CELLULOLOYTIC VESTIGES OF THE XYLANASE ACTIVITY IN A NEW STRICTLY XYLANOLYTIC, THERMOPHILIC Clostridium sp.

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SUMMARY

The xylanase activity of the new thermophilic, anaerobic, Clostridium sp. EPP100 was induced by xylan, cellobiose, and lactose, a pattern previously noted only in cellulolytic organisms. The lactose-induced xylanase had microcrystalline cellulose binding activity. Induction of xylanase activity was dependent on inducer concentration and was not fully repressible by glucose, xylose, or any mono-sugars tested. The β-glycosidases and xylanase were not induced coordinately by lactose and cellobiose.

Introduction

Cellulase-free xylanases are desirable for certain industrial applications, including the enzymatic bleaching of wood pulp. Organisms that produce high levels of xylanase commonly produce cellulases simultaneously, and these xylanases are often induced by the end product of cellulase action, cellobiose, and by lactose (DeBlois and Wiegel, 1990 and papers cited therein). We recently isolated a non-cellulolytic thermophilic bacterium from an alkaline pool at Mickey Hot Springs, Oregon (Clostridium sp. EPP100, taxonomic description in preparation; DeBlois and Wiegel, 1992) in which the xylanase is induced by cellobiose and lactose, as well as by xylan.

Clostridium sp. EPP100 is related to the cellulolytic species, C. thermocellum, C. thermolacticum, and C. stercorarium according to 16s rRNA sequence analysis (F. Rainey, personal communication). However, it does not grow on filter paper, produced no cellulase activity when grown with cellobiose, lactose or xylan (as detectable by reducing sugar assays of dialyzed supernatants incubated with carboxymethyl cellulose (CMC), or native gel overlays containing CMC), but the xylanase activity adsorbed to microcrystalline cellulose. The absence of cellulase activity together with the binding of xylanase activity to cellulose and the similarity of the xylanase induction to a pattern characteristic of cellulases, suggests that this strain is in transition between being cellulolytic and strictly xylanolytic.

Regulation of xylanase in response to different carbon sources and growth conditions is varied
among xylanolytic organisms, including thermophilic anaerobes (Wiegel et al., 1985). We show that the xylanase activity of the new non-cellulolytic thermophile, Clostridium sp. EPP100, has cellulose binding activity, is induced by cellobiose, lactose, and xylan, and is regulated separately from β-glycosidases.

Materials and Methods

Growth and xylanase activity
Xylanase activity was assayed by a modified version of Bailey et al. (1992). A 0.225 ml portion of 0.25% solution of Remazol Brilliant Blue (RBB) Xylan (Sigma), in 50 mM NaKPO4 buffer, pH 6.5, was mixed with culture supernatant (0.025 ml) in microfuge tubes, and incubated in a 60°C water bath for 30 min. The tubes were removed to an ice bath, and 0.52 ml of 0.95% ethanol was added to each, followed by 20 min incubation in the ice bath. Precipitated xylan was separated by centrifugation and the absorbance of the supernatant read at 595 nm. Activity units are expressed as μmol/min.

Anaerobic medium was prepared as described by Wiegel et al. (1979). Anaerobic technique was used for all manipulations of cells. Cell protein was measured by the method of Bradford (1976). For induction of xylanase activity in Clostridium sp. EPP100, cultures containing 5 ml were incubated at 60°C with xylobiose (0.3%), oat spelt xylan (0.2%), galactose (0.5%), maltose (0.6%), xylose (0.6%), L-arabinose (0.5%), N-acetyl glucosamine (0.6%), glucose (0.5%), salicin (0.45%), lactose (0.6%), or cellobiose (0.6%). Following growth, cultures were used for a 10% (v/v) inoculum into fresh tubes. Growth was estimated by measuring the increase in optical density at 600 nm using a Bausch and Lomb Spectronic 70 spectrophotometer.

Induction and repression studies
The effect of inducer concentration was measured in cells grown on glucose, concentrated by centrifugation and resuspended in fresh medium to an O.D.600 = 0.2. The medium contained 0.2% arabinose and 0.01, 0.05, 0.1, 0.5% of cellobiose, lactose, or oat spelt xylan. Incubation was at 60°C, growth was measured as O.D. 600 and aliquots were removed for xylanase assay at 3, 7.5, 16 and 24 h following transfer.

The effect of glucose or xylose on xylanase induction by cellobiose, lactose or birch xylan was determined similarly using medium with inducer + glucose or xylose ad indicated in the text.

Cellulose binding assay
A 5% solution of thrice washed microcrystalline cellulose (Avicel) in 75 mM Tris HCl, pH 7.5, was stirred for 1.5 h. Tris buffer was added to volumes of 0, 50, 100, 250, or 500 μl Avicel solution in microfuge tubes to make 500 μl. To these tubes was added 25 μl of partially purified xylanase from lactose grown Clostridium sp. EPP100. Incubation was at 25°C for 1 h with vortexing at 15 min intervals. After centrifugation, the supernatants were assayed for xylanase activity using the PAHBAH reducing sugar assay, while unbound protein was measured with the BCA reagent.

Glycosidase assays
Cultures of 75 ml were grown with 0.5% glucose and amended when the O.D. reached 0.15-0.2 (designated t0) with 0.05% cellobiose, 0.1% lactose, or 0.05% oat spelt xylan. Aliquots of 15 ml taken at intervals of 0, 1.5, 3.5, and 9.5 h from 75 ml cultures were centrifuged and the cells resuspended in 0.5 ml 0.1 M MOPS, pH 6.8. Portions of 0.325 ml of cell suspension were diluted with 0.25 ml 0.1 M citrate/phosphate (C/P) buffer, pH 6.6, for use in glycosidase assays. β-Xylosidase activity was calculated from assays with 3 mM p-nitrophenylxylopyranoside (pN PX), β-glucosidase activity with 4 mM pNPglucopyranoside (pNPG), and β-galactosidase with 4 mM pNPgalactopyranoside (pNP Gal). Assay mixtures contained 190 μl C/P buffer + 1 drop toluene + 50 μl pNPX, pNPG, or pNP Gal + 10 μl cells.