polyketides.**

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*Arthrobacter* sp. strain NRRL B-3381 was part of a 1970 U.S. patent issued for an erythromycin process (1). Unlike other erythromycin processes, the NRRL B-3381 strain (isolated from Lajas Valley, Cabo Rojo, Puerto Rico) was notable for producing only erythromycin A and not the related compounds erythromycin B and C. From a large collection of industrially relevant actinobacteria, Sydney Brenner, then of the Medical Research Council (MRC) Molecular Genetics Unit, initiated a research program to genetically manipulate this nonfilamentous bacterium for polyketide combinatorial chemistry (2, 3). Since then, strain NRRL B-3381 has been taxonomically reclassified as the type genus and species *Aeromicrobium erythreum* (4), methods of plasmid transformation and gene disruption were developed (3, 5), and cosmid clones of *ery* (erythromycin) genes were isolated and sequenced (6). Although interesting metabolic manipulations of *A. erythreum* have been performed (7, 8), extensive uses of its polyketide synthase and other erythromycin biosynthesis genes have not been reported. Access to the complete genome sequence of the NRRL B-3381 strain may facilitate macrolide antibiotic development and other biotechnological uses of this and related *Actinobacteria* (9).

Total DNA was prepared using the Qiagen Gentra Puregene yeast/bact kit with overnight cultures of *A. erythreum* (collection strain designated AR18) grown at 30°C in 2xYT medium with shaking. Ten micrograms of purified DNA was processed with the Pacific Biosciences (PacBio) 10-kbp library kit in the NC State University Genomic Sciences Laboratory and then analyzed by single-molecule real-time (SMRT) RS II sequencing. Genome assembly was done with PacBio SMRT HGAP 2/Quiver using 75,640 reads with  $N_{50}$  of 5,716 bases (mean, 3,737 bases; 0.83 quality) totaling 282,723,636 bases. A total of 70,568 reads were mapped at 62.4× coverage to an assembled contig of 3,629,239 bases. No plasmid DNA was assembled. The genome is circular (by read overlap observations and PCR confirmation), has 72% G+C content, and is oriented in the GenBank file starting at 103 bases preceding the *dnaA* coding sequence (CDS).

The 3.6-Mbp genome was annotated with the NCBI Prokaryotic Genome Annotation Pipeline (10), with some manual cura-

tion. There are 3,121 CDSs, 270 pseudogenes needing further analysis, two rRNA operons (16S, 23S, and 5S), at least 47 tRNAs, and one potential transfer-messenger RNA (tmRNA). Methylation kinetics revealed m6A (N<sup>6</sup>-methyladenine), primarily at CTCCAG and CTGGAG (a *BpmI*-like site).

Sequences of the erythromycin-related genes, including those encoding the methyltransferase resistance enzyme (*ermR*) and the 65-kbp *ery* gene cluster, are essentially as previously reported (2, 6) (accession no. AY623658). The *ery* gene cluster includes three polyketide (6-deoxyerythronolide B) synthase modules (*eryAI—AIII*) and the methyltransferases, dehydratase, isomerase, sugar transferases, etc. leading to erythromycin A. *Aeromicrobium marinum* DSM 15272 (accession no. CM001024) encodes the most proteins currently orthologous to proteins of *A. erythreum*, and orthologs are also found from *Nocardioides simplex* VKM Ac-2033D (11).

The available *A. erythreum* NRRL B-3381 genome sequence should provide a resource for comparative and evolutionary genomics and, as suggested by Sydney Brenner 30 years ago, facilitate metabolic and combinatorial engineering of polyketide biosynthesis in a genetically tractable unicellular actinobacterium.

**Nucleotide sequence accession number.** The genome sequence has been deposited in GenBank with accession no. [CP011502](https://www.ncbi.nlm.nih.gov/nuccore/CP011502). The version described in this paper is the first version.

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