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Near-Complete Genome Sequence of the Cellulolytic Bacterium *Bacteroides* (*Pseudobacteroides*) *cellulosolvens* ATCC 35603

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We report the single-contig genome sequence of the anaerobic, mesophilic, cellulolytic bacterium, *Bacteroides cellulosolvens*. The bacterium produces a particularly elaborate cellulosome system, wherein the types of cohesin-dockerin interactions are opposite of other known cellulosome systems: cell-surface attachment is thus mediated via type-I interactions, whereas enzymes are integrated via type-II interactions.

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The cellulosome is one of the most efficient systems known to biodegrade plant cell-wall polysaccharides and cellulosic wastes. This multi-enzyme extracellular complex incorporates multiple hydrolytic enzymes onto the bacterial cell-surface through dockerin modules that tightly bind to scaffoldin proteins via complementary cohesin modules (1–3). Additional carbohydrate-binding modules (CBM) attach the entire enzymatic complex to the cellulosic substrate (4). The biodegrading activity of cellulosomes has been studied extensively in related cellulolytic bacteria, such as *Clostridium (Ruminiclostridium) thermocellum, Acetivibrio cellulolyticus, Clostridium (Ruminiclostridium) clariflavum, Clostridium (Ruminiclostridium) cellulolyticum*, *Clostridium cellulovorans, Clostridium (Ruminiclostridium) papyrosolvens*, and *Ruminococcus flavefaciens* (5).

Bacteroides cellulosolvens ATCC 35603 (DSM 2933) is a cellulolytic bacterium, originally isolated from sewage sludge (6, 7) in co-culture with *Clostridium saccharolyticum*. Initially classified as a Gram-negative bacterium, analysis of the 16S RNA indicated that *B. cellulosolvens*, like *A. cellulolyticus*, is a member of the phylogenetically diverse clostridial assemblage (8, 9). Recently, *B. cellulosolvens* was renamed *Pseudobacteroides cellulosolvens* (10).

B. cellulosolvens was selected for its ability to grow under mesophilic, anaerobic conditions, and the bacterium was able to bind and degrade crystalline cellulose to cellobiose and glucose (11–13). Its cellulose-degrading activity was shown to be cell-associated (14), and elaborate cellulolytic cell-surface structures were subsequently demonstrated (15, 16). Cellulosome-like complexes were further identified in the bacterium (17), supported by the recognition of the major scaffoldin protein (CipBc, later renamed ScaA) (18, 19), which includes eleven type-II cohesin domains, a family-3a CBM, and a C-terminal dockerin domain. Its scaffoldin was shown to interact with a family-48 glycoside hydrolase (18), and the crystal structure of its type-II cohesin was determined (20).

The genome is reported as a large contig of 6,878,816 bp, translated into 5,897 predicted proteins. Sequencing was performed using PacBio RS-II technology and data from four SMRT cells was assembled using SMRTanalysis v2.2 (HGAP3 protocol). The initial assembly generated three contigs at ~65× raw read coverage, which were joined using Geneious R8 (21) and then validated by PCR and Sanger sequencing (22). Illumina reads (at ~200× coverage) also confirmed contiguity. The ends of the single contig were unable to be joined experimentally or *in silico*, possibly as a result of a misassembly or active mobile genetic element. Active transposase systems have been shown to interfere with closure previously (23). Therefore, the genome is reported as near-complete assembly. Gene prediction and annotation were performed as described previously (24, 25).

Intriguingly, the types of cohesin-dockerin interaction in *B. cellulosolvens* are reversed from those of all other known cellulosome systems, whereby cell-surface attachment of noncatalytic scaffoldins in *B. cellulosolvens* is mediated via type-I interactions, whereas the enzymes are integrated via type II-interactions (19, 26, 27). The genome codes for 75 cohesin modules (mostly type-II cohesins), packaged in more than two-dozen scaffoldins, and over 200 dockerin-containing proteins, including glycoside hydrolases, carbohydrate esterases, and polysaccharide lyases. Thus, *B. cellulosolvens* scaffoldins represent the largest noncatalytic cellulosomal subunits known to date, indicating the presence of a particularly elaborate cellulosome system.

Nucleotide sequence accession numbers. This whole-genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession number LGTC00000000. The version described in this paper is version LGTC01000000.

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