

## GENOME ANNOUNCEMENTS

### Complete Genome Sequence of *Bifidobacterium bifidum* S17<sup>∇</sup>

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**Here, we report on the first completely annotated genome sequence of a *Bifidobacterium bifidum* strain. *B. bifidum* S17, isolated from feces of a breast-fed infant, was shown to strongly adhere to intestinal epithelial cells and has potent anti-inflammatory activity *in vitro* and *in vivo*. The genome sequence will provide new insights into the biology of this potential probiotic organism and allow for the characterization of the molecular mechanisms underlying its beneficial properties.**

Bifidobacteria represent an important group of the intestinal microbiota of humans and are believed to be promising candidates for pharmaceutical applications and functional food products due to their ability to exclude intestinal pathogens, strengthen the intestinal barrier, and/or modulate the immune response in the intestine (8). In order to unravel the molecular mechanisms responsible for these beneficial effects, several bifidobacterial strains have recently been sequenced (17). However, while the genus *Bifidobacterium* comprises 31 species with nine subspecies, at present only nine whole-genome sequences of four species and two subspecies are publically available (9).

Here, we present the first fully annotated genome sequence for the species *Bifidobacterium bifidum*. The strain selected for sequencing (*B. bifidum* S17) was isolated from feces of a breast-fed infant. *B. bifidum* S17 was shown to display unusually strong adhesion to intestinal epithelial cells (IECs) (11, 12) and elicits a promising anti-inflammatory capacity both *in vitro* (11, 13) and *in vivo* in a murine model of colitis (11).

A long tag paired-end library was constructed from genomic DNA and sequenced using a Roche Genome Sequencer FLX Titanium by Eurofins MWG Operon (Ebersberg, Germany). A total of 372,681 reads with a total of 75,885,699 bp were obtained, giving 34.7-fold coverage. A total of 321,408 reads were marked as mate pairs. Sequences were assembled by gsAssembler (Roche Applied Science) and Staden (15) into a total of 48 contigs distributed over three scaffolds. Remaining inter- and intrascaffold gaps were closed by Sanger sequencing of PCR products. Potential frameshifts were identified using FSfind (7) and verified by Sanger sequencing. Protein-encoding open reading frames (ORFs) were identified by employing Prodigal (5) using standard settings. The resulting translations were

used for a BLASTP (1) search against the nonredundant GenBank database. All automatically annotated ORFs were manually corrected using the Artemis software (14) based on the presence of a potential ribosomal binding sites and alignments with homologous ORFs from other organisms, and the start codons were redefined where necessary. Initial automated functional assignment was done using TIGRFam (3), Pfam (2), and Interpro (4), as well as KEGG (6) and COG (16) predictions. Manual corrections of automatically assigned functions were verified on an individual gene-by-gene basis. The pSORT software (<http://psort.hgc.jp/>) was used to predict protein localization. tRNA genes were identified by tRNAscan-SE (10), and the rRNA genes were predicted based on the BLASTN searches and manually annotated.

The complete genomic information of the *B. bifidum* S17 is contained on a single circular chromosome of 2,186,882 bp with an average GC content of 62%. A total of 1,782 protein-coding genes, 53 tRNA genes for all amino acids, and three *rrn* operons were identified.

In summary, we report the first fully sequenced and completely annotated genome of a *B. bifidum* strain. The analysis of the *B. bifidum* S17 genome will provide the basis to elucidate the molecular mechanisms of host colonization and anti-inflammatory effects. Moreover, the genome sequence of *B. bifidum* extends the pangenome of the genus *Bifidobacterium*.

**Nucleotide sequence accession number.** The genome sequence of *B. bifidum* S17 was deposited in the GenBank under the accession number CP002220.

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#### REFERENCES

1. Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman. 1990. Basic local alignment search tool. *J. Mol. Biol.* **215**:403–410.

