

# 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 hydro-

lase (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](https://www.ncbi.nlm.nih.gov/nuccore/LGTC00000000). The version described in this paper is version [LGTC01000000](https://www.ncbi.nlm.nih.gov/nuccore/LGTC01000000).

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## REFERENCES

- Bayer EA, Morag E, Lamed R. 1994. The cellulosome—A treasure-trove for biotechnology. *Trends Biotechnol* 12:379–386. [http://dx.doi.org/10.1016/0167-7799\(94\)90039-6](http://dx.doi.org/10.1016/0167-7799(94)90039-6).
- Bayer EA, Belaich J, Shoham Y, Lamed R. 2004. The cellulosomes: Multienzyme machines for degradation of plant cell wall polysaccharides. *Annu Rev Microbiol* 58:521–554. <http://dx.doi.org/10.1146/annurev.micro.57.030502.091022>.
- Fontes CM, Gilbert HJ. 2010. Cellulosomes: highly efficient nanomachines designed to deconstruct plant cell wall complex carbohydrates. *Annu Rev Biochem* 79:655–681. <http://dx.doi.org/10.1146/annurev-biochem-091208-085603>.
- Boraston AB, Bolam DN, Gilbert HJ, Davies GJ. 2004. Carbohydrate-binding modules: fine-tuning polysaccharide recognition. *Biochem J* 382:769–781. <http://dx.doi.org/10.1042/BJ20040892>.
- Bayer EA, Lamed R, White BA, Flint HJ. 2008. From cellulosomes to cellulosomics. *Chem Rec* 8:364–377. <http://dx.doi.org/10.1002/tcr.20160>.
- Murray WD, Sowden LC, Colvin JR. 1984. *Bacteroides cellulosolvans* sp. nov., a cellulolytic species from sewage sludge. *Int J Syst Bacteriol* 34:185–187. <http://dx.doi.org/10.1099/00207713-34-2-185>.
- Murray WD, Sowden LC, Colvin JR. 1986. Symbiotic relationship of *Bacteroides cellulosolvans* and *Clostridium saccharolyticum* in cellulose fermentation. *Appl Environ Microbiol* 51:710–715.
- Lin C, Urbance JW, Stahl DA. 1994. *Acetivibrio cellulolyticus* and *Bacteroides cellulosolvans* are members of the greater clostridial assemblage. *FEMS Microbiol Lett* 124:151–155. <http://dx.doi.org/10.1111/j.1574-6968.1994.tb07277.x>.
- Yutin N, Galperin MY. 2013. A genomic update on clostridial phylogeny: gram-negative spore formers and other misplaced clostridia. *Environ Microbiol* 15:2631–2634. <http://dx.doi.org/10.1111/1462-2920.12173>.
- Horino H, Fujita T, Tonouchi A. 2014. Description of *Anaerobacterium chartisolvans* gen. nov., sp. nov., an obligately anaerobic bacterium from *Clostridium* rRNA cluster III isolated from soil of a Japanese rice field, and reclassification of *Bacteroides cellulosolvans* Murray et al. 1984 as *Pseudobacteroides cellulosolvans* gen. nov., comb. nov. *Int J Syst Bacteriol* 64:1296–1303. <http://dx.doi.org/10.1099/ijs.0.059378-0>.
- Giuliano C, Khan AW. 1984. Cellulase and sugar formation by *Bacteroides cellulosolvans*, a newly isolated cellulolytic anaerobe. *Appl Environ Microbiol* 48:446–448.
- Giuliano C, Khan AW. 1985. Conversion of cellulose to sugars by resting cells of a mesophilic anaerobe, *Bacteroides cellulosolvans*. *Biotechnol Bioeng* 27:980–983. <http://dx.doi.org/10.1002/bit.260270708>.
- Murray WD. 1985. Increased cellulose hydrolysis by *Bacteroides cellulosolvans* in a simplified synthetic medium. *J Biotechnol* 3:131–140. [http://dx.doi.org/10.1016/0168-1656\(85\)90014-8](http://dx.doi.org/10.1016/0168-1656(85)90014-8).
- Murray WD, Sowden LC, Colvin JR. 1986. Localization of the cellulase activity of *Bacteroides cellulosolvans*. *Lett Appl Microbiol* 3:69–72. <http://dx.doi.org/10.1111/j.1472-765X.1986.tb01550.x>.
