

Complete Genome Sequence of Thiostrepton-Producing *Streptomyces laurentii* ATCC 31255

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***Streptomyces laurentii* ATCC 31255 produces thiostrepton, a thiopeptide class antibiotic. Here, we report the complete genome sequence for this strain, which contains a total of 8,032,664 bp, 7,452 predicted coding sequences, and a G+C content of 72.3%.**

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Streptomyces species are Gram-positive aerobic mycelial bacteria belonging to the family *Streptomycetaceae* (1). These bacteria have the ability to produce a wide variety of secondary metabolites, including antibiotics (2), and are also interesting subjects with which to study morphological differentiation (3). Pock formation involving conjugative plasmids is a typical physiological characteristic used for morphological differentiation of *Streptomyces* species (4), which contain both plasmids and a large linear chromosome.

Streptomyces laurentii ATCC 31255 was classified as a thiostrepton producer not involving *Streptomyces azureus*, *Streptomyces hawaiiensis*, or *Streptomyces* sp. strain X-14b (5). It is distinct from the *Streptomyces fradiae* group (5), and *Streptomyces terrimum* and *Streptomyces roseofulvus* are contained within the *S. laurentii* group (1). The thiostrepton resistance gene (*tsr*) is a selective marker often used in the genetic engineering of actinomycetes and was isolated from the strain (6). We previously reported that *S. laurentii* contains a linear conjugative plasmid, pSLL (7), and circular integrative plasmid, pSLS (8). pSLL suppressed the injurious effects caused by pSLS, which include marked decreases in spore formation and thiostrepton productivity. The whole-genome sequence of *S. laurentii* may shed light on the mechanisms of spore formation and thiostrepton production and may also be useful for comparative studies of morphological and metabolic differentiation among thiostrepton-producing *Streptomyces* species.

A sample was prepared for sequencing by growing *S. laurentii* ATCC 31255 aerobically overnight at 28°C in tryptic soy broth (TSB) (Oxoid). The genomic DNA was then extracted and purified, as we described previously (9). The prepared genome was sequenced using the PacBio RSII platform; 150,292 raw reads resulted in 100,282 quality-filtered trimmed reads, yielding 741 Mb, with a mean genome-wide coverage of 77.43×. The filtered reads were assembled using HGAP version 2.3.0 (10) and resulted in a 1-contig scaffold. Annotation was performed using Microbial Genome Annotation Pipeline (MiGAP; <http://www.migap.org/>).

The genome of *S. laurentii* ATCC 31255 includes a linear chromosome, with a G+C content of 72.3%. Annotation using the COG, RefSeq, and TrEMBL databases with tRNAscan-SE version

1.23 and additional manual inspection revealed 7,452 predicted coding regions, 69 tRNA genes, and 7 rRNA genes.

Terminal inverted repeats are contained at the end of the chromosome of *S. laurentii*. In addition, a gene cluster for thiostrepton biosynthesis was annotated from genes SLA3859 (*tsrS*) to SLA3877 (*tsrA*) (11). This cluster shows strong similarity to the thiostrepton biosynthesis cluster of *S. azureus* (12). The *tsr* gene is located between the EF-Tu gene and the *N*-acetylmuramoyl-L-alanine amidase family protein-coding gene. One copy of the pSLS sequence is integrated between SLA2930 and SLA2952, and a second copy is between SLA3776 and SLA3796. Plasmid pSLL does not exist in free form within its host cell.

Nucleotide sequence accession number. The *S. laurentii* ATCC 31255 genome sequence and annotation data have been deposited in the DDBJ/EMBL/GenBank under accession no. AP017424.

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REFERENCES

- Kämpfer P, Glaeser SP, Parkes L, van Keulen G, Dyson P. 2014. The family *Streptomycetaceae*, p 889–1010. In Rosenberg E, DeLong E, Lory S, Stackebrandt E, Thompson F (ed), *The prokaryotes*. Springer Verlag, Heidelberg, Germany.
- Omura S. 1992. The expanded horizon for microbial metabolites—a review. *Gene* 115:141–149. [http://dx.doi.org/10.1016/0378-1119\(92\)90552-Z](http://dx.doi.org/10.1016/0378-1119(92)90552-Z).
- Chandra G, Chater KF. 2014. Developmental biology of *Streptomyces* from the perspective of 100 actinobacterial genome sequences. *FEMS Microbiol Rev* 38:345–379. <http://dx.doi.org/10.1111/1574-6976.12047>.
- Doi K, Ohyama Y, Yokoyama E, Nishiyama T, Fujino Y, Nagayoshi Y, Ohshima T, Ogata S. 2012. Expression analysis of the *spi* gene in the pock-forming plasmid pSA1.1 from *Streptomyces azureus* and localization of its product during differentiation. *Appl Microbiol Biotechnol* 95:707–716. <http://dx.doi.org/10.1007/s00253-012-4000-9>.

5. Trejo WH, Dean LD, Pluscec J, Meyers E, Brown WE. 1977. *Streptomyces laurentii*, a new species producing thiostrepton. *J Antibiot* **30**: 639–643. <http://dx.doi.org/10.7164/antibiotics.30.639>.
6. Wada K, Kobayashi J, Furukawa M, Doi K, Ohshiro T, Suzuki H. 2016. A thiostrepton resistance gene and its mutants serve as selectable markers in *Geobacillus kaustophilus* HTA426. *Biosci Biotechnol Biochem* **80**: 368–375. <http://dx.doi.org/10.1080/09168451.2015.1079478>.
7. Kinoshita-Iramina C, Kitahara M, Doi K, Ogata S. 1997. A conjugative linear plasmid in *Streptomyces laurentii* ATCC 31255. *Biosci Biotechnol Biochem* **61**:1469–1473. <http://dx.doi.org/10.1271/bbb.61.1469>.
8. Sakemi H, Nishitake S, Rodprapakorn M, Shirakami T, Takechi S, Doi K, Ogata S. 2003. Nucleotide sequence of conjugative and integrating plasmid pSLS from *Streptomyces laurentii* ATCC 31255. *J Fac Agric Kyushu Univ* **47**: 407–417.
9. Doi K, Sacki MC, Ono Y, Ogata S. 1995. Plasmid formation and its relation to the formation of spontaneously developing pocks in *Streptomyces azureus* ATCC 14921. *J Appl Microbiol* **79**:237–251.
10. Chin CS, Alexander DH, Marks P, Klammer AA, Drake J, Heiner C, Clum A, Copeland A, Huddleston J, Eichler EE, Turner SW, Korlach J. 2013. Nonhybrid, finished microbial genome assemblies from long-read SMRT sequencing data. *Nat Methods* **10**:563–569. <http://dx.doi.org/10.1038/nmeth.2474>.
11. Kelly WL, Pan L, Li C. 2009. Thiostrepton biosynthesis: prototype for a new family of bacteriocins. *J Am Chem Soc* **131**:4327–4334. <http://dx.doi.org/10.1021/ja807890a>.
12. Sakihara K, Maeda J, Tashiro K, Fujino Y, Kuhara S, Ohshima T, Ogata S, Doi K. 2015. Draft genome sequence of thiostrepton-producing *Streptomyces azureus* ATCC 14921. *Genome Announc* **3**(5):e01183-15. <http://dx.doi.org/10.1128/genomeA.01183-15>.