Complete Genome Sequence of *Amycolicococcus subflavus* DQS3-9A1^T, an Actinomycete Isolated from Crude Oil-Polluted Soil[∇]

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***Amycolicococcus subflavus* DQS3-9A1^T, isolated from crude oil-polluted soil in the Daqing Oilfield in China, is a type strain of a newly published novel species in the novel genus *Amycolicococcus*. Here we report the complete genome of DQS3-9A1^T and genes associated with oil-polluted environment.**

Crude oil contamination is a selective pressure to which microorganisms must adapt, since oil pollution in general causes low oxygen and water availability. Bacteria growing in oil-polluted environments are often thought to be useful for the bioremediation of hydrocarbon-polluted environments. Among the novel species isolated from two crude oil-polluted soils in the Daqing and Shengli oilfields in China (3, 5, 9, 10, 12) is an actinomycete, *Amycolicococcus subflavus* DQS3-9A1^T (= DSM 45089^T = CGMCC 4.3532^T), a type strain of a newly published novel species in the novel genus *Amycolicococcus*.

The complete genome sequence of *Amycolicococcus subflavus* DQS3-9A1^T was determined by a combined strategy of 454 sequencing (7) and Solexa paired-end sequencing technology (2). Genomic libraries containing 8-kb inserts were constructed. A total of 1,207,966 paired-end reads and 314,516 single-end reads were produced with the GS FLX system, giving a 74-fold coverage of the genome. Many (84.45%) of the reads, including 34 nonredundant contigs, were assembled into five large scaffolds by using the 454 Newbler assembler (454 Life Sciences, Branford, CT). A total of 3,948,580 reads (3-kb library) were generated to reach an 81-fold depth of coverage with an Illumina Solexa IIx genome analyzer (Illumina, San Diego, CA) and mapped to the scaffolds using the Burrows-Wheeler alignment (BWA) tool (6). The gaps between the scaffolds were filled by sequencing PCR products using an ABI 3730 capillary sequencer. The genome was analyzed as described previously (4).

The complete genome of *Amycolicococcus subflavus* DQS3-9A1^T consists of a circular chromosome of 4,738,809 bp, with a G+C content of 62.24% and two plasmids. One plasmid (pAS9A-1) contains 17,897 bp, with a G+C content of 63.87%, and the other plasmid (pAS9A-2) contains 106,784 bp, with a

G+C content of 61.86%. There are 4,585 putative open reading frames (ORFs) in the chromosome, with 27 and 121 putative ORFs in pAS9A-1 and pAS9A-2, respectively. Also, 45 tRNAs and 3 rRNA operons were identified in the chromosome.

Genes involved in hydrocarbon degradation pathways were found in the genome. A cluster of *alkBGT* genes was identified in DQS3-9A1^T. It encoded an integral membrane alkane hydroxylase (AlkB) (AS9A_3799), two rubredoxins (AlkG) (AS9A_3797, AS9A_3798), and a rubredoxin reductase (AlkT) (AS9A_3796), which showed the same arrangement of genes as that in *Rhodococcus* spp. (1, 8, 11). Two other *alkB*-homologous genes were also found in the genome (AS9A_2113, AS9A_2121), without genes encoding rubredoxin or rubredoxin reductase in the flanking region. In addition, DQS3-9A1^T contains genes encoding alcohol dehydrogenase (AlkJ), aldehyde dehydrogenase (AlkH), and fatty acid-coenzyme A ligase (AlkK), involved in the alkane degradation pathway. The results revealed that DQS3-9A1 might be an alkane-degrading strain and could be a putative candidate for bioremediation of crude oil-contaminated environments.

Nucleotide sequence accession numbers. The nucleotide sequence of *Amycolicococcus subflavus* DQS3-9A1^T has been deposited in the GenBank database under accession numbers CP002786 (chromosome), CP002787 (pAS9A-1), and CP002788 (pAS9A-2).

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REFERENCES

1. Amouric, A., et al. 2009. Identification of different alkane hydroxylase systems in *Rhodococcus ruber* strain SP2B, an hexane-degrading actinomycete. *J. Appl. Microbiol.* **108**:1903–1916.
2. Bentley, D. R., et al. 2008. Accurate whole human genome sequencing using reversible terminator chemistry. *Nature* **456**:53–59.
3. Cai, M., et al. 27 August 2010. *Salinarimonas ramus* sp. nov. and *Tessara-coccus oleiagri* sp. nov., isolated from a crude oil contaminated saline soil. *Int. J. Syst. Evol. Microbiol.* [Epub ahead of print.] doi:10.1099/ijs.0.025932-0.
4. Feng, L., et al. 2008. A recalibrated molecular clock and independent origins for the cholera pandemic clones. *PLoS One* **3**:e4053.
5. Gu, J., et al. 2007. *Marinobacter gudaonensis* sp. nov., isolated from an oil-polluted saline soil in a Chinese oilfield. *Int. J. Syst. Evol. Microbiol.* **57**:250–254.
6. Li, H., and R. Durbin. 2010. Fast and accurate long-read alignment with Burrows-Wheeler transform. *Bioinformatics* **26**:589–595.
7. Margulies, M., et al. 2005. Genome sequencing in microfabricated high-density picolitre reactors. *Nature* **437**:376–380.

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8. **McLeod, M. P., et al.** 2006. The complete genome of *Rhodococcus* sp. RHA1 provides insights into a catabolic powerhouse. *Proc. Natl. Acad. Sci. U. S. A.* **103**:15582–15587.
9. **Wang, Y. N., et al.** 2007. *Halomonas shengliensis* sp. nov., a moderately halophilic, denitrifying, crude-oil-utilizing bacterium. *Int. J. Syst. Evol. Microbiol.* **57**:1222–1226.
10. **Wang, Y. N., et al.** 2007. *Halomonas gudaonensis* sp. nov., isolated from a saline soil contaminated by crude oil. *Int. J. Syst. Evol. Microbiol.* **57**:911–915.
11. **Whyte, L. G., et al.** 2002. Gene cloning and characterization of multiple alkane hydroxylase systems in *Rhodococcus* strains Q15 and NRRL B-16531. *Appl. Environ. Microbiol.* **68**:5933–5942.
12. **Wu, X. L., et al.** 2009. *Filomicrobium insigne* sp. nov., isolated from an oil-polluted saline soil. *Int. J. Syst. Evol. Microbiol.* **59**:300–305.