CLONING AND EXPRESSION OF A $\beta$-1,3-GLUCANASE GENE FROM BACILLUS CIRCULANS KCTC3004 IN ESCHERICHIA COLI

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SUMMARY

A new gene encoding the $\beta$-1,3-glucanase (laminarinase) of Bacillus circulans KCTC3004 was cloned into Escherichia coli using pUC19 as a vector. The gene localized in the 5.3 kb PstI DNA fragment was expressed independently of its orientation in the cloning vector showing enzyme activity about 33 times greater than that produced by the original B. circulans. The optimum pH and temperature of the cloned enzyme were pH 5.4 and 50°C, respectively. The molecular weight of the enzyme was about 38,000 and the processing of the enzyme molecule within the E. coli cell was not observed. The enzyme hydrolyzed laminarin to produce laminaritriose, laminaribiose, and glucose as main products, but it was inactive for lichenan, CMC, or xylan.

INTRODUCTION

Application of recombinant DNA technology to hydrolysis of $\beta$-1,3-glucans, the main cell wall components of the yeasts and fungi, or the major structural and storage polysaccharides (laminarin) of the marine macro alga Laminaria saccharina has been initiated. In 1989, Watanabe et al. first isolated a $\beta$-1,3-glucanase gene from Bacillus circulans WL-12 and cloned in E. coli. Later, two $\beta$-1,3-glucanase genes of B. circulans WL-12 were cloned in E. coli(Fiske et al., 1990). By the way, the gene donor strain, B. circulans WL-12 had been isolated from soil as a yeast cell wall lytic bacterium(Tanaka and Phaff, 1965) and subsequent studies on the characterization of the $\beta$-1,3-glucanases or the genes encoding the enzymes were performed exclusively with the B. circulans WL-12 strain(Kobayashi et al., 1974; Fleet and Phaff, 1974; Tanaka et al., 1978; Esteban et al., 1984; Watanabe et al., 1989; Fiske et al., 1990). We attempted to isolate and study new genes encoding $\beta$-1,3-glucanase from other B. circulans strain, KCTC3004 than WL-12. We report here construction of a recombinant plasmid containing a new $\beta$-1,3-glucanase gene of B. circulans KCTC3004 strain. With this plasmid E. coli JM109 was transformed to produce laminarinase at high efficiency.
Materials and Methods

Bacterial strains, plasmids, and culture conditions

_Bacillus circulans_ KCTC3004(ATCC21783) was used as β-1,3-glucanase gene source, _Escherichia coli_ JM109 as cloning host, and pUC19 as cloning vector. _B. circulans_ was grown at 30°C in LN medium (g/l: beef extract, 3; peptone, 5; tryptone, 10; yeast extract, 5; NaCl, 10). _E. coli_ was grown in LB medium at 37°C. Ampicillin (50 μg/ml) was added to the medium when _E. coli_ carried plasmids.

Preparation of DNA

Chromosomal DNA of _B. circulans_ was isolated from exponential phase cultures (Rodriguez and Tait, 1983), and cloning vector pUC19 DNA was prepared from _E. coli_ JM109 by the alkaline lysis method (Maniatis et al., 1982).

Construction of recombinant plasmids

_B. circulans_ chromosomal DNA and pUC19 DNA were digested with PstI. The vector was further treated with calf intestinal alkaline phosphatase. Ligation of chromosomal DNA (3 μg) with pUC19 DNA (1 μg) was carried out with the aid of T4 DNA ligase in a final volume of 30 μl. _E. coli_ JM109 was transformed (Goodman and Macdonald, 1979) with the ligation mixture.

Detection of β-1,3-glucanase positive _E. coli_ transformants

White colonies of _E. coli_ transformants appearing on MacConkey plates were toothpicked onto LB plates containing laminarin (0.4%) and ampicillin (50 μg/ml). After growth at 37°C for 36 h, β-1,3-glucanase positive clones were detected using the Congo red dye method (Teather and Wood, 1982). Colonies surrounded by yellow halos were harvested as β-1,3-glucanase positive _E. coli_ clones.

Preparation of enzyme extracts

Whole cell extracts were prepared from cells grown in 10 ml broth. Harvested cells were washed with 0.05 M citrate buffer, pH 5.4, the pellet suspended in 10 ml of the citrate buffer and sonicated for 2 min (_E. coli_) and 5 min (_B. circulans_) with a Branson Sonifier (model 350) at 40% output. Cell debris was removed from the extract by centrifuging for 15 min at 9,000 g. Another 10 ml sample of the culture was centrifuged and supernatant was stored for extracellular β-1,3-glucanase assay.

Assay of β-1,3-glucanase activity

0.5 ml of appropriately diluted enzyme solution was mixed with 0.5 ml of 1% laminarin in 0.05 M citrate buffer, pH 5.4. After 10 min at 50°C, total released reducing sugar was determined by the dinitrosalicylic acid method (Miller, 1939). One unit of activity was defined as the amount of the enzyme which liberated 1 μmole of reducing sugar expressed as glucose per min from laminarin.

SDS-PAGE and zymogram

SDS-PAGE in 12.5% slab was carried out as described by Laemmli (1970). After electrophoresis was completed, renaturation of the enzymes in the gel and detection of β-1,3-glucanase activity in the gel were mainly performed as described by Watanabe et al. (1989) with minor modifications. SDS was removed from the gel by washing twice for 30 min in 0.05 M citrate buffer, pH 5.4 without isopropanol. Then, the gel was soaked for 30 min in the 2.5% Triton X-100 solution before activity staining.

Thin layer chromatography

To analyze hydrolyzed products of laminarin, a mixture consisting of 0.5 ml of enzyme fluid prepared from whole cell extracts of _E. coli_ transformant and 1 ml of 1% laminarin dissolved in 0.05 M citrate buffer, pH 5.4 was incubated at 45°C for a given period. The reaction mixture was then boiled for 10 min, spotted on silica gel 60 TLC plates (Merck No. 5724), and developed at room temperature with isoamyl alcohol-ethanol-ammonia-water (50 : 60 : 1 : 30, v/v). For the detection of carbohydrates, a mixture of 0.5 ml p-anisaldehyde, 0.5 ml concentrated H2SO4, and a few drops of glacial acetate in 9 ml of 95% ethanol was used as spray reagent. Glucose and cellobiose were used as standards.