

Complete Genome Sequence of Probiotic *Bifidobacterium animalis* subsp. *lactis* Strain V9[∇]

Zhihong Sun,^{1†} Xia Chen,^{1†} Jicheng Wang,¹ Pengfei Gao,² Zhemin Zhou,³ Yi Ren,⁴
Tiansong Sun,¹ Lei Wang,⁴ He Meng,⁵ Wei Chen,^{6*} and Heping Zhang^{1*}

Key Laboratory of Dairy Biotechnology and Engineering, Ministry of Education, School of Food Science and Engineering, Inner Mongolia Agricultural University, Hohhot 010018, China¹; Inner Mongolia Light Industrial Institute of Research, Hohhot 010050, China²; The Engineering and Research Center for Microbial Functional Genomics and Detection Technology, Ministry of Education, TEDA School of Biological Sciences and Biotechnology, Nankai University, Tianjin 300457, China³; Tianjin Biochip Corporation, Tianjin 300457, China⁴; School of Agriculture and Biology, Shanghai Jiao Tong University, Shanghai 200240, China⁵; and State Key Laboratory of Food Science and Technology, School of Food Science and Technology, Jiangnan University, Wuxi 214122, China⁶

Received 2 April 2010/Accepted 19 May 2010

***Bifidobacterium animalis* subsp. *lactis* strain V9 is a Chinese commercial bifidobacteria with several probiotic functions. It was isolated from a healthy Mongolian child in China. We present here the complete genome sequence of V9 and compare it to 3 other published genome sequences of *B. animalis* subsp. *lactis* strains. The result indicates the lack of polymorphism among strains of this subspecies from different continents.**

Bifidobacterium animalis subsp. *lactis* strain V9 was isolated from the feces of a healthy Mongolian child in China (5). It has shown a high level of tolerance to gastric acid and bile acids (5). This strain has been implemented in the industrial production of dairy starter cultures by Inner Mongolia Yili Industrial Group Company Limited, the largest dairy corporation in China.

Whole-genome sequencing of *B. animalis* subsp. *lactis* V9 was performed with a combined strategy of 454 sequencing (8) and Solexa paired-end sequencing technology (2). Genomic libraries containing 7-kb inserts were constructed, and 325,824 paired-end reads and 67,177 single-end reads were generated using the GS FLX system, giving 36.0-fold coverage of the genome. A total of 96.0% of the reads were assembled into four large scaffolds, including 163 nonredundant contigs, using the 454 Newbler assembler (454 Life Sciences, Branford, CT). A total of 8,953,102 reads (2-kb library) were generated to reach a depth of 335-fold coverage with an Illumina Solexa Genome Analyzer Iix and mapped to the scaffolds using the Burrows-Wheeler Alignment (BWA) tool (7). The gaps between scaffolds were filled by sequencing PCR products using an ABI 3730 capillary

sequencer. The analysis of the genome was performed as described previously (3, 4).

The complete genome sequence of V9 contains a circular 1,944,050-bp chromosome, with a GC content of 60.5%. The genome size is slightly larger than the sequenced genome sizes of *B. animalis* subsp. *lactis* strains DSM 10140^T (1), BI-04 (1), and AD011 (6) due to a unique insertion of 4,037 bp. The V9 genome contains 1,636 genes in total, including 1,572 coding genes, 4 rRNA operons, and 52 tRNAs.

Comparison of the four *B. animalis* subsp. *lactis* genomes revealed nearly perfect synteny. AD011 is the most diverged strain, with more single nucleotide polymorphisms (SNPs) and indels than the other three strains. There are 197 SNPs in AD011, with 70 synonymous and 16 nonsynonymous SNPs, which means that there is only 1 SNP per 10 kb, indicating the high consistency within this subspecies. The other three strains are almost identical, with only 25 SNPs in V9, 13 SNPs in BI-04, and 44 SNPs in DSM 10140^T. Strain V9 was isolated from the feces of a Mongolian child in Inner Mongolia, China, where traditional fermented milk has been consumed for thousands of years, and the other three strains were originally isolated from fecal samples (1, 6) or yogurt (1) in the United States of America, France, and Korea. The result indicated the lack of polymorphism among multiple lineages from different continents (1).

Interestingly, compared to the other three sequenced *B. animalis* subsp. *lactis* strains, V9 has a large insertion, which encodes one putative transposase (BaV_1091) and two sugar metabolism-related proteins, an alpha-1,4-glucosidase (BaV_1092) and an ABC transporter solute-binding protein (BaV_1093). This insertion is a copy of the region at positions 1,860,164 to 1,864,073, which is commonly shared by all four *B. animalis* subsp. *lactis* strains.

* Corresponding author. Mailing address for Heping Zhang: Key Laboratory of Dairy Biotechnology and Engineering, Ministry of Education, College of Food Science and Engineering, Inner Mongolia Agricultural University, 306 Zhaowuda Road, Hohhot 010018, China. Phone: 86-471-4319940. Fax: 86-471-4300122. E-mail: hepingdd@vip.sina.com. Mailing address for Wei Chen: State Key Laboratory of Food Science and Technology, School of Food Science and Technology, Jiangnan University, 1800 Lihu Avenue, Wuxi 214122, China. Phone and Fax: 86-510-85912155. E-mail: weichen@jiangnan.edu.cn.

† These authors contributed equally to this work.

∇ Published ahead of print on 28 May 2010.

Nucleotide sequence accession number. The sequence and annotation of the *B. animalis* subsp. *lactis* V9 genome is available in GenBank under accession number CP001892.

