

Genome Sequence of *Streptomyces griseus* Strain XylebKG-1, an Ambrosia Beetle-Associated Actinomycete[∇]

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***Streptomyces griseus* strain XylebKG-1 is an insect-associated strain of the well-studied actinobacterial species *S. griseus*. Here, we present the genome of XylebKG-1 and discuss its similarity to the genome of *S. griseus* subsp. *griseus* NBRC13350. XylebKG-1 was isolated from the fungus-cultivating *Xyleborinus saxeseni* system. Given its similarity to free-living *S. griseus* subsp. *griseus* NBRC13350, comparative genomics will elucidate critical components of bacterial interactions with insects.**

Streptomyces griseus is a soil bacterium known for its production of secondary metabolites, including streptomycin, the first effective antibiotic for tuberculosis (19). Here, we present the genome sequence of *Streptomyces griseus* strain XylebKG-1, which, to our knowledge, is the first strain of *S. griseus* associated with an insect. XylebKG-1 was isolated from the ambrosia beetle *Xyleborinus saxeseni*, which cultivates a fungus for food (2). *Actinobacteria*-specific isolations from both beetles and their fungal galleries resulted in isolation of XylebKG-1.

DNA from pure isolates was extracted using a bead-beating protocol (10), and the genome was sequenced at the DOE Joint Genome Institute (JGI). A noncontiguous finished genome of XylebKG-1 was generated using a shotgun approach employing a combination of Illumina (3) and 454 sequencing technologies (15). An Illumina shotgun library (69,927,062 reads totaling ~5.3 Gbp) and two 454 GS (FLX Titanium) shotgun libraries (688,595 standard reads and 172,566 20-kbp paired-end reads totaling ~352 Mbp) were sequenced and assembled. The 454 data were assembled using Newbler, version 2.3 (Roche), and Illumina sequencing data were assembled with Velvet, version 0.7.63 (20). All assemblies were integrated using parallel Phrap, version SPS D 4.24 (High Performance Software, LLC). Illumina data were used to correct potential base errors and increase consensus quality using the software program Polisher, developed at the JGI (Alla Lapidus, unpublished). Possible misassembled regions were corrected using gapResolution (Cliff Han, unpublished) or Dupfinisher (11) or by sequencing cloned bridging PCR fragments with subcloning. Gaps between contigs were closed by

editing in Consed (6–8), by PCR, and by Bubble PCR (J.-F. Cheng, unpublished) primer walks. The total size of the genome is 8,727,768 bp, and the final assembly is based on 352.4 Mbp of 454 sequence (38× coverage) and 5.3 Gbp of Illumina sequence (257× coverage).

The overall genome G+C content is 72.1%. Generation (<http://compbio.ornl.gov/generation/>), Glimmer (5), and Critica (version 1.05) (1) were used to predict a total of 7,265 candidate protein-encoding gene models. RNAmmer (13) annotated six 16S rRNAs and six 23S rRNAs. A tRNAscan-SE (14) search revealed 66 tRNAs corresponding to all 20 standard amino acids. Additional PRIAM (4), KEGG (12), and COG (18) analyses were completed, and the results can be accessed at <http://genome.ornl.gov/microbial/streACT1>.

Evidence for XylebKG-1 as a strain of *S. griseus* is based on similarity between the genomes of XylebKG-1 and the type strain *S. griseus* subsp. *griseus* NBRC13350 (16). Both have similar genome sizes (8.7 Mbp to 8.5 Mbp) and G+C contents (72.1% to 72.2%), six rRNA operons, and 66 tRNAs. An average nucleotide identity analysis conducted between both genomes using Jspecies (version 1.2.1) (17) revealed a 98.98 ANI_b value (91.68% genome alignment) and a 98.95 ANI_m value (94.13% genome alignment), indicating a species level degree of similarity (9). Given this similarity, XylebKG-1 represents a unique opportunity to study genetic elements involved in *Actinobacteria*-insect associations.

