



# Whole-Genome Sequence of *Streptomyces kaniharaensis* Shomura and Niida SF-557

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**ABSTRACT** *Streptomyces kaniharaensis* is a Gram-positive bacterium that produces formycin A 5'-phosphate, a C nucleotide with antimicrobial and anticancer activity. Here, we report the sequencing, assembly, and annotation of the draft genome sequence of *Streptomyces kaniharaensis* Shomura and Niida.

*Streptomyces kaniharaensis*, isolated from soil, produces formycin A 5'-phosphate, a C nucleotide exhibiting antimicrobial activity (1). The metabolic pathway to formycin A 5'-phosphate is interesting because little is known about C nucleotide biosynthesis (2). Here, we present the draft genome sequence of *Streptomyces kaniharaensis* Shomura and Niida.

*Streptomyces kaniharaensis* Shomura and Niida (ATCC 21070) was purchased from ATCC (Middlesex, UK). Freeze-dried cells were dissolved in autoclaved tryptic soy broth (TSB) medium containing 25% glycerol. The culture (10  $\mu$ l) was plated onto TSB agar and incubated at 26°C for 15 days. After this time, one 0.5-inch-diameter mycelium was used to inoculate TSB liquid medium (50 ml). Incubation at 26°C for 5 more days and centrifugation (3,500 rpm) at 4°C gave cell pellets that were washed (2 $\times$ ) with deionized water and stored at -80°C. Genomic DNA extraction was performed using a PureLink genomic DNA minikit (Thermo Fisher, UK) following the protocol for Gram-positive bacteria. Repeating this procedure gave 17  $\mu$ g of genomic DNA ( $A_{260}/A_{280}$  1.9;  $A_{260}/A_{230}$  2.0), which was not sheared because the size range was suitable for library preparation. Genomic DNA (5  $\mu$ g) was size selected (6 to 50 kb) on a 0.75% cassette using the Sage Blue Pippin system, purified using AMPure beads (1:1 vol/vol), and checked for concentration (Qubit), and the size distribution was determined (Fragment Analyzer, Agilent). A PacBio DNA library was generated, which was sequenced on an RSII single-molecule real-time (SMRT) cell. PacBio reads were quality controlled on the basis of (i) productivity or yield of the cell, (ii) loading of zero-mode wave guides (ZMWs) based on a Poisson distribution, (iii) the length and quality of polymerase reads, and (iv) ensurance that the reads of each insert were equal to its expected size. PacBio reads were assembled using the Hierarchical Genome Assembly Process (HGAP) pipeline as embedded in the PacBio SMRT link software. Default parameters were used except where otherwise noted. Sequencing generated 13,396 preassembled reads with a mean read length of 7,063 bp. Raw reads were trimmed and filtered for high-quality bases. In total, 130,203 reads were aligned and mapped, leading to 1,470,565,355 mapped subread bases. A draft genome sequence of *Streptomyces kaniharaensis* was assembled from 19 polished contigs with an  $N_{50}$  contig length of 6,153,183 bp. The size of the assembled genome is 8,774,359 bp, and the G+C content is 72.15%. Annotation was carried out using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (3), which identified 8,035 genes, of which 7,532 coding DNA sequences (CDSs) were associated with proteins.

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Putative biosynthetic gene clusters (BGCs) were identified using PATRIC database tools (<https://www.patricbrc.org/>) (4) by searching the proteome (PATRIC identification number 212423.6) for ForH homologs (PDB number 6NKO) (5). The top hit was annotated as IMP cyclohydrolase (EC 3.5.4.10) (locus tag F7Q99\_03110), and analyses of proteins encoded by neighboring genes (locus tags F7Q99\_03100 to F7Q99\_03210) allowed us to identify the BGC for formycin A 5'-phosphate. Our assignment was later verified independently (6). Some enzymes in the BGC have been recently characterized, including ForT, which couples 4-amino-1H-pyrazole-3,5-dicarboxylic acid and phosphoribosyl pyrophosphate (PRPP) (7, 8), and ForI, a pyridoxal 5-phosphate (PLP)-dependent aminotransferase involved in pyrazole synthesis (9).

