



Complete Genome Sequence of *Bifidobacterium longum* 105-A, a Strain with High Transformation Efficiency

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Bifidobacterium longum 105-A shows high transformation efficiency and allows for the generation of gene knockout mutants through homologous recombination. Here, we report the complete genome sequence of strain 105-A. Genes encoding at least four putative restriction-modification systems were found in this genome, which might contribute to its transformation efficiency.

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ifidobacteria are naturally found in the human large intestine Band are used as probiotic bacteria due to their beneficial effects on human health (1). Bifidobacteria are known for their difficulties with gene manipulation, especially in the generation of gene knockout mutants (2-4). This is mainly caused by the low transformation efficiencies demonstrated by Bifidobacterium strains, in part due to their restriction-modification (R-M) systems (2-6). Among the bifidobacteria, Bifidobacterium longum 105-A (7), isolated from human feces, has shown exceptionally high transformation efficiency (approximately 10^4 to 10^6 transformants/µg DNA) with several plasmid DNAs (6-9). Moreover, gene knockout mutants of B. longum 105-A and its derivative strain have been successfully generated using homologous recombination systems (8, 10–12). Thus, strain 105-A has become a representative host strain for functional genomics studies of bifidobacteria. Here, we deciphered the complete genome sequence of *B. longum* 105-A.

Genomic DNA was isolated from *B. longum* 105-A as described previously (8) and sequenced through the massively parallel sequencing method using a PacBio RS II (Pacific Biosciences of California, Inc., Menlo Park, CA, USA) and the Genome Analyzer IIx (GAIIx; Illumina, Inc., San Diego, CA, USA). Approximately 740and 253-fold sequence coverages were obtained by the PacBio RS II and GAIIx, respectively. Sequence reads from the PacBio RS II and GAIIx, respectively. Sequence assembler version 7.0 and Velvet 0.7.55, respectively. The Celera Assembler generated short contigs and a super contig with a gap region. The gap was covered by a combination of two contigs assembled from the GAIIx data and a short contig assembled from the PacBio RS II data. The junction regions among the contigs were confirmed by Sanger sequencing. Error correction of the homopolymer region was performed by mapping the short read data obtained by the GAIIx.

The circular chromosome of B. longum 105-A contains 2,290,145 bp, for which 1,878 open reading frames (ORFs), 56 tRNA genes, 1 transfer-messenger RNA (tmRNA) gene, and 4 rRNA operons were predicted by g-MiGAP (13). The average G+C content of the genome is 60.06%. The total number of ORFs in the B. longum 105-A genome is higher than that in the representative B. longum strain, NCC2705 (14), which has 1,728 ORFs, 57 tRNA genes, 1 tmRNA gene, and 4 rRNA operons. In addition, local BLASTx analysis using REBASE entries (15) revealed that the B. longum 105-A genome contains genes encoding at least four types of putative R-M systems: a type I system comprising BL105A_1442 (a methyltransferase), BL105A_1441 (a specificity subunit), and BL105A_1439 (an endonuclease); one type II system comprising BL105A_1060 (an endonuclease) and BL105A_1059 (a methyltransferase); another type II system comprising BL105A_0366 (an endonuclease/methyltransferase); and a type IV system comprising BL105A_0073 (an endonuclease). Further functional analysis of the R-M system genes will clarify their contributions to transformation efficiency. The complete genome sequence of *B. longum* 105-A will largely contribute to postgenomic or functional genomics studies of bifidobacteria.

Nucleotide sequence accession number. The complete genome sequence of *B. longum* 105-A has been deposited in the DDBJ/EMBL/GenBank database under accession no. AP014658.

