

Complete Genome Sequence of *Bifidobacterium longum* JDM301[∇]

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Bifidobacteria, known as probiotic bacteria, are high-G+C Gram-positive bacteria which naturally inhabit the human gastrointestinal tract and vagina. Recently, we completely sequenced *Bifidobacterium longum* JDM301, which is a widely used Chinese commercial strain with several probiotic properties.

Bifidobacterium spp., which are considered model probiotic bacteria like *Lactobacillus* spp., play an important role in the stability of the intestinal microflora, the modulation of the immune response, and so on (6, 8). We determined the complete genome sequence of *B. longum* JDM301, which is commercially used in China as a probiotic strain, using the GS 20 system (454 Life Science Corporation) (7). A total of 192,888 reads with an average length of 210 bp were assembled into 112 contigs by the 454 assembly tool. Among these, 92 large contigs were larger than 500 bp. We determined the order of the largest contigs through BLAST analysis with the reference strain *B. longum* ATCC 15697 (GenBank accession number CP001095) (10) and arranged the others by multiplex PCR. Gaps were closed by sequencing gap-spanning PCR products or clones using ABI 3730 xl DNA sequencers. Primer design and sequence assembly were performed with the Phred/Phrap/Consed software package (2, 3).

The complete genome of *B. longum* JDM301 is composed of a 59.8% G+C circular chromosome of 2,477,838 bp without any plasmid. The genome of JDM301 (2.48 Mb) is smaller than that of *B. longum* ATCC 15697 (2.83 Mb) and slightly larger than the complete genomes of *B. longum* NCC2705 (2.26 Mb; GenBank accession number AE014295) and DJO10A (2.38 Mb; GenBank accession number CP000605). The JDM301 genome contains 1,959 protein-coding genes, three rRNA operons, and 55 tRNA genes. There are four rRNA operons with two cascaded in the three reference *B. longum* strains, while there are three in JDM301. We resequenced the three regions containing rRNA operons in the genome of JDM301 and found no cascaded rRNA operon. However, the locations of the three rRNA operons in JDM301 are the same as in the other three *B. longum* strains. No complete prophages were found in the genome sequence, but 12 phage-related fragments were identified. The genome also contains 15 pseudogenes, which is evidence of the recent and ongoing genome reduction of lactic acid bacilli (9). In addition, 15 complete or disrupted insertion sequence (IS) elements were found in the entire

genome, which were identified as 5 derivatives of ISBlo5, 3 complete copies of ISBlo3, and 7 other ISs or derivatives belonging to the IS256, IS3, IS21, and IS30 family elements, respectively. One of them, named ISBad1, was 98.08% identical to that of *B. adolescentis*, and the others have been reported in the *B. longum* NCC2705 genome (8).

Genome analysis revealed 14 response regulators and 14 sensor histidine kinases throughout the JDM301 chromosome, which may suggest a less complex regulatory network in JDM301 than that in ATCC 15697 (10). JDM301 has been grown as a commercial strain in stable and rich nutritional medium for such a long time that the regulatory networks in the genome may degenerate slowly, which is required for responses to dynamic environmental cues in the gastrointestinal tract.

A gene (for BLJ_1359) encoding a serpin (serine protease inhibitor) with 92.69% identity to that of NCC2705 (4) was found in the genome sequence. The serpin is a potential probiotic effector molecule, and it may contribute to the immunomodulation of this *B. longum* strain. As we know, modulation of the immune system is one of probiotic functions of lactic acid bacteria (5).

As model probiotic bacteria, *Bifidobacterium* spp. attract more and more interest since the molecular mechanisms of their probiotic activities remain unclear to a large degree (11). On the other hand, the biosafety of probiotics has attracted more attention with their enlarged and wide applications in food and drug products (1). We believe that this work will bring us deeper insight into the probiotic activities and safety of the strain widely used in China.

Nucleotide sequence accession number. The genome sequence of *B. longum* JDM301 was deposited in GenBank under the accession number CP002010.

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REFERENCES

1. Ammor, M. S., A. B. Florez, and B. Mayo. 2007. Antibiotic resistance in non-enterococcal lactic acid bacteria and bifidobacteria. *Food Microbiol.* **24**:559–570.
2. Ewing, B., L. Hiller, M. C. Wendl, and P. Green. 1998. Base-calling of

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- automated sequencer traces using *Phred*. I. Accuracy assessment. *Genome Res.* **8**:175–185.
3. **Gordon, D., C. Abajian, and P. Green.** 1998. *Consed*: a graphical tool for sequence finishing. *Genome Res.* **8**:195–202.
 4. **Ivanov, D., C. Emonet, F. Foata, M. Affolter, M. Delley, M. Fisseha, S. Blum-Sperisen, S. Kochhar, and F. Arigoni.** 2006. A serpin from the gut bacterium *Bifidobacterium longum* inhibits eukaryotic elastase-like serine proteases. *J. Biol. Chem.* **281**:17246–17252.
 5. **Lee, B., J. H. Lee, H. S. Lee, E. A. Bae, C. S. Huh, Y. T. Ahn, and D. H. Kim.** 2009. Glycosaminoglycan degradation-inhibitory lactic acid bacteria ameliorate 2,4,6-trinitrobenzenesulfonic acid-induced colitis in mice. *J. Microbiol. Biotechnol.* **19**:616–621.
 6. **Marco, M. L., S. Pavan, and M. Kleerebezem.** 2006. Towards understanding molecular modes of probiotic action. *Curr. Opin. Biotechnol.* **17**:204–210.
 7. **Margulies, M., M. Egholm, W. E. Altman, S. Attiya, J. S. Bader, L. A. Bembien, 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 microfabricated high-density picolitre reactors. *Nature* **437**:376–380.
 8. **Schell, M. A., M. Karmirantzou, B. Snel, D. Vilanova, B. Berger, G. Pessi, M. C. Zwahlen, F. Desiere, P. Bork, M. Delley, R. D. Pridmore, and F. Arigoni.** 2002. The genome sequence of *Bifidobacterium longum* reflects its adaptation to the human gastrointestinal tract. *Proc. Natl. Acad. Sci. U. S. A.* **99**:14422–14427.
 9. **Schroeter, J., and T. Klaenhammer.** 2009. Genomics of lactic acid bacteria. *FEMS Microbiol. Lett.* **292**:1–6.
 10. **Sela, D. A., J. Chapman, A. Adeuya, J. H. Kim, F. Chen, T. R. Whitehead, A. Lapidus, D. S. Rokhsar, C. B. Lebrilla, J. B. German, N. P. Price, P. M. Richardson, and D. A. Mills.** 2008. The genome sequence of *Bifidobacterium longum* subsp. *infantis* reveals adaptations for milk utilization within the infant microbiome. *Proc. Natl. Acad. Sci. U. S. A.* **105**:18964–18969.
 11. **Ventura, M., S. O’Flaherty, M. J. Claesson, F. Turrone, T. R. Klaenhammer, D. van Sinderen, and P. W. O’Toole.** 2009. Genome-scale analyses of health-promoting bacteria: probionomics. *Nat. Rev. Microbiol.* **7**:61–71.