**Data availability.** The draft genome sequence of *Streptomyces kaniharaensis* Shomura and Niida (BioProject number PRJNA574488) has been deposited in GenBank under the accession number WBOF00000000. The raw reads (SRA numbers SRX7765393 and SRX7765394) are also available. DNA sequences for enzymes in the formycin A 5'-phosphate BGC can be found under the accession number NZ\_WBOF01000001 and spanned from RefSeq locus tag F7Q99\_03050 to F7Q99\_03210 (PATRIC fig|212423.6.regulatory.2 to fig|212423.6.peg.674).

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## REFERENCES

- Suhadolnik RJ. 1970. Nucleoside antibiotics. John Wiley & Sons, New York, NY.
- Ochi K, Yashima S, Eguchi Y, Matsushita K. 1979. Biosynthesis of formycin. Incorporation and distribution of  $^{13}\text{C}$ -,  $^{14}\text{C}$ -, and  $^{15}\text{N}$ -labeled compounds into formycin. *J Biol Chem* 254:8819–8824.
- Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Pruitt KD, Borodovsky M, Ostell J. 2016. NCBI Prokaryotic Genome Annotation Pipeline. *Nucleic Acids Res* 44: 6614–6624. <https://doi.org/10.1093/nar/gkw569>.
- Wattam AR, Brettin T, Davis JJ, Gerdes S, Kenyon R, Machi D, Mao C, Olson R, Overbeek R, Pusch GD, Shukla MP, Stevens R, Vonstein V, Warren A, Xia F, Yoo H. 2018. Assembly, annotation, and comparative genomics in PATRIC, the all bacterial bioinformatics resource center. *Methods Mol Biol* 1704:79–101. [https://doi.org/10.1007/978-1-4939-7463-4\\_4](https://doi.org/10.1007/978-1-4939-7463-4_4).
- Wang S-A, Ko Y, Zeng J, Geng Y, Ren D, Ogasawara Y, Irani S, Zhang Y, Liu H-W. 2019. Identification of the formycin A biosynthetic gene cluster from *Streptomyces kaniharaensis* illustrates the interplay between biological pyrazolopyrimidine formation and *de novo* purine biosynthesis. *J Am Chem Soc* 141:6127–6131. <https://doi.org/10.1021/jacs.9b00241>.
- Ko Y, Wang S-A, Ogasawara Y, Ruszczycky M, Liu H-W. 2017. Identification and characterization of enzymes catalyzing pyrazolopyrimidine formation in the biosynthesis of formycin A. *Org Lett* 19:1426–1429. <https://doi.org/10.1021/acs.orglett.7b00355>.
- Ren D, Wang S-A, Ko Y, Geng Y, Ogasawara Y, Liu H-W. 2019. Identification of the C-glycoside synthases during biosynthesis of the pyrazole-C-nucleosides formycin and pyrazofurin. *Angew Chem Int Ed Engl* 58: 16512–16516. <https://doi.org/10.1002/anie.201910356>.
- Zhang M, Zhang P, Xu G, Zhou W, Gao Y, Gong R, Cai Y-S, Cong H, Deng Z, Price NPJ, Mao X, Chen W. 2019. Comparative investigation into formycin A and pyrazofurin A biosynthesis reveals branch pathways for the construction of C-nucleoside scaffolds. *Appl Environ Microbiol* 86: e01971-19. <https://doi.org/10.1128/AEM.01971-19>.
- Gao S, Liu H, de Crécy-Lagard V, Zhu W, Richards NGJ, Naismith JH. 2019. PMP-diketopiperazine adducts form at the active site of a PLP dependent enzyme involved in formycin biosynthesis. *Chem Commun (Camb)* 55: 14502–14505. <https://doi.org/10.1039/c9cc06975e>.