JOURNAL OF BACTERIOLOGY, Sept. 2010, p. 4526–4527 Vol. 192, No. 17
0021-9193/10/$12.00 doi:10.1128/JB.00596-10

GENOME ANNOUNCEMENTS

Genome Sequence of the Milbemycin-Producing Bacterium *Streptomyces bingchenggensis*▽

Xiang-Jing Wang,[1]† Yi-Jun Yan,[1]† Bo Zhang,[1] Jing An,[1] Ji-Jia Wang,[1] Jun Tian,[1] Ling Jiang,[1] Yi-Hua Chen,[2] Sheng-Xiong Huang,[2] Min Yin,[2] Ji Zhang,[1] Ai-Li Gao,[1] Chong-Xi Liu,[1] Zhao-Xiang Zhu,[1] and Wen-Sheng Xiang[1]*

School of Life Science, Northeast Agricultural University, Harbin 150030, China,[1] and Division of Pharmaceutical Sciences and Department of Chemistry, University of Wisconsin—Madison, Madison, Wisconsin 53705[2]

Received 25 May 2010/Accepted 15 June 2010

Streptomyces bingchenggensis **is a soil-dwelling bacterium producing the commercially important anthelmintic macrolide milbemycins. Besides milbemycins, the insecticidal polyether antibiotic nanchangmycin and some other antibiotics have also been isolated from this strain. Here we report the complete genome sequence of *S. bingchenggensis*. The availability of the genome sequence of *S. bingchenggensis* should enable us to understand the biosynthesis of these structurally intricate antibiotics better and facilitate rational improvement of this strain to increase their titers.**

Streptomyces bingchenggensis was isolated from a soil sample collected in Harbin, China. It can efficiently produce the commercialized anthelmintic macrolide compounds milbemycins A3 and A4 together with 10 novel milbemycin analogs (10–14). Another insecticidal antibiotic, the polyether ionophore nanchangmycin, can also be produced by *S. bingchenggensis* (8). Furthermore, two novel cyclic pentapeptides, bingchamides A and B, have also been isolated from this strain (9). Considering the fertility of the secondary metabolites produced by *S. bingchenggensis*, its genome sequence should provide fundamental understanding of the biosynthesis of these compounds and facilitate the bioengineering efforts involving this species.

The genome sequencing of *S. bingchenggensis* was performed with the Illumina Genome Analyzer (Illumina, San Diego, CA) according to the manufacturer's instructions. High-molecular-mass genomic DNA isolated from *S. bingchenggensis* was used to construct small (200- to 500-bp) and large (2- to 3-kb) random sequencing libraries. The reads were first filtered and assembled into 274 contigs using SOAPdenovo (http://soap.genomics.org.cn/). We then used the paired-end information, step by step from the shortest (224 bp) to the longest (2,000 bp) insert size, to join the contigs into 17 scaffolds. During the finishing phase, gaps between scaffolds were filled by primer walking, subcloning, and multiplex PCR. Putative protein-coding sequences were predicted using the GLIMMER program (2) trained on annotated open reading frames (ORFs) of *Streptomyces coelicolor* A3(2) and *Streptomyces avermitilis*. The annotation was accomplished by BlastP analysis of sequences in the Nr, Nt, and SwissProt databases and by manual curation of the outputs of a variety of similarity searches.

S. bingchenggensis has a single linear chromosome composed of 11,936,683 bp (70.8% G+C) with no plasmids and is likely the largest bacterial genome sequenced to date. It is much longer than the other three sequenced streptomycete linear genomes [*S. coelicolor* A3(2) (8.7 Mbp), *S. avermitilis* MA-4680 (9.0 Mbp), and *S. griseus* IFO13350 (8.5 Mbp)] (1, 5, 6). Comparison with the other streptomycete genomes demonstrated a 7.2-Mb core region of *S. bingchenggensis* spanning from 3.2 Mb to 10.4 Mb and two "arms" 3.2 Mb (right) and 1.5 Mb (left) in size. Analysis of the *S. bingchenggensis* genome revealed that its chromosome contains 10,023 predicted protein-coding sequences with 6,419 being assigned known or putative functions. The majority of the genes (83%) predicted to be essential are located in the core region; while most of the secondary metabolite genes are found in the two noncore arms.

