Improved De Novo Draft Genome Sequence of the Nocavionin-Producing Type Strain *Nocardia terpenica* IFM 0706 and Comparative Genomics with the Closely Related Strain *Nocardia terpenica* IFM 0406

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ABSTRACT We report an improved de novo draft genome sequence of the human-pathogenic strain *Nocardia terpenica* IFM 0706 T. The resequencing unveiled that the genome size is larger than anticipated, reducing significantly the number of contigs and building a basis for comparison with the closely related strain *N. terpenica* IFM 0406.

Strain IFM 0706 T (=JCM13033 T=DSM44935 T=NBRC100888 T) was isolated in the 1990s from a nocardiosis patient and was originally identified as a *Nocardia brasiliensis* strain. Together with the strain *N. brasiliensis* IFM 0406 (1), it was recognized as a new species and reclassified as *Nocardia terpenica*, with IFM 0706 representing the corresponding type strain of this new species (2). Recently, IFM 0706 T was shown to produce the antibiotic nocavionin (3).

Within the course of our genome-driven investigations of *Nocardia* strains (4–6), we noted that a genome sequence of IFM 0706 T was available under its synonymous designation *N. terpenica* NBRC 100888 T (GenBank accession number BAI10000000.1). However, an annotation was missing, and the genome is highly fragmented. In addition, in comparison with closely related strains (4, 7), we hypothesized that the genome size of 8.63 Mbp might be too small. In order to close the significant genomic gaps and to increase the genomic resolution focused on secondary metabolism, we resequenced the genome of strain IFM 0706 T.

For genomic DNA isolation, a ZR Quick-DNA fungal/bacterial DNA miniprep kit (Zymo Research, Irvine, CA, USA) was used according to the manufacturer’s protocol, except that the vortexing step was reduced from 15 to 5 min and conducted at maximum speed. The DNA was sheared using a Covaris g-TUBE, and the genomic library was prepared according to the standard PacBio 6-kb multiplex protocol, followed by size selection with the BluePippin size selection system (Sage Science, Inc.). The library was sequenced on a PacBio Sequel instrument using v3.0 chemistry, including Sequel Polymerase v3.0 and one single-molecule real-time (SMRT) cell v3, resulting in 321,329 reads with a median read length of 4,523 bp. No quality filtering was conducted; however, subreads shorter than 50 bp were discarded. The remaining PacBio long reads were assembled using SMRTLink v7.0.1 and HGAP4 (8, 9). All software settings were kept at their default, except for the HGAP4 genome size estimate parameter, which was set to 9 Mbp. Overall, the reads were assembled to a 9,269,950-nucleotide draft genome at 142-fold coverage. The resulting sequence consists of 5 contigs with a G+C content of 68.52%. Gene functional annotation using PGAP v4.11 (10) identified 8,402 coding genes.

In summary, the resequencing of strain IFM 0706 T enabled us to increase the quantity (from 8.63 Mbp to 9.27 Mbp) and quality of genomic information, to signifi-
cantly reduce the number of contigs (from 4,460 down to 5), to correct the G+C content (from 68.30 to 68.52%), and to provide the annotation.

Sequence alignment of IFM 0706\(^*\) with its closely related strain \(N.\) \textit{terpenica} IFM 0406 (GenBank accession number LWGR00000000.1\), employing Mauve \(\text{snapshot}_{2015-02-25}\) (using the progressiveMauve algorithm) (11, 12), showed six large blocks of correspondence, confirming the overall relatedness of the two genomes, with the exception of multiple deletion/insertion segments (Fig. 1). In comparison to IFM 0406, the genome of IFM 0706\(^*\) contains an additional 95,215 bases (1.03%) and lacks 114,674 bases (1.24%). Further genomic indices were determined as follows: the average nucleotide identity (using autoMLST \([1.24\%]\)). Further genomic indices were determined as follows: the average nucleotide identity (using autoMLST \([13]\), digital DNA-DNA hybridization, and difference in G+C content (Genome-to-Genome Distance Calculator v2.1, applying formula 2 \([14]\)) between the strains were 100% (Mash distance, 0.0002), 99.50%, and 0.01% (68.51 versus 68.52%), respectively. This corroborated the similarities between the strains and confirmed that they belong to the same species. These findings are also reflected in the biosynthetic potential of both strains to produce secondary metabolites. Bioinformatics analysis using antiSMASH v5.1 (15) revealed that IFM 0706\(^*\) shares largely the same type of biosynthetic gene clusters (BGCs) with IFM 0406. In addition to 35 orphan BGCs, these include the BGCs for brasilinolide (16), terpenibactin (6), nocavionin (3), and basilicardin A (1, 5).

Data availability. This whole-genome sequencing project has been deposited at DDBJ/ENA/GenBank under the accession number \(\text{JABMCZ0000000000}\). The corresponding raw sequencing data set has been registered in the NCBI SRA database under the accession number \(\text{SRR11861893}\).

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