A Genomic Comparison of Two Gram Positive Aerobes Reveals Different Strategies for Carbohydrate Degradation

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Project Goals: Produce new cellulytic and hemicellulytic enzymes for GLBRC partners.

New and improved biomass-degrading enzymes are a major requirement to achieving a viable cellulosic fuels business. *Cellulomonas fimi* ATCC 484 (Celf) and *Streptomyces flavogriseus* ATCC 33331 (Sfla) are high GC actinobacteria reported as cellulolytic organisms. Preliminary results indicate that both microorganisms are capable of hydrolyzing crystalline cellulose. Genomic DNA was prepared for sequencing, assembly and annotation by the DOE Joint Genome Institute. Genomic analysis indicates that both of these soil microbes utilize a secretory model for producing most of their carbohydrate active enzymes (CAZymes); there is no evidence for cellulosomes and little evidence for membrane bound CAZymes. Sfla has almost twice as many genes (6858) compared to Celf (3864) but roughly the same number of CAZymes (220 vs 173). The number and type of glycosyl hydrolases (GH), carbohydrate esterases (CE), pectate lyases (PL), and carbohydrate binding modules (CBM) and the structural families they belonged to were compared. Numerically, the most abundant CBM families are 2, 13, and 32 for Sfla compared to family 2 and 13 for Celf. CMB family 32 binds preferentially to galactosyl saccharides, family 2 binds cellulose, and family 13 binds xylan. Celf has 5 genes and Sfla has 15 genes containing annotated CBMs attached to unknown domains. The Sfla genome contains 5 CBM33 members, compared to only one in Celf. CBM33 proteins may be functionally similar to the GH61 present in fungi, suggesting a more oxidative route to cellulose degradation in Sfla. The Sfla genome contains twelve GH23 and two GH25 genes, compared to two GH23 and zero GH25 genes in Celf; GH23 and GH25 genes code for lysozyme-type enzymes. Sfla possesses eight GH18 family members compared to only one in Celf; GH18 genes typically code for chitinase or cellulase activities. Celf contains slightly more GH13 family members (18 vs. 13); GH13 genes code for amylases. The potency and utility of Celf and Sfla cellulosic enzymes are being studied by GLBRC research scientists.

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