The *S. bingchenggensis* genome contains a very high number of insertion sequences, almost one-third of which are associated with transposases. About half of the insertion sequence elements are found in two large regions in the right arm (1.6 to 2.3 Mbp) and in the core region (9.9 to 10.4 Mbp), and transcriptional regulators are also highly distributed in those regions. The substantially lower GC content of these two regions indicates that they were acquired by horizontal gene transfer, which serves as a main strategy for genome expansion in actinomycetes. Approximately 400 kb at both ends also show remarkably low GC content, implying another exogenic gene acquisition event, which is reminiscent of the proposal that streptomycetes gain their linear ends from linear plasmids. Many transposase genes are found in the subterminal invert repeat regions, suggesting a high frequency of insertions here and offering another clue to genome expansion.

Preliminary analyses of the *S. bingchenggensis* genome revealed at least 23 gene clusters for polyketide, nonribosomal

* Corresponding author. Mailing address: School of Life Science, Northeast Agricultural University, Harbin 150030, China. Phone: 13159802118. Fax: 86-451-55190413. E-mail: xiangwensheng@yahoo.com.cn.

† These authors contributed equally to this work.

▽ Published ahead of print on 25 June 2010.

peptide, or terpene biosynthesis. Not surprisingly, 14 of the biosynthetic clusters are located in the 3.2-Mbp extralarge right arm, including the putative clusters for milbemycins and bingchamides; the nanchangmycin biosynthetic cluster, together with the other five cryptic gene clusters, are found in the core region; only three clusters are in the left arm. Milbemycins have an aglycone similar to that of other anthelmintic compounds, avermectins (4). Accordingly, its putative biosynthetic enzymes contain four large type I polyketide synthases (PKSs) possessing modules similar to those of avermectin synthases from *S. avermitilis* (3). Specifically, the gene *milA1*, which encodes the loading module and the first elongation module of milbemycin, is 62 kb away from the other three milbemycin PKS genes, revealing a noteworthy example of a nonclustered biosynthetic pathway in actinomycetes. The nanchangmycin cluster is very similar to the cloned nanchangmycin type I PKS cluster from *Streptomyces nanchangensis* (7). It contains all of the ORFs of the *S. nanchangensis* nanchangmycin biosynthetic cluster, and all of the putative proteins show extremely high similarity (most of them >95%) to their counterparts. Bingchamides A and B are proposed to be biosynthesized by nonribosomal peptide synthetases (NRPSs), and their postulated cluster contains four genes encoding NRPSs with five modules. The deduced substrate specificities of the five adenylation domains are in accordance with the solved structures of bingchamides. The other 20 gene clusters are proposed to dictate the biosynthesis of siderophore, geosmin, or absolutely unknown compounds waiting to be exploited by further endeavors.

Nucleotide sequence accession number. Genome information for the chromosome of *S. bingchenggensis* has been deposited in GenBank under accession number CP002047.

We thank Guan-Yu Zhou, Li-Zhi Xu, and Nan-Nan Qin (Beijing Genomics Institute) for technical assistance in genome sequencing.

This research was supported by the National Key Technology R&D Program (2006BAD31B02), the Program for New Century Excellent Talents in University (NCET-08-0668, 1154-NCET-002), the Outstanding Youth Foundation of Heilongjiang Province (JC200706), the National Key Project for Basic Research (2010CB126102), and the Program for New Teachers in University (20092325120007).

