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The cellulosome of *Clostridium cellulolyticum*

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Abstract

Clostridium cellulolyticum ATCC 35319 has been extensively studied in the past few years on both the enzymatic and metabolic aspects of cellulose degradation and is considered as the model of mesophilic cellulolytic *clostridia*. As is true of most cellulolytic *clostridia*, this bacterium possesses an extracellular multi-enzymatic complex, the cellulosome. Cellulosomes are highly efficient in the degradation of plant cell wall polysaccharides. In *C. cellulolyticum*, most of the cellulosomal genes are localised in an approximately 26 kb DNA fragment, the *cel* cluster, where 12 genes have been identified to date *cipC-cel48F-cel8C-cel9G-cel9E-orfX-cel9H-cel9J-man5K-cel9M-rgl11Y-cel5N*. The *C. cellulolyticum* cellulosome is organised around the scaffolding protein CipC, which permits the binding of the different cellulosomal enzymes via interactions of dockerin–cohesin domains. Twelve cellulosomal enzymes have been identified including cellulases, hemicellulase and pectinase. The state of knowledge about the structure, ultrastructure, enzymatic activity, regulation and extracellular assembly of the *C. cellulolyticum* cellulosome are reviewed, and potential biotechnological exploitations discussed.

Keywords: Cellulolytic clostridia; Cellulose degradation; Cellulosome; Bacterial metabolism; Protein engineering

1. Introduction

Cellulose is a linear insoluble glucan biopolymer composed of the repeating structural unit cellobiose [1]. The association of these glucan chains leads to the formation a microfibril in a regular crystalline arrangement [2]. At the microscopic scale, the association of these microfibrils forms a cellulose fibril also called macrofibril or fibre at the macroscopic scale [3]. Native cellulose is paracrystalline; amorphous and crystalline regions alternate within the microfibril [4]. In nature, cellulose is essentially synthesised by plants [5,6] but also by some animals, algae and bacteria [7,8]. In plants, cellulose is associated with other biopolymers, i.e. hemicelluloses, pectins, glycoproteins and lignin, in various proportions; it is therefore designated lignocellulose [1,9,10]. In terrestrial ecosystems, the net primary production of biomass is 60 milliard tonnes of carbon per year and about half of this carbon is fixed in the form of cellulose [11].

Even with the existence of cellulases of animal origin now firmly established [12], the cellulose degradation in the environment is still considered primarily as a microbiological process, involving protozoa, fungi and bacteria [13,14]. Micro-organisms have developed several strategies to digest the cellulose [7]. Some produce a large amount of cellulases as single enzymes released in the extracellular medium. Others produce single polypeptides containing several cellulosic domains. An even greater level of complexity has been attained by some anaerobic micro-organisms where several cellulases are regrouped into an extracellular enzymatic complex, called cellulosome. The concept of cellulosome was firstly introduced with the thermophilic cellulolytic and anaerobic bacterium, *Clostridium thermocellum* [15,16].

Abbreviations: CBD, cellulose-binding domain; CBM, carbohydrate-binding domain; CbpA, cellulose binding protein A; Cc, *Clostridium cellulolyticum*; Cel, cellulase; CipA, cellulosome integrating protein A; CipC, cellulosome integrating protein C; Coh, cohesin domain; Doc, dockerin domain; EngE, endoglucanase E; GH, glycoside hydrolase; HLD, hydrophilic domain; Ig, immunoglobulin-like module; Man, mannanase; OlpB, outer layer protein B; ORF, open-reading frame; ORFXp, ORF X polypeptide; ORF2p, ORF 2 polypeptide; Pel, pectate lyase; PL, polysaccharide lyase; PTS, proline–threonine–serine; Rlg, rhamnogalacturonase; SdbA, scaffoldin dockerin binding A; SLH, S-layer homology

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Cellulosic activities allow the release of soluble cellodextrins from cellulose, which permits in return microbial growth. In cellulolytic ecosystems, complex interactions exist between the different microbial communities [3]. The final products of cellulose digestion are water and carbon dioxide in aerobic conditions, and also methane in anaerobiosis [3,17]. Therefore, mineralisation of cellulose plays a key role in carbon cycling within the biosphere [3,17]. From a biotechnological point of view, enzymatic and/or microbiological processes seem extremely promising approaches for the valorisation of cellulosic compounds [18]. Since the first step in cellulose metabolisation or degradation involves the action of cellulases, a lot of research has focused on these enzymes in the past few years [19]. Among the different cellulosic systems, the cellulosome received particular attention since it permits a highly efficient degradation of crystalline cellulose and offers exceptional biotechnological applications [20].

