

# Complete Sequence of Probiotic Symbioflor 2 *Escherichia coli* Strain G3/10 and Draft Sequences of Symbioflor 2 *E. coli* Strains G1/2, G4/9, G5, G6/7, and G8

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**The complete genome of probiotic *Escherichia coli* strain G3/10 is presented here. In addition, the probiotic *E. coli* strains G1/2, G4/9, G5, G6/7, and G8 are presented in draft form. These six strains together comprise the probiotic product Symbioflor 2 (DSM 17252).**

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Although probiotic activity is more often associated with lactic acid bacteria, some *Escherichia coli* strains have also been shown to have beneficial effects on human health. We consider it an essential step in the safety evaluation of probiotics to analyze their complete genomic content. The six probiotic *E. coli* strains presented here are all components of the probiotic product Symbioflor 2 (DSM 17252), provided by SymbioPharm (Herborn, Germany). The product is recommended for the treatment of irritable bowel syndrome (IBS) in adults and children (1, 2). The

*E. coli* strains comprising Symbioflor 2 were originally isolated from a single healthy human donor and stored separately since their isolation in 1956. Based on decades of experience of clinical use in humans, and in the absence of severe adverse reactions, Symbioflor 2 can be considered safe. In February 2011, all six Symbioflor 2 *E. coli* strains were assigned to risk group 1 by the Central Committee on Biological Safety (ZKBS) of the German Federal Office of Consumer Protection and Food Safety (BVL).

The genome of *E. coli* G3/10 was sequenced at CeBiTec (Uni-

TABLE 1 Characteristics of the six genomes

<i>E. coli</i> strain (DSMZ no.) <sup>a</sup>	Chromosome size (bp)	No. of contigs	Accession no. <sup>b</sup>	Plasmid(s) (size [bp])	Accession no.
G1/2 (DSM 16441)	5,090,326	252	<a href="https://www.ncbi.nlm.nih.gov/nuccore/JPKH000000000">JPKH000000000</a>	pSYM10 (1,549), pSYM12 (7,111)	<a href="https://www.ncbi.nlm.nih.gov/nuccore/KM107846">KM107846</a> (pSYM10), <a href="https://www.ncbi.nlm.nih.gov/nuccore/KM107848">KM107848</a> (pSYM12)
G3/10 (DSM 16443)	4,999,267	12	<a href="https://www.ncbi.nlm.nih.gov/nuccore/JPKI000000000">JPKI000000000</a>	pSYM1 (50,572), pSYM2 (4,197), pSYM3 (1,934), pSYM4 (1,304), pSYM5 (6,567), pSYM6 (10,433)	<a href="https://www.ncbi.nlm.nih.gov/nuccore/JN887338">JN887338</a> (pSYM1), <a href="https://www.ncbi.nlm.nih.gov/nuccore/KM107838">KM107838</a> (pSYM2), <a href="https://www.ncbi.nlm.nih.gov/nuccore/KM107839">KM107839</a> (pSYM3), <a href="https://www.ncbi.nlm.nih.gov/nuccore/KM107840">KM107840</a> (pSYM4), <a href="https://www.ncbi.nlm.nih.gov/nuccore/KM107841">KM107841</a> (pSYM5), <a href="https://www.ncbi.nlm.nih.gov/nuccore/KM107842">KM107842</a> (pSYM6)
G4/9 (DSM 16444)	4,545,818	79	<a href="https://www.ncbi.nlm.nih.gov/nuccore/JPJ000000000">JPJ000000000</a>	pSYM4	<a href="https://www.ncbi.nlm.nih.gov/nuccore/KM107843">KM107843</a> (pSYM7), <a href="https://www.ncbi.nlm.nih.gov/nuccore/KM107844">KM107844</a> (pSYM8), <a href="https://www.ncbi.nlm.nih.gov/nuccore/KM107845">KM107845</a> (pSYM9), <a href="https://www.ncbi.nlm.nih.gov/nuccore/KM107847">KM107847</a> (pSYM11)
G5 (DSM 16445)	4,787,583	151	<a href="https://www.ncbi.nlm.nih.gov/nuccore/JPJK000000000">JPJK000000000</a>	pSYM3, pSYM7 (4,452), pSYM8 (2,353), pSYM9 (12,686), pSYM11 (3,215)	
G6/7 (DSM 16446)	5,236,262	185	<a href="https://www.ncbi.nlm.nih.gov/nuccore/JPMZ000000000">JPMZ000000000</a>	pSYM10, pSYM12	
G8 (DSM 16448)	5,160,208	300	<a href="https://www.ncbi.nlm.nih.gov/nuccore/JPKL000000000">JPKL000000000</a>	pSYM10, pSYM12	

<sup>a</sup> Strain number in the Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany.

<sup>b</sup> The contigs are merged into a single accession number.

versity of Bielefeld, Bielefeld, Germany) using the Roche 454 Genome Sequencer FLX system (Roche Diagnostics, Mannheim, Germany). Its chromosome was initially assembled into ~190 contigs, flanked by repeated *rrn* loci, which were assembled into four large scaffolds, using the GS *de novo* assembler. Eight contigs with a total size of about 64 kb could not be assembled into any of these scaffolds. Gap closure on the scaffolds was performed by the sequencing of PCR products amplified with terminal primers and fosmid walking, resulting in four contiguous finished sequences. The three gaps between these sequences could not be closed by PCR. The strain contains one 50-kb plasmid and five smaller plasmids (Table 1). The other Symbioflor 2 *E. coli* strains, G1/2, G4/9, G5, G6/7, and G8, were sequenced at the Helmholtz Centre for Infection Research (Braunschweig, Germany) using the Genome Analyzer IIx (Illumina, San Diego, CA, USA). These strains contain between 1 and 5 plasmids (Table 1). *De novo* assembly was performed with Velvet (3). Gap closure was achieved by sequencing the PCR products generated with terminal primers.

The annotation of open reading frames (identified in the genomes by various automated protocols) was performed using the GenDB 2.4 annotation pipeline (4). tRNA genes were predicted using tRNAscan (5). The automated annotation was revised by BLAST analysis (6) using publicly available databases. The Symbioflor 2 strains G6/7 and G8 are closely related and may have originated from the same clone, as few differences were detected between the two draft genome sequences.

Of note is the production of microcin S by *E. coli* G3/10, a microcin encoded by a plasmid-carried operon (7). Microcins are peptides that function as bacteriocins against closely related species (8), and microcin S of strain G3/10 may play a role in its probiotic activity.

**Nucleotide sequence accession numbers.** This whole-genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession numbers given in Table 1. The version described in this paper is the first version.

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A.Z., K.Z., and F.G. filed a patent, P31501-EP: "Bacterially formed microcin S, a new antimicrobial peptide, effective against pathogenic microorganisms, e.g. enterohemorrhagic *Escherichia coli* (EHEC)." The other authors have no conflict of interest to declare.

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