

# Genome Sequence of *Chthoniobacter flavus* Ellin428, an Aerobic Heterotrophic Soil Bacterium<sup>∇</sup>

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***Chthoniobacter flavus* Ellin428 is the first isolate from the class *Spartobacteria* of the bacterial phylum *Verrucomicrobia*. *C. flavus* Ellin428 can metabolize many of the saccharide components of plant biomass but is incapable of growth on amino acids or organic acids other than pyruvate.**

*Chthoniobacter flavus* Ellin428 is a nonmotile, rod-shaped, yellow Gram-negative aerobic bacterium, isolated from a rye-grass-clover pasture in Victoria, Australia (8). It is the first isolate of the class *Spartobacteria* within the *Verrucomicrobia*. *C. flavus* Ellin428 is able to grow on many of the saccharides that can be found in plant biomass. It is likely that strain Ellin428 is involved in the transformation of organic carbon compounds in the soil, as has been suggested previously (8). Ellin428 does not seem to have the capacity to fix nitrogen. Whereas bacteria from the *Verrucomicrobia* have in the past only rarely been detected by cultivation, molecular surveys mostly targeting 16S rRNA or the encoding gene have indicated that members of this deeply branching phylum are abundant in a large variety of different ecosystems (1, 7, 10, 11).

Compartmentalization has been reported to occur in several *Verrucomicrobia* species, such as *Chthoniobacter flavus*, *Pedosphera parvula*, and *Verrucomicrobium spinosum* (4). Membrane coat-like proteins are known to be important in shaping eukaryotic membranes; however, they have evaded detection in prokaryotic genomes based on sequence similarity (9). A total of 14 membrane coat proteins were detected in *C. flavus* Ellin428 in a survey of available genomes of members of the *Planctomycetes-Verrucomicrobia-Chlamydiae* superphylum, using coat-protein-specific fold types rather than sequence-based searches (9).

The genome of *C. flavus* Ellin428 was sequenced using a combination of Sanger (3-kb pUC) and 454 sequencing plat-

forms (GS 20). All general aspects of library construction and sequencing can be found at the JGI website (<http://www.jgi.doe.gov/>). Pyrosequencing reads were assembled using the Newbler assembler (Roche). Possible misassemblies were corrected, and gaps between contigs were closed by editing in Consed, by custom primer walks from subclones or PCR products. Together, the combination of the Sanger and 454 sequencing platforms provided 25.6-fold coverage of the genome. The gene-modeling program Prodigal (3) was used for the prediction of protein-coding sequences (CDS) from the unclosed permanent draft genome, using default settings that permit overlapping genes and using ATG, GTG, and TTG as potential starts. The resulting protein translations were compared to GenBank's nonredundant database (NR) and the Swiss-Prot/TrEMBL, PRIAM, Pfam, TIGRFam, Interpro, KEGG, and COGs (clusters of orthologous groups of proteins) databases using BLASTP or HMMER. From these results, product assignments were made, followed by manual corrections (6).

The unclosed draft genome of *C. flavus* Ellin428 contains 7,848,700 nucleotides. The overall G+C content of the chromosome is 61.1%. The chromosome contains 6,716 CDS, 58 tRNA genes, and 4 rRNAs. A putative function could be predicted for 3,584 CDS (53%), and 3,658 CDS (54%) were assigned to COGs. For 30% of all CDS (2,007), signal peptides were predicted.

**Nucleotide sequence accession number.** The draft genome sequence of *C. flavus* Ellin428 has been assigned GenBank accession number ABVL000000000.

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## REFERENCES

1. **Derrien, M., M. C. Collado, K. Ben-Amor, S. Salminen, and W. M. de Vos.** 2008. The mucin degrader *Akkermansia muciniphila* is an abundant resident of the human intestinal tract. *Appl. Environ. Microbiol.* **74**:1646–1648.
2. Reference deleted.
3. **Hyatt, D., et al.** 2010. Prodigal: prokaryotic gene recognition and translation initiation site identification. *BMC Bioinformatics* **11**:119.
4. **Lee, K. C., et al.** 2009. Phylum *Verrucomicrobia* representatives share a compartmentalized cell plan with members of bacterial phylum Planctomycetes. *BMC Microbiol.* **9**:5.
5. Reference deleted.
6. **Markowitz, V. M., et al.** 2006. The integrated microbial genomes (IMG) system. *Nucleic Acids Res.* **34**:D344–D348.
7. **Sangwan, P., S. Kovac, K. E. R. Davis, M. Sait, and P. H. Janssen.** 2005. Detection and cultivation of soil verrucomicrobia. *Appl. Environ. Microbiol.* **71**:8402–8410.
8. **Sangwan, P., X. Chen, P. Hugenholtz, and P. H. Janssen.** 2004. *Chthoniobacter flavus* gen. nov., sp. nov., the first pure-culture representative of subdivision two, *Spartobacteria* classis nov., of the phylum *Verrucomicrobia*. *Appl. Environ. Microbiol.* **70**:5875–5881.
9. **Santarella-Mellwig, R., et al.** 2010. The compartmentalized bacteria of the planctomycetes-verrucomicrobia-chlamydiae superphylum have membrane coat-like proteins. *PLoS Biol.* **8**:e1000281.
10. **van Passel, M. W. J., et al.** 2011. The genome of *Akkermansia muciniphila*, a dedicated intestinal mucin degrader, and its use in exploring intestinal metagenomes. *PLoS One.* **6**:e16876.
11. **Wagner, M., and M. Horn.** 2006. The *Planctomycetes*, *Verrucomicrobia*, *Chlamydiae* and sister phyla comprise a superphylum with biotechnological and medical relevance. *Curr. Opin. Biotechnol.* **17**:241–249.