MOLECULAR CLONING AND CHARACTERIZATION OF CELLULASE GENES OF *RUMINOCOCCUS FLAVEFACIENS* STRAIN 186

by

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ABSTRACT

A genomic library of *Ruminococcus flavefaciens* 186 was constructed using the lambda vector λNM1149. It constituted 2.1 x 10^4 recombinant clones which was large enough to represent the entire genome of this bacterium. From this library, 26 CMC^+ clones were identified after screening about 2500 recombinant clones. These CMC^+ clones were divided into four groups according to their insertion fragment size. Partial restriction maps of these clones have been achieved. Eight representative clones from these four groups showed different mapping patterns. One of the three 9 kb insert clones (λCM903) was selected for further study based on its ability to express all three types of cellulase activities.

The locations of endoglucanase and exoglucanase genes in λCM903 were determined. Two separate fragments from λCM903 were subcloned and named λCMEH1 (CMC^+) and λCMEH2 (MUC^+). Cross hybridization experiment using *cel*A gene of *C. thermocellum* and the 26 recombinant clones showed no significant homology. However, different degrees of homology were found among the 26 ruminococcal clones.

The difficulty of subcloning the ruminococcal DNA fragments into plasmid vectors was one of the major obstacles in the study of cellulase gene in foreign hosts. Vectors with different functions were tried but all the recombinant plasmid clones showed instability.

The cellular location of cellulase enzymes in *E. coli* cell was determined. Most of the endo- and exo-glucanases were found in the periplasmic space. Partial purification of these cellulase enzymes from *E. coli* cells using chromatography was then performed and the characterization of these enzymes was achieved.

Using *Exo*III deletion, the locations of endoglucanase and exoglucanase genes were determined in λCM903. The internal *Hin*dIII-*Hin*dIII fragment of 7.3 kb from λCM903 was sequenced. Five ORFs were detected using computer software (UWGCG) analyses.

The first ORF which coded for an endoglucanase gene (*ren*A) was 2157 bp long with putative 680 amino acid residue. The SD sequence and promoter sequence were present. The best fitting cellulase gene tested was that of the *cen*A gene of *Ce. fimi.* An unusual structure of Pro-Thr-Ser rich region, which had 38 out of 42 a.a. residue of proline, threonine or serine, was found in the N-terminal of the putative peptide. The second ORF which was 1821 bp long coded for an exoglucanase gene (*rex*). The putative amino acid sequence had 572 a.a. residue, also a SD sequence and a promoter sequence were found. A Pro-Thr-Ser rich region, which was highly conserved with PTS of *ren*A, was found at the C-terminal of the putative peptide. Again, the cellulase gene, *cex* gene of *Ce. fimi* showed the best similarity. It is suggested that the gene structure of cellulase in *R. flavefaciens* strain 186 was similar to cellulase genes in family A as described by Ong et al, 1989. The third ORF was found overlapping with ORF1 using the transcriptional second reading frame. This ORF had a putative SD sequence but lacked a promoter sequence. The coding region of this ORF has the characteristics of a β-glucosidase gene which was 1300 bp long (a putative sequence of 443 amino acid residues). The fourth ORF which used the second reading frame was 1300 bp long with a SD sequence 5' upstream of the ATG codon, overlapped with ORF1 gene. This ORF coded for a protease gene. The fifth ORF located closely to the 3' end of the 7.3 kb fragment used the second reading frame. This ORF had both putative SD sequence and promoter sequence. It was 1080 bp long with putative 341 amino acid residues and showed the structure of a xylanase gene. A short PTS region was also found in this ORF.

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LIST OF PUBLICATIONS

Publications or abstracts arising from this thesis

1. Molecular cloning and expression of multiple cellulase genes of *Ruminococcus flavefaciens* strain 186 in *Escherichia coli*. Huang CM, Kelly WJ, Asmundson RV and Yu PL, Appl. Microbiol. Biotechnol. (1989) 31: 265-271.
2. Purification and cloning of cellulase from *Ruminococcus flavefaciens*. Huang CM, Kelly WJ, Yu PL and Asmundson RV, Collogue FEMS symposium, Paris. 1987.
3. Molecular cloning of cellulase genes of *Ruminococcus flavefaciens* strain 186 in *E. coli*. Huang CM, Kelly WJ, Asmundson RV and Yu PL, New Zealand, Australia Microbiological combined meeting, Auckland. 1987.
4. Characterization of the cellulases and cellulase genes of *Ruminococcus flavefaciens* involved in cellulolytic fermentation. Huang CM, Asmundson RV and Yu PL, Fermentation technologies: Industrial application. (P.L. Yu ed.) Elsevier Applied Science, UK. 1990.
5. Molecular analyses of cellulase genes and cellulase complexes from *Ruminococcus flavefaciens*. Huang CM, Asmundson RV and Yu PL, Microbiological Society annual meeting, New Zealand. Lincoln, 1990.
6. Structure of a cellulase gene from *Ruminococcus flavefaciens*. Huang CM, Yu PL and Asmundson RV, 6th International Symposium on the Genetics of Industrial Microorganisms. Paris. 1990.
7. Structure of the genes encoding endo-, exo-glucanase and β-glucosidase of *Ruminococcus flavefaciens* strain 186. (in preparation)
8. Characterization of endo- and exo-glucanase and β-glucosidase of *Ruminococcus flavefaciens* strain 186 expressed in *E. coli*. (in preparation)
9. Structure of the gene encoding xylanase of *Ruminococcus flavefaciens* strain 186. (in preparation)

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