- Lamed R, Naimark J, Morgenstern E, Bayer EA. 1987. Specialized cell surface structures in cellulolytic bacteria. *J Bacteriol* 169:3792–3800.
- Morag E, Halevy I, Bayer EA, Lamed R. 1991. Isolation and properties of a major cellobiohydrolase from the cellulosome of *Clostridium thermocellum*. *J Bacteriol* 173:4155–4162.
- Lamed R, Morag (Morgenstern) E, Mor-Yosef O, Bayer EA. 1991. Cellulosome-like entities in *Bacteroides cellulosolvans*. *Curr Microbiol* 22:27–33. <http://dx.doi.org/10.1007/BF02106209>.
- Ding S-Y, Bayer EA, Steiner D, Shoham Y, Lamed R. 2000. A scaffoldin of the *Bacteroides cellulosolvans* cellulosome that contains 11 type II cohesins. *J Bacteriol* 182:4915–4925. <http://dx.doi.org/10.1128/JB.182.17.4915-4925.2000>.
- Xu Q, Bayer EA, Goldman M, Kenig R, Shoham Y, Lamed R. 2004. Architecture of the *Bacteroides cellulosolvans* cellulosome: description of a cell-surface-anchoring scaffoldin and a family-48 cellulase. *J Bacteriol* 186:968–977. <http://dx.doi.org/10.1128/JB.186.4.968-977.2004>.
- Noach I, Frolow F, Jakoby H, Rosenheck S, Shimon LJW, Lamed R, Bayer EA. 2005. Crystal structure of a type-II cohesin module from the *Bacteroides cellulosolvans* cellulosome reveals novel and distinctive secondary structural elements. *J Mol Biol* 348:1–12. <http://dx.doi.org/10.1016/j.jmb.2005.02.024>.
- Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A. 2012. Geneious basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28:1647–1649. <http://dx.doi.org/10.1093/bioinformatics/bts199>.
- Utturkar SM, Klingeman DM, Land ML, Schadt CW, Doktycz MJ, Pelletier DA, Brown SD. 2014. Evaluation and validation of *de novo* and hybrid assembly techniques to derive high-quality genome sequences. *Bioinformatics* 30:2709–2716. <http://dx.doi.org/10.1093/bioinformatics/btu391>.
- De Leon KB, Utturkar SM, Camilleri LB, Elias DA, Arkin AP, Fields MW, Brown SD, Wall JD. 2015. Complete genome sequence of *Pelosinus fermentans* JBW45, a member of a remarkably competitive group of *Negativicutes* in the *Firmicutes* phylum. *Genome Announc* 2(5):01090–15. <http://dx.doi.org/10.1128/genomeA.01090-15>.
- Woo HL, Utturkar S, Klingeman D, Simmons BA, DeAngelis KM, Brown SD, Hazen TC. 2014. Draft genome sequence of the lignin-degrading *Burkholderia* sp. strain lig30, isolated from wet tropical forest soil. *Genome Announc* 2(3):00637–14. <http://dx.doi.org/10.1128/genomeA.00637-14>.
- Hyatt D, Chen G-L, LoCascio PF, Land ML, Larimer FW, Hauser LJ. 2010. Prodigal: prokaryotic gene recognition and translation initiation site identification. *BMC Bioinformatics* 11:119. <http://dx.doi.org/10.1186/1471-2105-11-119>.
- Haimovitz R, Barak Y, Morag E, Voronov-Goldman M, Shoham Y, Lamed R, Bayer EA. 2008. Cohesin-dockerin microarray: diverse specificities between two complementary families of interacting protein modules. *Proteomics* 8:968–979. <http://dx.doi.org/10.1002/pmic.200700486>.
- Cameron K, Weinstein JY, Zhivin O, Bule P, Fleishman SJ, Alves VD, Gilbert HJ, Ferreira LMA, Fontes CMGA, Bayer EA, Najmudin S. 2015. Combined crystal structure of a type-I cohesin: mutation and affinity-binding studies reveal structural determinants of cohesin-dockerin specificity. *J Biol Chem* 290:16215–16225. <http://dx.doi.org/10.1074/jbc.M115.653303>.