

## Heterologous Expression of a *Clostridium thermocellum* Proteins and Assembly of Cellulosomes 'in vitro' for Biotechnology Applications

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**Abstract :** The utilization of lignocellulosic biomass as source of polysaccharides for industrial applications requires an arsenal of enzymes with different mode of action able to hydrolyze its complex and recalcitrant structure. *Clostridium thermocellum* is gram-positive, thermophilic bacterium producing lignocellulosic hydrolyzing enzymes in the form of multi-enzyme complex, termed celulosomes. This complex has several hydrolytic enzymes attached to a large and enzymically inactive protein known as Cellulosome-integrating protein (CipA), which serves as a scaffolding protein for the complex produced. This attachment occurs through specific interactions between cohesin modules of CipA and dockerin modules in enzymes. The present work aims to construct celulosomes in vitro with the structural protein CipA, a xylanase called Xyn10D and a cellulose called CelJ from *C.thermocellum*. A mini-scaffoldin was constructed from modules derived from CipA containing two cohesion modules. This was cloned and expressed in *Escherichia coli*. The other two genes were cloned under the control of the alcohol oxidase 1 promoter (AOX1) in the vector pPIC9 and integrated into the genome of the methylotrophic yeast *Pichia pastoris* GS115. Purification of each protein is being carried out. Further studies regarding enzymatic activity of the celulosome is going to be evaluated. The celulosome built in vitro and composed of mini-CipA, CelJ and Xyn10D, can be very interesting for application in industrial processes involving the degradation of plant biomass.

**Keywords :** celulosome, CipA, *Clostridium thermocellum*, cohesin, dockerin, yeast

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