Nucleotide sequence accession number. The genome sequence of *Streptomyces griseus* strain XylebKG-1 has been deposited in GenBank under accession no. ADFC00000000.

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REFERENCES

1. **Badger, J. H., and G. J. Olsen.** 1999. CRITICA: coding region identification tool invoking comparative analysis. *Mol. Biol. Evol.* **16**:512–524.
2. **Batra, L. R.** 1967. *Ambrosia* fungi: a taxonomic revision, and nutritional studies of some species. *Mycologia* **59**:976–1017.
3. **Bennett, S.** 2004. Solexa Ltd. *Pharmacogenomics* **5**:433–438.
4. **Claudel-Renard, C., C. Chevalet, T. Faraut, and D. Kahn.** 2003. Enzyme-specific profiles for genome annotation: PRIAM. *Nucleic Acids Res.* **31**:6633–6639.
5. **Delcher, A. L., K. A. Bratke, E. C. Powers, and S. L. Salzberg.** 2007. Identifying bacterial genes and endosymbiont DNA with Glimmer. *Bioinformatics* **23**:673–679.
6. **Ewing, B., and P. Green.** 1998. Base-calling of automated sequencer traces using Phred. II. Error probabilities. *Genome Res.* **8**:186–194.
7. **Ewing, B., L. Hillier, M. C. Wendl, and P. Green.** 1998. Base-calling of automated sequencer traces using Phred. I. Accuracy assessment. *Genome Res.* **8**:175–185.
8. **Gordon, D., C. Abajian, and P. Green.** 1998. Consed: A graphical tool for sequence finishing. *Genome Res.* **8**:195–202.
9. **Goris, J., et al.** 2007. DNA-DNA hybridization values and their relationship to whole-genome sequence similarities. *Int. J. Syst. Evol. Microbiol.* **57**:81–91.
10. **Graff, A., and R. Conrad.** 2005. Impact of flooding on soil bacterial communities associated with poplar (*Populus* sp.) trees. *FEMS Microbiol. Ecol.* **53**:401–415.
11. **Han, C., and P. Chain.** 2006. Finishing repetitive regions automatically with Dupfinisher, p. 142–147. *In* H. Valafar (ed.), *Proceedings of the 2006 International Conference on Bioinformatics Computational Biology, BIOCOMP'06*. CSREA Press, Las Vegas, NV.
12. **Kanehisa, M., S. Goto, M. Furumichi, M. Tanabe, and M. Hirakawa.** 2010. KEGG for representation and analysis of molecular networks involving diseases and drugs. *Nucleic Acids Res.* **38**:D355–D360.
13. **Lagesen, K., et al.** 2007. RNAmmer: consistent and rapid annotation of ribosomal RNA genes. *Nucleic Acids Res.* **35**:3100–3108.
14. **Lowe, T. M., and S. R. Eddy.** 1997. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. *Nucleic Acids Res.* **25**:0955–0964.
15. **Margulies, M., et al.** 2005. Genome sequencing in microfabricated high-density picolitre reactors. *Nature* **437**:376–380.
16. **Ohnishi, Y., et al.** 2008. Genome sequence of the streptomycin-producing microorganism *Streptomyces griseus* IFO 13350. *J. Bacteriol.* **190**:4050–4060.
17. **Richter, M., and R. Rosselló-Móra.** 2009. Shifting the genomic gold standard for the prokaryotic species definition. *Proc. Natl. Acad. Sci. U. S. A.* **106**:19126–19131.
18. **Tatusov, R. L., M. Y. Galperin, D. A. Natale, and E. V. Koonin.** 2000. The COG database: a tool for genome-scale analysis of protein functions and evolution. *Nucleic Acids Res.* **28**:33–36.
19. **Waksman, S. A.** 1953. Streptomycin: background, isolation, properties, and utilization. *Science* **118**:259–266.
20. **Zerbino, D. R., and E. Birney.** 2008. Velvet: algorithms for de novo short read assembly using de Bruijn graphs. *Genome Res.* **18**:821–829.