Genome Sequence of the Abyssomicin- and Proximicin-Producing Marine Actinomycete *Verrucosispora maris* AB-18-032[∇]

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Verrucosispora maris AB-18-032 is a marine actinomycete that produces atrop-abyssomicin C and proximicin A, both of which have novel structures and modes of action. In order to understand the biosynthesis of these compounds, to identify further biosynthetic potential, and to facilitate rational improvement of secondary metabolite titers, we have sequenced the complete 6.7-Mb genome of Verrucosispora maris AB-18-032.

Verrucosispora maris AB-18-032 was isolated from a deep marine sediment sample collected from the East Sea (Sea of Japan) (3, 9). It produces unique polycyclic type 1 polyketide antibiotics, namely, the abyssomicins, which are the first known natural-product inhibitors of the para-aminobenzoic acid biosynthetic pathway (6, 7). In particular, atrop-abyssomicin C is active against Gram-positive bacteria, including Bacillus subtilis, methicillin-resistant Staphylococcus aureus and vancomycin-resistant S. aureus (6, 7, 9), and Mycobacterium tuberculosis (5). The strain also produces proximicin A, a furan analogue of netropsin with novel antitumor properties (4, 10). Here, we announce the genome sequence of Verrucosispora maris AB-18-032, the first member of this genus to be sequenced, which should provide fundamental understanding of the biosynthesis of these antibiotics and facilitate bioengineering and biodiscovery efforts involving this strain.

The complete genome of *Verrucosispora maris* AB-18-032 was sequenced using Roche 454 genome sequencer (GS) FLX (9.4-, 9.9-, 6.7-, and 6.8-times coverage of single-end reads and 13.3-times coverage of paired-end reads having 3-kb inserts) and Solexa (Illumina genome analyzer [GA], 43-times coverage with ~350-bp inserts and paired-end reads) sequencing technology. To combine the GA library with 454 GS FLX libraries, reads were assembled into 1,730 contigs using the ABySS 1.20 assembler (11), and the contigs were shredded into 15,379 fake reads. The fake reads and 454 GS FLX reads were assembled into 5 scaffolds (116 contigs) by using a combination of Newbler gsAssembler 2.3 (454 Life Sciences, Branford, CT) and CABOG assembler (8). The actual order of the scaffolds was determined by a series of PCRs based on a permutation table, and gaps were filled by primer-walking PCR followed by sequencing using an ABI 3730

system (Applied Biosystems, CA). Finally, Polisher software (Alla Lapidus, unpublished data) was used with the Illumina data to correct for homopolymers in the 454 reads and increase consensus quality. Annotation was performed by the RAST server (2) and the NCBI Prokaryotic Genomes Automatic Annotation Pipeline (PGAAP) (http://www.ncbi.nlm.nih.gov/genomes/static /Pipeline.html), and both annotations were scrutinized by manual inspection based on BLAST searches (1).

The genome of *Verrucosispora maris* AB-18-032 has a single circular chromosome of 6,673,976 bp with a GC content of 70.9%. A circular plasmid of 58,295 bp with a GC content of 70.3%, pVMKU, was also identified. The chromosome has 5,947 coding sequences (CDS), 51 tRNA genes, and 9 rRNA genes, and the plasmid has 55 CDS.

Nucleotide sequence accession numbers. The complete genome sequence of *Verrucosispora maris* AB-18-032 has been deposited in NCBI GenBank under accession numbers CP002638 and CP002639.

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REFERENCES

- 1. Altschul, S. F., et al. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25:3389–3402
- ation of protein database search programs. Nucleic Acids Res. 25:3389–3402.

 2. Aziz, R. K., et al. 2008. The RAST Server: rapid annotations using subsystems technology. BMC Genomics 9:75.
- Bister, B., et al. 2004. Abyssomicin C-A polycyclic antibiotic from a marine *Verrucosispora* strain as an inhibitor of the *p*-aminobenzoic acid/ tetrahydrofolate biosynthesis pathway. Angew. Chem. Int. Ed. Engl. 43: 2574–2576.
- Fiedler, H-P., et al. 2008. Proximicin A, B and C, novel aminofuran antibiotic and anticancer compounds isolated from marine strains of the actinomycete Verrucosispora. J. Antibiot. (Tokyo) 61:158–163.
- Freundlich, J. S., et al. 2010. The abyssomicin C family as in vitro inhibitors of Mycobacterium tuberculosis. Tuberculosis 90:298–300.
- 6. Keller, S., et al. 2007. Abyssomicins G and H and atrop-abyssomicin C from

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3392 GENOME ANNOUNCEMENTS J. BACTERIOL.

the marine Verrucosispora strain AB-18-032. J. Antibiot. (Tokyo) 60:391-

- Keller, S., H. S. Schadt, I. Ortel, and R. D. Sussmuth. 2007. Action of atrop-abyssomicin C as an inhibitor of 4-amino-4-deoxychorismate synthase
- PabB. Angew. Chem. Int. Ed. Engl. 46:8284–8286.
 Miller, J. R., et al. 2008. Aggressive assembly of pyrosequencing reads with mates. Bioinformatics 24:2818–2824.
 Riedlinger, J., et al. 2004. Abyssomicins, inhibitors of the para-aminobenzoic
- acid pathway produced by the marine Verrucosispora strain AB-18-032. J. Antibiot. (Tokyo) 57:271-279.
- Schneider, K., et al. 2008. Proximicins A, B, and C—antitumor furan analogues of netropsin from the marine actinomycete *Verrucosispora* induce upregulation of p53 and the cyclin kinase inhibitor p21. Angew. Chem. Int. Ed. Engl. 47:3258–3261.
- Ed. Engr. 47.3236–3201.
 Simpson, J. T., et al. 2009. ABySS: a parallel assembler for short read sequence data. Genome Res. 19:1117–1123.