Preliminary construction of a chimeric cellulose operon containing two structural genes coding for CMCase and cellobiase

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CMCase, a member of cellulose decomposing enzymes, hydrolyze cellulose up to cellobiose. Cellobiase splits cellobiose to glucose units. Therefore, a linkage of the twogenes coding for CMCase and cellobiase on the same plasmid is needed to produce a cellulase complex which can produce glucose from cellulose. A genetic operon in which the two structural genes are under the control of a single promoter would be ideal for this purpose. The present report is on the linking of the two cellulase genes in one plasmid as a preliminary step of the operon construction.

Localization of *Bacillus* CMCase gene in pBSl cloned in *Escherichia coli*

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The *Bacillus* CMCase gene we have previously cloned in E. coli is contained in the 3.2 Kb chromosomal insert of the 7.5 Kb pBSl plasmid. We have also found that the CMCase produced by this gene has molecular weight of about 32,000 suggesting that the CMCase coding region lies on about 0.8 Kb fragment. The present report deals with a series of subclonings to localize more precisely the region coding for the CMCase production.

Physical and catalytic properties of CMCase encoded by *Bacillus subtilis gene in B. megaterium*

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Carboxymethyl cellulase (CMCase) produced by cloned B. *megaterium* was found to contain 5.2% carbohydrate but no metal ion. The enzyme was isoelectric at pH 7.23 and was high is basic amino acids. The N-terminal of the enzyme was glutamic acid. The cellulolytic activity of this enzyme was extended to the small molecular substrates such as from cellotriose to cellopentaose. In additon, the enzyme showed transglycoslation activity. The pK values of the enzyme we estimated to be 4.4 and 6.7, andthat of the enzyme-substrate complex were 4.2 and 7.2, respectively. The enzyme was not affected by the treatment with iodoacetic acid, but the modification of enzyme with carbodiimide and diethyl pyrocarbonate resulted in a marked loss of the enzyme activity. These results suggest that the active site of enzyme essentially contains carboxylic and imidazole group of amino acid residues.