Contribution of scaffoldins to biomass degradation by *Clostridium thermocellum*: the effect of scaffoldin-deletions on expression of other genes

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Background: *Clostridium thermocellum* cellulosomes are supramolecular machines that digest plant cell walls very efficiently. One of our goals is to understand the working mechanism of the *C. thermocellum* cellulosome system, which consists of numerous different cellulosomal cellulases that are organized, through specialized binding domains, onto non-catalytic scaffoldin peptides. In order to create tools for studies of the individual functions and contributions to overall activity of the different scaffoldin proteins, we are systematically creating mutants in which we have knocked out the genes for individual scaffoldins or combinations thereof. The knockout mutants created thus far are being investigated by transcriptomic profiling to elucidate regulatory and functional relationships among the scaffoldins and cellulases.

I. Materials and Methods:

- Genetic system used for gene knockout: a system for multi-gene knockout in *C. thermocellum*, provided to NREL by Dartmouth College (Lynd Laboratory).
- Gene deletion followed Dan Olson's protocol [1]; correct gene deletion was confirmed by both PCR, DNA sequencing and genome sequencing.
- Strains were grown at 50 ml in anaerobic serum bottles on 5 g/L Avicel (Sigma, PH-101) in MTC medium (5 g/L MOPS). Cultures were grown at 60°C and shaken at 180 RPM. Cells were collected when the Avicel conversion reached 50 %, frozen immediately in liquid nitrogen, and then stored at -80°C for further transcriptomic study.
- RNA was isolated from 25 ml of culture using the TriZol reagent and a bead beating method, as described previously [2]. Labeled cDNA was prepared and hybridized to a NimbleGen 12-plex array following the manufacturer's protocols for gene expression. Probe intensity values were $\log_2$ transformed on importation into JMP Genomics version 6 (SAS Institute, Cary, NC, USA). Data were normalized using the Loess method and significant differential expression determined by the ANOVA method with a 5% False Discovery Rate.

II. Results:

Table 1. *C. thermocellum* Gene-Knockout Strains

Strain	Gene(s) Deleted
CTN4	CipA, OlpB, Orf2P, OlpA and SdbA
CTN5	OlpB, Orf2p, OlpA and SdbA
CTN7	CipA, OlpB, Orf2p, OlpA, SdbA and OlpC
DS11	CipA
DS3	Parent

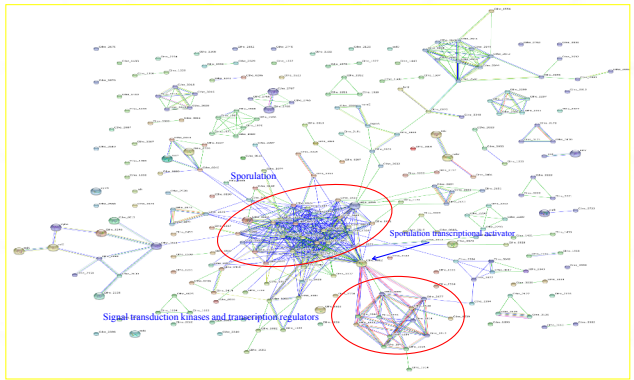

Figure 2. Relationships among proteins encoded by up-regulated genes in DS11 versus parent DS3

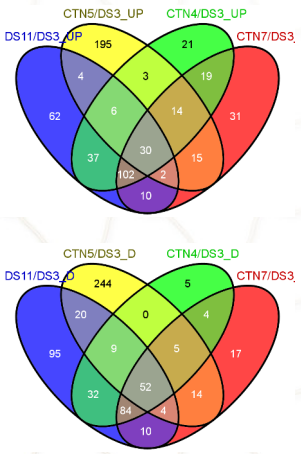

Figure 1. Venn diagram of up-regulated (top) and down-regulated (bottom) genes in four mutant strains compared to DS3.

