The CBMomes of cellulolytic bacteria colonizing different ecological niches present distinct carbohydrate specificities

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Background

The energetic constraints posed by anaerobic ecosystems lead to the evolution of remarkable highly efficient supramolecular multi-enzyme complexes of Carbohydrate Active Enzymes (CAZymes), termed Cellulosomes (Figure 1).

Cellulosomal enzymes are often appended to other non-catalytic domains, such as Carbohydrate Binding Modules (CBMs), which participate in pivotal protein-carbohydrate interactions, thus targeting the catalytic module to specific carbohydrate substrates.

Clostridium thermocellum and Ruminococcus flavefaciens FD-1 are two highly efficient cellulose-producing cellulolytic bacteria that colonize different ecological niches, the soil and the rumen of mammals, respectively.

Aims

An High throughput Cloning, Expression and Protein purification platform was used to produce the CBMomes of R. flavefaciens and C. thermocellum.

Microarray technology was used to uncover oligosaccharide and polysaccharide binding of the CBMs from the different organisms.

Cloning, Expression and Purification of 150 CBMs

Figure 2. An High-Throughput cloning and expression platform was used to produce 150 CBMs, 30 from C. thermocellum and 50 from R. flavefaciens. A: The genes were amplified and cloned in an Escherichia coli expression vector. B. The majority of the CBMs were readily expressed and purified using IMAC.

C. thermocellum

R. flavefaciens FD-1

Concluding Remarks

C. thermocellum contains a larger number of CBMs than R. flavefaciens, maybe reflecting its thermophilic origin.

The two bacteria present CBMomes expressing different carbohydrate-binding specificities.

Overall this work suggests that the physico-chemical properties of different ecological niches modulate the evolution of CAZymes presenting distinct ligand specificities.

CBM ligand specificity by Carbohydrate Microarray Analysis

Figure 3. Representation and imaging of the microarray. Polysaccharide and oligosaccharide probes in the form of microplates are robotically spotted into 16-petri microculture-based glass slides. In this array format binding of up to 16 CBMs to 64 different probes can be analyzed on the same slide.

Figure 4. Summary of the microarray analyses of both organisms CBMomes. Initial screening analyses were performed for 65 C. thermocellum and 40 R. flavefaciens CBMs. Data showed that 32 C. thermocellum and 23 R. flavefaciens CBMs bind to at least one of the carbohydrate probes included in the microarray.

Figure 5. Initial microarray screening analyses of the C. thermocellum and R. flavefaciens FD-1 CBM modules belonging to different CAZomes. The relative intensities of binding to the carbohydrate probes in the microarray are shown as the percentage of the fluorescence signal intensity relative to the probe that binds more strongly to each protein. For each family a representative CBM of these analyzed is shown. The carbohydrate microarray is comprised of polysaccharide samples from different sources - representative of major sequences found in fungal and plant cell walls, and a set of oligosaccharides prepared as oligosaccharidyl (OSG) probes. The sequence-defined OSG probes included in the microarray are depicted at the left.

REFERENCES:


ACKNOWLEDGMENTS:

The authors are grateful to D. Robert Doble from the Glycosciences Laboratory, Imperial College London for assistance in the robotic microarray printing and to Professor Manuel Coimbra (University of Aveiro) for kindly providing some of the polysaccharides.