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Whole Genome Sequence of the Rifamycin B-Producing Strain *Amycolatopsis mediterranei* S699

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***Amycolatopsis mediterranei* S699 is an actinomycete that produces an important antibiotic, rifamycin B. Semisynthetic derivatives of rifamycin B are used for the treatment of tuberculosis, leprosy, and AIDS-related mycobacterial infections. Here, we report the complete genome sequence (10.2 Mb) of *A. mediterranei* S699, with 9,575 predicted coding sequences.**

Amycolatopsis mediterranei S699 is derived from the original strain that was isolated in 1957 from a soil sample from a pine arboretum, France (9). Rifamycins were isolated from the fermentation culture of this strain in 1957 at Lepetit Laboratory in Milan (11). It was first named *Streptomyces mediterranei* (9), later *Nocardia mediterranea* (13), and finally *Amycolatopsis mediterranei* (8). The significance of *A. mediterranei* S699 lies in its ability to produce an important antibiotic, rifamycin B. While semisynthetic derivatives of rifamycin B are used against *Mycobacterium tuberculosis* and *Mycobacterium leprae*, the causative agents of tuberculosis and leprosy, respectively, they are also active against variety of other organisms, including those causing AIDS-related mycobacterial infections (4, 14).

A. mediterranei S699 has been the focus of extensive research to explore the genetics of rifamycin biosynthesis (1, 2, 5, 7, 10, 12, 15), making it one of the most thoroughly studied strains. However, as of today, the genome sequence of only one rifamycin producer, *A. mediterranei* U32, which produces rifamycin SV, is available (16). Here we present the complete genome sequence of *A. mediterranei* S699.

Whole-genome sequencing of strain S699 was performed by using the Roche 454 system (GS20 version) and Sanger shotgun sequencing. The hybrid assembly of Sanger reads and pyrosequencing data using Phrap resulted in 386 contigs. Subsequently, sequence gaps were filled by primer walking, transposon mutagenesis, and complete sequencing of linker clones using Roche 454 (GS FLX). The final assembly, which consisted of 1,993,024 reads, was done with the MIRA3 assembler by mapping to the reference genome of *A. mediterranei* U32 (GenBank accession number CP002000). The assembly with an average sequencing depth of ~23× was validated *in silico* using BACCardI (3). Annotation was done at the NCBI-Prokaryotic Genomes Automatic Annotation Pipeline (PGAAP) (http://www.ncbi.nlm.nih.gov/genomes/static/Pipeline.html).

The complete genome sequence of *A. mediterranei* S699 contains a single circular 10,236,779-bp chromosome with a GC content of 71.3%. There are 9,575 coding sequences (CDSs) falling into 6,883 functional COGs (clusters of orthologous groups). The coding density is 90%, with an average CDS length of 954 bp. A total of 52 tRNA and 4 rRNA operons are present. Besides the published 90-kb cluster of *rif* genes, which includes a set of five large open reading frames encoding type I polyketide synthase (PKS) (2, 10), five other PKS clusters were observed. Twelve nonribosomal peptide synthases (NRPSs) and three hybrid NRPS/PKS clusters were also identified. Additionally, 341 CpG islands, 58 transposons, 1,148 tandem repeats, and five CRISPR elements are present. The genome sequence of *A. mediterranei* S699 is highly similar to that of *A. mediterranei* U32 with respect to nucleotide identity (>99%) and gene order.

Overproduction of rifamycin in *A. mediterranei* using traditional approaches of strain improvement appears to have attained a plateau (6). However, the availability of genome sequences of S699 and U32 will further improve our understanding of rifamycin biosynthesis, which in turn may provide new knowledge that will help improve rifamycin production and generate rifamycin analogs to combat rapidly emerging multidrug-resistant microorganisms.

Nucleotide sequence accession number. The genome sequence of *Amycolatopsis mediterranei* S699 has been deposited in GenBank under accession number CP002896.

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