Xylanase Activities Associated with the *Clostridium thermocellum* Cellulosome

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The cellulosome produced by *Clostridium thermocellum* is a multienzyme complex having strong activity against crystalline cellulose. The presence of xylanase activity has been often reported associated with the cellulosome of *C. thermocellum*, although this bacterium is unable to grow on xylan and xylose. In agreement with these observations, several xylanase genes have been cloned and characterized along with their translated products by our group and others, i.e., *xynA*, *xynB*, *xynC*, *XynX*, *xynY*, and *xynZ*. XynA and XynB contain a family 11 catalytic domain and the other xylanases, family 10. In addition, the endoglucanase CelJ encoded by *celJ* has an activity toward xylan as well as cellulosic materials. Among these enzymes XynC and CelJ were identified as major components of the *C. thermocellum* cellulosome. In this paper, we attempt to summarize xylanase species associated with the *C. thermocellum* cellulosome and their genes.

INTRODUCTION

Clostridium thermocellum is well-known to produce the cellulosome, a multienzyme complex having strong activity against crystalline cellulose. Molecular aspects of the *C. thermocellum* cellulosome will be described in detail by other authors in this book. Although this bacterium is unable to grow on xylan and xylose (1), the presence of xylanase activities has been often reported associated with the cellulosome, e.g., Morag et al. (2) showed that several major xylanase activities could be detected, corresponding to molecular weights of 170,000, 84,000 67,000, and 54,000, by activity staining of the regenerated xylanase activities after SDS-PAGE separation. They also found that several xylanase activities existed in noncellulosomal proteins. Similar results were obtained by Kohring et al. (3). When the cellulosome attacks natural plant fibers, xylanase species should play a role for facilitating hydrolysis of them, because cellulose coexists with hemicellulose such as xylan, which will prohibit cellulases from approaching the cellulose in plant cell walls. For understanding the function of the cellulosome, therefore, it is important to characterize xylanase species present in it. From this point of view, xylanase genes were cloned from *C. thermocellum* and characterized along with their gene products by us and other groups. In this paper, we attempt to summarize xylanase species associated with the *C. thermocellum* cellulosome and their genes.

MATERIALS AND METHODS

Strains and growth conditions. The *C. thermocellum* strain F1 was used for the isolation of the genomic DNA (30). The *E. coli* strains used in this study were XL1-Blue MRF' (Stratagene) and JM109 (Stratagene). Recombinant *E. coli* strains were cultured in Luria broth supplemented with ampicillin (50 □g/ml) at 37 ˚C.

Plasmids and plasmid constructions. The plasmid vectors used in this study were pT7 Blue (Novagen), pBluescript II KS(+) and KS(-) (Stratagene), and pQE-30T (3). The plasmids used to produce the truncated derivatives of CelJ were constructed as follows: DNA

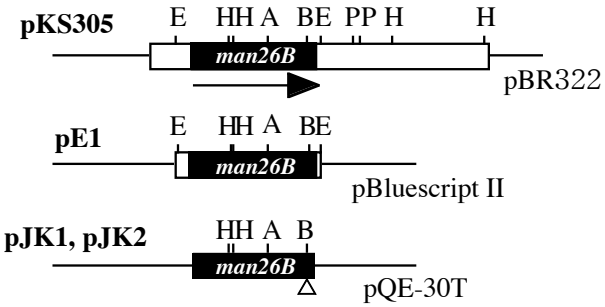

Fig. 1. Restriction maps of pKS305 and its derivatives.

fragments encoding respective derivatives were amplified by PCR from *C. thermocellum* F1 genomic DNA with LA *Taq* DNA polymerase (Takara) and an appropriate combination of primers (Fig. 1) containing artificial *Bgl*II or *Sal*I restriction sites for cloning the PCR fragments into plasmid vectors. The resulting PCR fragments were cloned into pT7 Blue according to the supplier's protocol. After sequencing the inserted DNA fragments for confirmation of the absence of mutation, the inserted fragments were transferred to pQE-30T. The combinations of the primers were as follows: celJF-Bgl706 and celJR-Sal2868 to construct pCBM-CM yielding a polypeptide composed of the family 30 CBM, the Ig-like module, and the family 9 catalytic module (CBM30-Ig-CM9); celJF-Bgl706 and celJR-Sal1248 to construct pCBM yielding a polypeptide composed of the CBM (CBM30); celJF-Bgl706 and ce lJR-Sal1561 to construct pCBM-Ig yielding a polypeptide composed of the CBM and the Ig-like module (CBM30-Ig); celJF-Bgl1269 and celJR-Sal2868 to construct pIg-CM yielding a polypeptide composed of the Ig-like module and the catalytic module (Ig-CM9); ----------.

RESULTS

Nucleotide and deduced amino acid sequences of the *cel99A* gene. The plasmid pKS305 was constructed from pBR322 and the genomic DNA of *C. thermocellum* F1 as a recombinant plasmid that conferred endoglucanase activity on *E. coli*. DNA sequencing sequencing of the inserted fragment in this plasmid showed that there was another open reading frame encoding----------.

DISCUSSION

Strong consumers of cellulose are known to produce a number of cellulases with different enzyme properties, -----.

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