CLONING AND EXPRESSION OF A BACILLUS SP. 79-23 CELLULASE GENE

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SUMMARY

A gene for encoding cellulase was cloned from Bacillus sp. 79-23 into Escherichia coli and the nucleotide sequence was determined. The cellulase gene, designated as celS, was composed of 1,497 base pairs and the nucleotide sequence of the celS gene was highly homologous to those of other B. subtilis cellulase genes. The enzyme encoded by celS was highly active on carboxymethylcellulose but also exhibited activity towards avicel and p-nitrophenyl-β-D-cellobiopyranoside. When its native promoter was replaced with a strong B. subtilis promoter, the extracellular cellulase was produced up to 8.5 units per ml in B. subtilis DB104.

INTRODUCTION

Cellulases have been studied mainly with respect to their potential industrial use for the exploitation of biomass resources. However, we have used cellulases to improve the texture as well as bleaching efficiency in the textile industry. A neutral action is essential for enzymes in the textile industry because an acidic pH may make blotches on the fabric. However, most known fungal and bacterial cellulases act at an acidic pH. We have therefore endeavored to find a cellulase acting at a neutral pH.

We have isolated a Bacillus strain as a producer of the neutral cellulase for potential use in the textile industry. The cellulases of this isolate, Bacillus sp. 79-23, have a broad pH optimum and maximum activities at about pH 6.0 with more than 85% of the maximum activity at pH 7.0 (data not shown). We now cloned a cellulase gene from Bacillus sp. 79-23 into Escherichia coli and have achieved overproduction of the extracellular cellulase in B. subtilis DB104.
MATERIALS AND METHODS

**Bacterial strains and culture conditions:** *Bacillus* sp. 79-23 was used as a cellulase gene source. *Escherichia coli* DH5α and *B. subtilis* DB104 (Kawamura and Doi, 1984) were used as host strains for expression of the cellulase gene. *E. coli* and *B. subtilis* were grown in LB medium at 37 °C. Ampicillin (100 μg/ml) and kanamycin (50 μg/ml) were added to the medium when *E. coli* and *B. subtilis* carrying recombinant plasmids were selected, respectively.

**Cloning and determination of nucleotide sequence of the *Bacillus* sp. 79-23 cellulase gene:** Standard recombinant DNA techniques (Sambrook et al., 1989) were used. The chromosomal DNA of *Bacillus* sp. 79-23 was digested partially with *Sau3AI* and fractionated the fragments having 3~7 kb sizes by using GENECLEAN II kit (Bio 101 Inc.). Those fragments were ligated with *BamHI*-digested and dephosphorylated pUC19 and then transferred into *E. coli* DH5α cells by the electro-transformation method (Dower et al., 1988). From among approximately 10,000 transformants grown on LB agar plates containing 100 μg/ml of ampicillin, a transformant having cellulase activity was isolated byoverlaying 0.6% (w/v) soft agar containing 1 mM 4-methylumberriferyl-β-D-celllobioside (MUC) and scoring fluorescence under UV light after 2 h incubation at 50 °C. For the determination of the nucleotide sequence of the *Bacillus* sp. 79-23 cellulase gene, the DNA fragment harboring the cellulase gene was further fragmented by various restriction enzymes and introduced into pUC19 in *E. coli* DH5α cells. The nucleotide sequence was determined by the dideoxy chain termination method with Sequenase kit (U.S. Biochemical Corp.).

**Transformation of *B. subtilis***: The transformation of *B. subtilis* cells was performed by the electro-transformation method (Goodfellow, 1990).

**Enzyme assays:** The cellulase activities were determined by measuring the amount of releasing sugars by using dinitrosalicylic acid (Miller et al., 1960). The reaction mixtures containing 1% (w/v) of the polysaccharide substrates in 50 mM sodium phosphate buffer (pH 7.0) were incubated at 50 °C. The enzyme activities towards pNP derivatives were determined by measuring the amount of releasing p-nitrophenol (Kim and Pack, 1989). One unit of the enzyme activity was defined as the amount of enzyme that produced 1 μmol of reducing sugar/p-nitrophenol per minute. Protein concentrations were determined by the Bradford method (Bradford, 1976). The detection of cellulase activity in polyacrylamide gels was performed as described by Jung et al. (Jung and Pack, 1993).

RESULTS AND DISCUSSIONS

**Cloning and Nucleotide Sequence of a *Bacillus* sp. 79-23 Cellulase Gene:** A *E. coli* clone showing MUCase activity was isolated from the *Bacillus* sp. 79-23 genomic library and this *E. coli* clone gave a large halo around the colony by staining with Congo Red. The recombinant plasmid, designated as pSW794, contains a 3 kb *Sau3AI* fragment (Fig. 1) and the nucleotide sequence of the cellulase gene was