REFERENCES

1. **Bentley, S. D., K. F. Chater, A. M. Cerdeno-Tarraga, G. L. Challis, N. R. Thomson, K. D. James, D. E. Harris, M. A. Quail, H. Kieser, D. Harper, A. Bateman, S. Brown, G. Chandra, C. W. Chen, M. Collins, A. Cronin, A. Fraser, A. Goble, J. Hidalgo, T. Hornsby, S. Howarth, C. H. Huang, T. Kieser, L. Larke, L. Murphy, K. Oliver, S. O'Neil, E. Rabbinowitsch, M. A. Rajandream, K. Rutherford, S. Rutter, K. Seeger, D. Saunders, S. Sharp, R. Squares, S. Squares, K. Taylor, T. Warren, A. Wietzorrek, J. Woodward, B. G. Barrell, J. Parkhill, and D. A. Hopwood.** 2002. Complete genome sequence of the model actinomycete *Streptomyces coelicolor* A3(2). Nature **417:**141–147.
2. **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.
3. **Ikeda, H., T. Nonomiya, M. Usami, T. Ohta, and S. Ōmura.** 1999. Organization of the biosynthetic gene cluster for the polyketide anthelmintic macrolide avermectin in *Streptomyces avermitilis*. Proc. Natl. Acad. Sci. U. S. A. **96:**9509.
4. **McKellar, Q. A., and H. A. Benchaoui.** 1996. Avermectins and milbemycins. J. Vet. Pharmacol. Ther. **19:**331–351.
5. **Ohnishi, Y., J. Ishikawa, H. Hara, H. Suzuki, M. Ikenoya, H. Ikeda, A. Yamashita, M. Hattori, and S. Horinouchi.** 2008. Genome sequence of the streptomycin-producing microorganism *Streptomyces griseus* IFO 13350. J. Bacteriol. **190:**4050–4060.
6. **Omura, S., H. Ikeda, J. Ishikawa, A. Hanamoto, C. Takahashi, M. Shinose, Y. Takahashi, H. Horikawa, H. Nakazawa, T. Osonoe, H. Kikuchi, T. Shiba, Y. Sakaki, and M. Hattori.** 2001. Genome sequence of an industrial microorganism *Streptomyces avermitilis*: deducing the ability of producing secondary metabolites. Proc. Natl. Acad. Sci. U. S. A. **98:**12215–12220.
7. **Sun, Y. H., X. F. Zhou, H. Dong, G. Q. Tu, M. Wang, B. F. Wang, and Z. X. Deng.** 2003. A complete gene cluster from Streptomyces nanchangensis NS3226 encoding biosynthesis of the polyether ionophore nanchangmycin. Chem. Biol. **10:**431–441.
8. **Wang, X. J., J. D. Wang, W. S. Xiang, and J. Zhang.** 2009. Three new milbemycin derivatives from Streptomyces bingchenggensis. J. Asian Nat. Prod. Res. **11:**597–603.
9. **Wang, X. J., S. L. Guo, W. Q. Guo, D. Xi, and W. S. Xiang.** 2009. Role of nsdA in negative regulation of antibiotic production and morphological differentiation in Streptomyces bingchengensis. J. Antibiot. **62:**309–313.
10. **Xiang, W. S., J. D. Wang, H. M. Fan, X. J. Wang, and J. Zhang.** 2008. New seco-milbemycins from *Streptomyces bingchenggensis*: fermentation, isolation and structure elucidation. J. Antibiot. **61:**27–32.
11. **Xiang, W. S., J. D. Wang, X. J. Wang, J. Zhang, and Z. Wang.** 2007. Further new milbemycin antibiotics from *Streptomyces bingchenggensis*. Fermentation, isolation, structural elucidation and biological activities. J. Antibiot. **60:**608–613.
12. **Xiang, W. S., J. D. Wang, X. J. Wang, and J. Zhang.** 2007. Two new beta-class milbemycins from *Streptomyces bingchenggensis*: fermentation, isolation, structure elucidation and biological properties. J. Antibiot. **60:**351–356.
13. **Xiang, W. S., J. D. Wang, X. J. Wang, and J. Zhang.** 2009. A novel macrolide compound from Streptomyces bingchenggensis: fermentation, isolation, structure elucidation and biological properties. J. Antibiot. **62:**229–231.
14. **Xiang, W. S., J. D. Wang, X. J. Wang, and J. Zhang.** 2009. Bingchamides A and B, two novel cyclic pentapeptides from the *Streptomyces bingchenggensis*: fermentation, isolation, structure elucidation and biological properties. J. Antibiot. **62:**501–505.