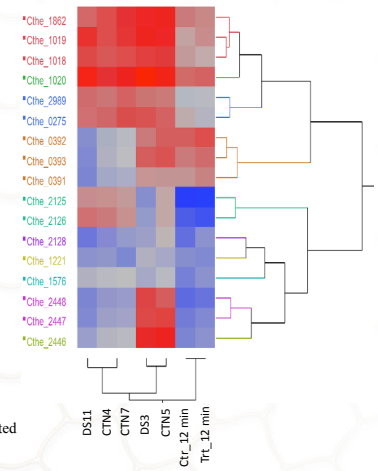

Figure 3. Hierarchical clustering of the gene expression of sugar transporter genes in various strains.

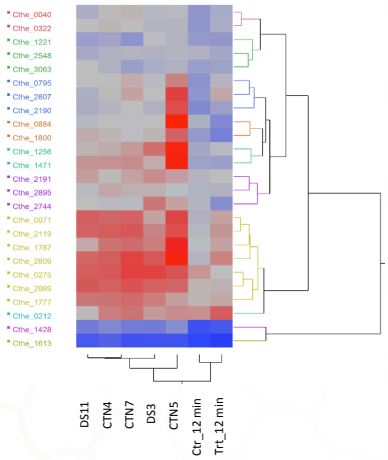

Figure 4. Hierarchical clustering of the gene expression of non-cellulosomal cellulase and hemicellulase genes in various strains

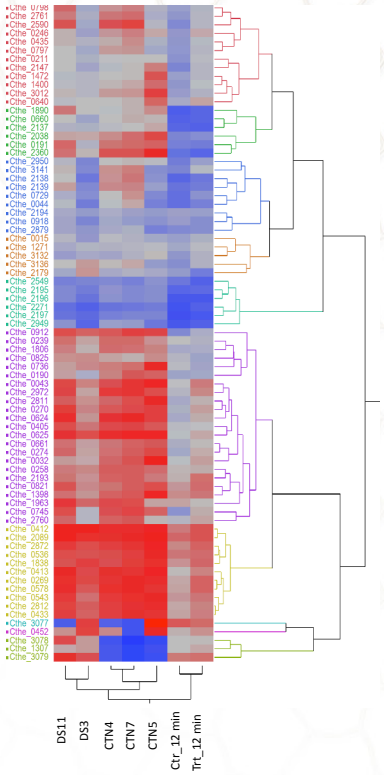

Figure 5. Hierarchical clustering of the gene expression of cellulosomal cellulase and hemicellulase genes in various strains.

III. Summary, Conclusions and Discussion:

1. Various strains with deletion of primary scaffoldin cipA and other scaffoldins or their combinations have been used for a transcription profile study (Table 1). Large numbers of genes, not only the genes related to biomass degradation and utilization, but also genes for other pathways (for example,,sporulation), have been up- or down-regulated (Figure 1 and 2).
2. After deletion of cipA (individually or combined with other scaffoldin genes), 1) the sugar transporters used in these deletion strains are different from the ones used in its parent; 2) the free (non-cellulosomal) enzyme system has not been up-regulated significantly to compensate for disruption of the cellulosome system by deletion of cipA (Figure 4), and 3) some individual cellulosomal cellulase and hemicellulase genes are up-regulated significantly (Figure 5), but secretome activity of the system has been dramatically reduced (previous result), demonstrating that the formation of the complete cellulosome is crucial for ***C. thermocellum's*** extremely high performance in Avicel degradation, as reported before.

IV. References:

1.Daniel G. Olson and Lee R. Lynd (2012). Transformation of Clostridium thermocellum by Electroporation. Methods in Enzymology 510, 317-330.
2.Yang S, Giannone RJ, Dice L, Yang ZK, Engle NL, Tschaplinski TJ, Hettich RL, Brown SD (2012): Elucidation of the Clostridium thermocellum ATCC27405 ethanol shock responses using an integrated transcriptomic, proteomic and metabolomic profiling approach. BMC Genomics 13, 336.

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