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REFERENCES

- Lee JH, O'Sullivan DJ. 2010. Genomic insights into bifidobacteria. Microbiol. Mol. Biol. Rev. 74:378-416. http://dx.doi.org/10.1128/ MMBR.00004-10.
- Fukiya S, Suzuki T, Kano Y, Yokota A. 2011. Current status of *Bifidobacterium* gene manipulation technologies, p 33–51. *In* Sonomoto K, Yokota A (ed), Lactic acid bacteria and bifidobacteria: current progress in advanced research. Caister Academic Press, Norfolk, United Kingdom.
- Guglielmetti S, Mayo B, Álvarez-Martín P. 2013. Mobilome and genetic modification of bifidobacteria. Benef. Microbes 4:143–166. http:// dx.doi.org/10.3920/BM2012.0031.
- 4. Brancaccio VF, Zhurina DS, Riedel CU. 2013. Tough nuts to crack: site-directed mutagenesis of bifidobacteria remains a challenge. Bioengineered 4:197–202. http://dx.doi.org/10.4161/bioe.23381.
- O'Connell Motherway M, Watson D, Bottacini F, Clark TA, Roberts RJ, Korlach J, Garault P, Chervaux C, van Hylckama Vlieg JET, Smokvina T, van Sinderen D. 2014. Identification of restrictionmodification systems of *Bifidobacterium animalis* subsp. *lactis* CNCM I-2494 by SMRT sequencing and associated methylome analysis. PLoS One 9:e94875. http://dx.doi.org/10.1371/journal.pone.0094875.
- Yasui K, Kano Y, Tanaka K, Watanabe K, Shimizu-Kadota M, Yoshikawa H, Suzuki T. 2009. Improvement of bacterial transformation efficiency using plasmid artificial modification. Nucleic Acids Res. 37:e3. http://dx.doi.org/10.1093/nar/gkn884.
- Matsumura H, Takeuchi A, Kano Y. 1997. Construction of Escherichia coli-Bifidobacterium longum shuttle vector transforming B. longum 105-A and 108-A. Biosci. Biotechnol. Biochem. 61:1211–1212. http:// dx.doi.org/10.1271/bbb.61.1211.
- 8. Hirayama Y, Sakanaka M, Fukuma H, Murayama H, Kano Y, Fukiya S, Yokota A. 2012. Development of a double-crossover markerless gene deletion system in *Bifidobacterium longum*: functional analysis of the

α-galactosidase gene for raffinose assimilation. Appl. Environ. Microbiol. 78:4984–4994. http://dx.doi.org/10.1128/AEM.00588-12.

- Tanaka K, Samura K, Kano Y. 2005. Structural and functional analysis of pTB6 from *Bifidobacterium longum*. Biosci. Biotechnol. Biochem. 69: 422–425. http://dx.doi.org/10.1271/bbb.69.422.
- Sakaguchi K, He J, Tani S, Kano Y, Suzuki T. 2012. A targeted gene knockout method using a newly constructed temperature-sensitive plasmid mediated homologous recombination in *Bifidobacterium longum*. Appl. Microbiol. Biotechnol. 95:499–509. http://dx.doi.org/10.1007/ s00253-012-4090-4.
- Sakaguchi K, Funaoka N, Tani S, Hobo A, Mitsunaga T, Kano Y, Suzuki T. 2013. The *pyrE* gene as a bidirectional selection marker in *Bifidobacterium longum* 105-A. Biosci. Microbiota Food Health 32:59–68. http:// dx.doi.org/10.12938/bmfh.32.59.
- Sakurama H, Kiyohara M, Wada J, Honda Y, Yamaguchi M, Fukiya S, Yokota A, Ashida H, Kumagai H, Kitaoka M, Yamamoto K, Katayama T. 2013. Lacto-*N*-biosidase encoded by a novel gene of *Bifidobacterium longum* subspecies *longum* shows unique substrate specificity and requires a designated chaperone for its active expression. J. Biol. Chem. 288: 25194–25206. http://dx.doi.org/10.1074/jbc.M113.484733.
- Sugawara H, Ohyama A, Mori H, Kurokawa K. 2009. Microbial genome annotation pipeline (MiGAP) for diverse users, abstr S-001. Abstr. 20th Int. Conf. Genome Informatics, Kanagawa, Japan.
- Schell MA, Karmirantzou M, Snel B, Vilanova D, Berger B, Pessi G, Zwahlen MC, Desiere F, Bork P, Delley M, Pridmore RD, Arigoni F. 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. http://dx.doi.org/10.1073/pnas.212527599.
- Roberts RJ, Vincze T, Posfai J, Macelis D. 2010. REBASE a database for DNA restriction and modification: enzymes, genes and genomes. Nucleic Acids Res. 38:D234–D236. http://dx.doi.org/10.1093/nar/gkp874.