0273–2289/84/0600–0349$02.00

Cloning and Expression of Two *Clostridium thermocellum* Endoglucanase Genes in *Escherichia coli*

P. CORNET, J. MILLET, P. BEGUIN, AND J.-P. AUBERT

Unité de Physiologie Cellulaire, Département de Biochimie et Génétique Moléculaire, Institut Pasteur, 75724 Paris Cedex 15, France

ABSTRACT

Along with the biochemical characterization of the enzymes involved in the process of cellulose degradation by *Clostridium thermocellum*, a genetic approach based on the cloning of the genes has been developed. From a gene bank of *C. thermocellum* DNA in cosmid pHC79 (*1*), two *E. coli* clones were isolated that produced endoglucanase activity, as demonstrated by the ability of crude extracts to release reducing sugars from soluble carboxymethylcellulose (CMC) while strongly reducing the viscosity.

The first clone, whose endoglucanase gene is termed *celA*, produced an enzyme sharing immunological and catalytic properties with the M_r 56,000 endoglucanase (designated endoglucanase A) purified from *C. thermocellum* culture supernatant by Pètre et al. (*2*). The second clone, carrying a gene termed *celB*, produced a different enzyme, which failed to cross-react immunologically with anti-endoglucanase A antiserum and had a lower activity toward trinitrophenylated CMC (1).

Both genes were subcloned in vector pBR322 to yield plasmids that carry *C. thermocellum* DNA insertions of about 2.7 kb. The cloned sequences were used as probes to analyze restriction fragments of total *C. thermocellum* DNA by Southern hybridization. Such studies demonstrated that the two genes share no homology and that they are not contiguous on the *C. thermocellum* chromosome.

For both genes, the level of endoglucanase activity was independent of their orientation, indicating that *E. coli* transcription and translation systems recognize sequences of *C. thermocellum* DNA and RNA that are required for proper gene expression. From the specific activity of the purified enzymes, it was calcu-

lated that each represent 0.2–0.4% of total *E. coli* protein, corresponding to at least 20–40% of their expression level in *C. thermocellum.*

Labeling of minicells containing the appropriate plasmids, followed by SDS-PAGE, demonstrated the synthesis of three immunologically related polypeptides encoded by the *celA* gene, with M_r 56,000, 55,000, and 50,200, and one polypeptide of M_r 66,000 encoded by the *celB* gene. In normal *E. coli* cells, the latter undergoes partial proteolysis and yields two active fragments of M_r 55,000 and 53,000. These fragments were purified and a specific antiserum was prepared. Ouchterlony double immunodiffusion tests and Western blot analysis showed that *C. thermocellum* culture supernatant contains a polypeptide of M_r 66,000 cross-reacting with antiserum made against *celB* protein synthesized in *E. coli.*

REFERENCES

1. Cornet, P., Tronik, D., Millet, J., and Aubert, J.-P. (1983), *FEMS Microbiol. Lett.* **16,** 137.
2. Pètre, J., Longin, R., and Millet, J. (1981), *Biochimie* **63,** 629.