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GENOME ANNOUNCEMENT

Draft Genome of *Streptomyces tsukubaensis* NRRL 18488, the Producer of the Clinically Important Immunosuppressant Tacrolimus (FK506)

Carlos Barreiro,[a] Carlos Prieto,[a] Alberto Sola-Landa,[a] Elena Solera,[a] Miriam Martínez-Castro,[b] Rosario Pérez-Redondo,[a] Carlos García-Estrada,[a] Jesús F. Aparicio,[a,b] Lorena T. Fernández-Martínez,[a] Javier Santos-Aberturas,[b] Zahra Salehi-Najafabadi,[b] Antonio Rodríguez-García,[a,b] Andreas Tauch,[c] and Juan F. Martín[b]

INBIOTEC (Instituto de Biotecnología de León), Parque Científico de León, León, Spain[a]; Área de Microbiología, Departamento de Biología Molecular, Universidad de León, Campus de Vegazana, León, Spain[b]; and Institute for Genome Research and Systems Biology, Center for Biotechnology, Bielefeld University, Bielefeld, Germany[c]

The macrocyclic polyketide tacrolimus (FK506) is a potent immunosuppressant that prevents T-cell proliferation produced solely by *Streptomyces* species. We report here the first draft genome sequence of a true FK506 producer, *Streptomyces tsukubaensis* NRRL 18488, the first tacrolimus-producing strain that was isolated and that contains the full tacrolimus biosynthesis gene cluster.

The genus *Streptomyces* includes high G+C Gram-positive bacteria, which present a characteristic life cycle closely connected with their ability to produce secondary metabolites (5). These bacteria are among the most ubiquitous soil microorganisms (1). *Streptomyces tsukubaensis* was isolated in 1984 from a soil sample obtained from the Tsukuba region of Japan, and it became relevant due to its ability to produce the potent immunosuppressant agent tacrolimus (FK506) (3), which is a commercially available and clinically used anti-allograft rejection drug. Initially, it was deposited as strain no. 9993, and later it was redeposited in the ARS culture collection under the accession number NRRL 18488. Tacrolimus yielded $2,340 million in profits in 2011 (http://www.evaluatepharma.com).

Genomic DNA from *S. tsukubaensis* NRRL 18488 was extracted according to the Kirby method (2) from a 60-h culture (stationary phase) in order to avoid bias in the distribution of reads through the chromosome due to its replication. The sequencing process was done by means of a 3-kbp paired-end library on the GS FLX sequencer (Roche). Two runs were carried out that yielded 158.8 and 153.4 Mbp. Gap closure was performed by means of a 1/4 plate shotgun Titanium 454 sequencing round (Roche), which yielded 86.0 Mbp of DNA sequence. In total, it gives a 52× genome final coverage. Reads were assembled using the Newbler Assembler software (version 2.3; Roche) into 14 scaffolds. A manual validation was carried out for specific genes (e.g., the tacrolimus gene cluster) and repetitive regions (e.g., rRNAs) using DNA sequences from an ordered cosmid library of *S. tsukubaensis*, as described by Martínez-Castro and coworkers for *Streptomyces tacrolimicus* (4). Thus, a final unique linear chromosome of 7.62 Mbp (71.52% G+C content), which is within the size range reported for other streptomycetes, and two circular plasmids, pSTS1 and pSTS2, of 24.7 (70.04% G+C) and 31.1 (71.13% G+C) kbp, respectively, were obtained. In addition, the 5′-telomere was defined by rapid amplification of cDNA ends (RACE) methodology and showed a conformation similar to that of other *Streptomyces* species (7) but with a more robust seven-hairpin structure. Genome annotation was performed at the NCBI Prokaryotic Genomes Automatic Annotation Pipeline (PGAAP) (http://www.ncbi.nlm.nih.gov/genomes/static/Pipeline.html), with the identification of 6,623 protein-coding genes, 6 rRNA operons (18 genes), 68 tRNAs, and 52 sigma factors.

The genome mining showed, in addition to the tacrolimus gene cluster that is fully sequenced and publicly available for the first time, several secondary metabolite gene clusters, including four type I PKS (polyketide synthase), two type II PKS, one type III PKS, three NRPS (non ribosomal peptide synthetase), three hybrid PKS/NRPS, eight terpene, six lantibiotic, and three siderophore gene clusters. They were identified and annotated by means of antiSMASH and NRPSsp servers (6, 8). This vast arsenal of secondary metabolite genes points to *S. tsukubaensis* as a highly profitable industrial microorganism, even beyond its use in tacrolimus production.

Nucleotide sequence accession numbers. This Whole Genome Shotgun project (chromosome and both plasmids) has been deposited at DDBJ/EMBL/GenBank under the accession number AJSZ00000000. The version described in this paper is the first version, AJSZ01000000.

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Address correspondence to Juan F. Martín, jf.martin@unileon.es, or Carlos Barreiro, c.barreiro@unileon.es.

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REFERENCES

1. **Hodgson DA.** 2000. Primary metabolism and its control in streptomycetes: a most unusual group of bacteria. Adv. Microb. Physiol. **42**:47–238.
2. **Kieser T, Bibb MJ, Buttner MJ, Chater KF, Hopwood DA.** 2000. Practical *Streptomyces* genetics. The John Innes Foundation, Norwich, United Kingdom.
3. **Kino T, et al.** 1987. FK-506, a novel immunosuppressant isolated from a *Streptomyces*. I. Fermentation, isolation, and physico-chemical and biological characteristics. J. Antibiot. (Tokyo) **40**:1249–1255.
4. **Martínez-Castro M, Solera E, Martín JF, Barreiro C.** 2009. Efficient pyramidal arrangement of an ordered cosmid library: rapid screening of genes of the tacrolimus-producer *Streptomyces* sp. ATCC 55098. J. Microbiol. Methods **78**:150–154.
5. **Martín JF, Liras P.** 2010. Engineering of regulatory cascades and networks controlling antibiotic biosynthesis in *Streptomyces*. Curr. Opin. Microbiol. **13**:263–273.
6. **Medema MH, et al.** 2011. antiSMASH: rapid identification, annotation and analysis of secondary metabolite biosynthesis gene clusters in bacterial and fungal genome sequences. Nucleic Acids Res. **39**(Web Server issue): W339–W346.
7. **Ohnishi Y, et al.** 2008. Genome sequence of the streptomycin-producing microorganism *Streptomyces griseus* IFO 13350. J. Bacteriol. **190**:4050–4060.
8. **Prieto C, García-Estrada C, Lorenzana D, Martín JF.** 2012. NRPSsp: non-ribosomal peptide synthase substrate predictor. Bioinformatics **28**: 426–427.