This research was supported by the National Natural Science Foundation of China (grants 30660135, 30760156, 30800861, and 30860219), the Hi-Tech Research and Development Program of China (863 Program) (grant 2007AA10Z353), the earmarked fund for the Modern Agro-industry Technology Research System, and the National Key Technologies R&D Program (grant 2009BAD1B01).

REFERENCES

- Barrangou, R., E. P. Briczinski, L. L. Traeger, J. R. Loquasto, M. Richards, P. Horvath, A. C. Coute-Monvoisin, G. Leyer, S. Rendulic, J. L. Steele, J. R. Broadbent, T. Oberg, E. G. Dudley, S. Schuster, D. A. Romero, and R. F. Roberts. 2009. Comparison of the complete genome sequences of *Bifidobacterium animalis* subsp. *lactis* DSM 10140 and BI-04. *J. Bacteriol.* **191**:4144–4151.
- Bentley, D. R., S. Balasubramanian, H. P. Swerdlow, G. P. Smith, J. Milton, C. G. Brown, K. P. Hall, D. J. Evers, C. L. Barnes, H. R. Bignell, J. M. Boutell, J. Bryant, R. J. Carter, R. Keira Cheetham, A. J. Cox, D. J. Ellis, M. R. Flatbush, N. A. Gormley, S. J. Humphray, L. J. Irving, M. S. Karbelashvili, S. M. Kirk, H. Li, X. Liu, K. S. Maisinger, L. J. Murray, B. Obradovic, T. Ost, M. L. Parkinson, M. R. Pratt, I. M. Rasolonjatovo, M. T. Reed, R. Rigatti, C. Rodighiero, M. T. Ross, A. Sabot, S. V. Sankar, A. Scally, G. P. Schroth, M. E. Smith, V. P. Smith, A. Spiridou, P. E. Torrance, S. S. Tzonev, E. H. Vermaas, K. Walter, X. Wu, L. Zhang, M. D. Alam, C. Anastasi, I. C. Aniebo, D. M. Bailey, I. R. Bancarz, S. Banerjee, S. G. Barbour, P. A. Baybayan, V. A. Benoit, K. F. Benson, C. Bevis, P. J. Black, A. Boodhun, J. S. Brennan, J. A. Bridgham, R. C. Brown, A. A. Brown, D. H. Buermann, A. A. Bundu, J. C. Burrows, N. P. Carter, N. Castillo, E. C. M. Chiara, S. Chang, R. Neil Cooley, N. R. Crake, O. O. Dada, K. D. Diakoumakos, B. Dominguez-Fernandez, D. J. Earnshaw, U. C. Egbujor, D. W. Elmore, S. S. Echin, M. R. Ewan, M. Fedurco, L. J. Fraser, K. V. Fuentes Fajardo, W. Scott Furey, D. George, K. J. Gietzen, C. P. Goddard, G. S. Golda, P. A. Granieri, D. E. Green, D. L. Gustafson, N. F. Hansen, K. Harnish, C. D. Haudenschild, N. I. Heyer, M. M. Hims, J. T. Ho, A. M. Horgan, et al. 2008. Accurate whole human genome sequencing using reversible terminator chemistry. *Nature* **456**:53–59.
- Feng, L., P. R. Reeves, R. Lan, Y. Ren, C. Gao, Z. Zhou, Y. Ren, J. Cheng, W. Wang, J. Wang, W. Qian, D. Li, and L. Wang. 2008. A recalibrated molecular clock and independent origins for the cholera pandemic clones. *PLoS One* **3**:e4053.
- Ferenci, T., Z. Zhou, T. Betteridge, Y. Ren, Y. Liu, L. Feng, P. R. Reeves, and L. Wang. 2009. Genomic sequencing reveals regulatory mutations and recombinational events in the widely used MC4100 lineage of *Escherichia coli* K-12. *J. Bacteriol.* **191**:4025–4029.
- Gao, P., Z. Sun, S. Ma, Q. Wang, J. Gao, C. Deng, and H. Zhang. 2009. Screening and identification of probiotic *Bifidobacterium* from Mongolian children. *Wei Sheng Wu Xue Bao* **49**:210–216.
- Kim, J. F., H. Jeong, D. S. Yu, S. H. Choi, C. G. Hur, M. S. Park, S. H. Yoon, D. W. Kim, G. E. Ji, H. S. Park, and T. K. Oh. 2009. Genome sequence of the probiotic bacterium *Bifidobacterium animalis* subsp. *lactis* AD011. *J. Bacteriol.* **191**:678–679.
- Li, H., and R. Durbin. 2009. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* **25**:1754–1760.
- Margulies, M., M. Egholm, W. E. Altman, S. Attiya, J. S. Bader, L. A. Bemben, J. Berka, M. S. Braverman, Y. J. Chen, Z. Chen, S. B. Dewell, L. Du, J. M. Fierro, X. V. Gomes, B. C. Godwin, W. He, S. Helgesen, C. H. Ho, G. P. Irzyk, S. C. Jando, M. L. Alenquer, T. P. Jarvie, K. B. Jirage, J. B. Kim, J. R. Knight, J. R. Lanza, J. H. Leamon, S. M. Lefkowitz, M. Lei, J. Li, K. L. Lohman, H. Lu, V. B. Makhijani, K. E. McDade, M. P. McKenna, E. W. Myers, E. Nickerson, J. R. Nobile, R. Plant, B. P. Puc, M. T. Ronan, G. T. Roth, G. J. Sarkis, J. F. Simons, J. W. Simpson, M. Srinivasan, K. R. Tartaro, A. Tomasz, K. A. Vogt, G. A. Volkmer, S. H. Wang, Y. Wang, M. P. Weiner, P. Yu, R. F. Begley, and J. M. Rothberg. 2005. Genome sequencing in micro-fabricated high-density picolitre reactors. *Nature* **437**:376–380.