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2. Finn, R. D., J. Mistry, J. Tate, P. Coghill, A. Heger, J. E. Pollington, O. L. Gavin, P. Gunasekaran, G. Ceric, K. Forslund, L. Holm, E. L. Sonnhammer, S. R. Eddy, and A. Bateman. 2010. The Pfam protein families database. *Nucleic Acids Res.* **38**:D211–D222.
3. Haft, D. H., J. D. Selengut, and O. White. 2003. The TIGRFAMs database of protein families. *Nucleic Acids Res.* **31**:371–373.
4. Hunter, S., R. Apweiler, T. K. Attwood, A. Bairoch, A. Bateman, D. Binns, P. Bork, U. Das, L. Daugherty, L. Duquenne, R. D. Finn, J. Gough, D. Haft, N. Hulo, D. Kahn, E. Kelly, A. Laugraud, I. Letunic, D. Lonsdale, R. Lopez, M. Madera, J. Maslen, C. McAnulla, J. McDowall, J. Mistry, A. Mitchell, N. Mulder, D. Natale, C. Orengo, A. F. Quinn, J. D. Selengut, C. J. Sigrist, M. Thimma, P. D. Thomas, F. Valentin, D. Wilson, C. H. Wu, and C. Yeats. 2009. InterPro: the integrative protein signature database. *Nucleic Acids Res.* **37**:D211–D215.
5. Hyatt, D., G. L. Chen, P. F. Locascio, M. L. Land, F. W. Larimer, and L. J. Hauser. 2010. Prodigal: prokaryotic gene recognition and translation initiation site identification. *BMC Bioinformatics* **11**:119.
6. Kanehisa, M., M. Araki, S. Goto, M. Hattori, M. Hirakawa, M. Itoh, T. Katayama, S. Kawashima, S. Okuda, T. Tokimatsu, and Y. Yamanishi. 2008. KEGG for linking genomes to life and the environment. *Nucleic Acids Res.* **36**:D480–D484.
7. Kislyuk, A., A. Lomsadze, A. L. Lapidus, and M. Borodovsky. 2009. Frame-shift detection in prokaryotic genomic sequences. *Int. J. Bioinform. Res. Appl.* **5**:458–477.
8. Leahy, S. C., D. G. Higgins, G. F. Fitzgerald, and D. van Sinderen. 2005. Getting better with bifidobacteria. *J. Appl. Microbiol.* **98**:1303–1315.
9. Lee, J. H., and D. J. O'Sullivan. 2010. Genomic insights into bifidobacteria. *Microbiol. Mol. Biol. Rev.* **74**:378–416.
10. 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**:955–964.
11. Preising, J., D. Philippe, M. Gleinser, H. Wei, S. Blum, B. J. Eikmanns, J. H. Niess, and C. U. Riedel. 2010. Selection of bifidobacteria for amelioration of murine colitis based on adhesion and anti-inflammatory capacity in vitro. *Appl. Environ. Microbiol.* **76**:3048–3051.
12. Riedel, C. U., F. Foata, D. R. Goldstein, S. Blum, and B. J. Eikmanns. 2006. Interaction of bifidobacteria with Caco-2 cells—adhesion and impact on expression profiles. *Int. J. Food Microbiol.* **110**:62–68.
13. Riedel, C. U., F. Foata, D. Philippe, O. Adolfsson, B. J. Eikmanns, and S. Blum. 2006. Anti-inflammatory effects of bifidobacteria by inhibition of LPS-induced NF- $\kappa$ B activation. *World J. Gastroenterol.* **12**:3729–3735.
14. Rutherford, K., J. Parkhill, J. Crook, T. Horsnell, P. Rice, M. A. Rajandream, and B. Barrell. 2000. Artemis: sequence visualization and annotation. *Bioinformatics* **16**:944–945.
15. Staden, R., K. F. Beal, and J. K. Bonfield. 2000. The Staden package, 1998. *Methods Mol. Biol.* **132**:115–130.
16. 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.
17. 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: probiogenomics. *Nat. Rev. Microbiol.* **7**:61–71.