Cellulosomes have only been described in anaerobic micro-organisms, i.e. some fungi and bacteria [21]. In the domain *Bacteria*, organisms possessing cellulosomes are only found in the phylum *Firmicutes*, class *Clostridia*, order *Clostridiales* and in the families *Lachnospiraceae* and *Clostridiaceae*. In this latter family, bacteria with cellulosomes are found in various clusters of the genus *Clostridium*. From a new phylogenetic arrangement, this genus has been divided into 19 different clusters [22–24]; clusters I, III–V, X and XIV contains cellulolytic clostridia [25]. Most cellulolytic clostridia are suspected to possess a cellulosome; most of this cellulolytic species are found in cluster III. In this cluster, experimental evidence of the presence of a cellulosome exists for the species *C. cellulolyticum*, *C. josui*, *C. papyrosolvens* and *C. thermocellum* [21,26]. For *C. aldrichii*, *C. cellobioparum* and *C. termitidis*, consistent experimental evidences are still awaited. However, for *C. stercorarium* neither genetic nor biochemical approaches could reveal its presence [7,27]. Interestingly, for *C. cellulovorans* and *C. acetobutylicum*, which belong to cluster I, genetic analyses have shown that the synteny and sequence homology of the cellulosomal genes are very similar to those of *C. cellulolyticum* [28–30]. Taking into account the phylogenetic distance between these three species, horizontal gene transfer rather than a common bacterial ancestor seems a most probable hypothesis explaining the close taxonomic relatedness of those cellulosomal genes [31,32].

Cellulosomes are present on the bacterial cell surface and are dedicated to cellulose depolymerisation. For the bacterial cell, the biosynthesis of a cellulosome presents several advantages: (i) a direct and specific adhesion to the substrate of interest permitting efficient competition with other micro-organisms present in the same biota and (ii) the proximity of the cell to the cellulose insures an efficient cellular uptake of the soluble cellodextrins by avoiding their diffusion in the extracellular medium [16]. From an enzymatic point of view, the cellulosome (i) allows optimum concerted activity and synergism of the cellulases, (ii) avoids non-productive adsorption of the cellulases, (iii) limits competition between cellulases for the sites of adsorption and (iv) allows optimal processivity of the cellulase all along the cellulose fibre [7].

C. cellulolyticum ATCC 35319, formerly identified as the strain H_{10}, was isolated from decayed grass compost at the Université de Nancy, France, in autumn 1982 by Petitdemange et al. [33]. In the following years, the genetic, structure, function and interaction of the cellulosic system of this bacterium have been the subject of considerable research. In light of new findings in this field, this review will describe the state of knowledge about the cellulosic system of *C. cellulolyticum* and its potential biotechnological exploitations.

2. Structure and ultrastructure of the cellulosome

In *C. cellulolyticum*, most of the cellulosomal genes are clustered in an approximately 26 kb long DNA fragment in which 12 genes have been identified, i.e. *cipC-cel48F-cel8C-cel9G-cel9E-orfX-cel9H-cel9J-man5K-cel9M-rgl11Y-cel5N* (Fig. 1) [34,35]. This cluster represents the largest *cel* cluster described in cellulosome-producing *clostridia* [34]. Such a genetic organisation is not always the rule since in *C. thermocellum* the cellulosomal genes seem scattered all over the genome [36].

cipC, the first gene of the *cel* cluster, encodes a specialized integrating protein without any catalytic activity, called scaffolding protein or scaffoldin. Cellulosome integrating protein C (CipC) permits binding of the different catalytic cellulosomal components. CipC has a modular organisation consisting of eight cohesin domains of type I (Coh_I) numbered from 1 to 8 from the N- to the C-terminus (Fig. 2). The cohesins permit the tight binding of complementary dockerin domain of type I (Doc_I) borne by the cellulosomal enzymes. In addition, a carbohydrate-binding module (CBM) permitting adhesion to the cellulose [37,38] and two X-modules, also called X2-module or hydrophilic domains (HLDs), are also present [39]. The CBM of CipC belongs to the family IIIa and allows the tight binding of the entire cellulosome to cellulosic substrate. The exact function of the X-module is still speculative (see Section 4.2). The crystallisation of CipC has proved extremely difficult [40]. However, the structural representatives of each of the cellulosome

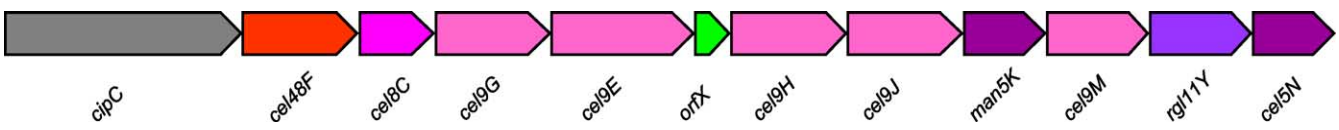

Fig. 1. Schema of the *cel* cluster in *C. cellulolyticum*.