The cellodextrinase from *Pseudomonas fluorescens* subsp. *cellulosa* consists of multiple functional domains

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A genomic library of *Pseudomonas fluorescens* subsp. *cellulosa* DNA was constructed in pUC18 and *Escherichia coli* recombinants expressing 4-methylumbelliferyl β -D-cellobioside-hydrolysing activity (MUCase) were isolated. Enzyme produced by MUCase-positive clones did not hydrolyse either cellobiose or cellotriose but converted cellotetraose into cellobiose and cleaved cellopentaose and cellohexaose, producing a mixture of cellobiose and cellotriose. There was no activity against CM-cellulose, insoluble cellulose or xylan. On this basis, the enzyme is identified as an endo-acting cellodextrinase and is designated cellodextrinase C (CELC). Nucleotide sequencing of the gene (*celC*) which directs the synthesis of CELC revealed an open reading frame of 2153 bp, encoding a protein of M_r 80189. The deduced primary sequence of CELC was confirmed by the M_r of purified CELC (77000) and by the experimentally determined *N*-terminus of the enzyme which was identical with residues 38–47 of the translated sequence. The *N*-terminal region of CELC showed strong homology with endoglucanase, xylanases and an arabinofuranosidase of *Ps. fluorescens* subsp. *cellulosa*; homologous sequences included highly conserved serine-rich regions. Full-length CELC bound tightly to crystalline cellulose. Truncated forms of *celC* from which the DNA sequence encoding the conserved domain had been deleted, directed the synthesis of a functional cellodextrinase that did not bind to crystalline cellulose. This is consistent with the *N*-terminal region of CELC comprising a non-catalytic cellulose-binding domain which is distinct from the catalytic domain. The role of the cellulose-binding region is discussed.

INTRODUCTION

Cellulose, one of the most abundant renewable terrestrial organic molecules, is hydrolysed by a repertoire of microbial enzymes which recognize β -1,4-glycosidic linkages; these enzymes are encoded by cellulase gene families (Hazlewood *et al.*, 1988; Gilbert *et al.*, 1987; Béguin, 1990). Aerobic fungi digest cellulose by the synergistic action of endoglucanase, cellobiohydrolase and β -glucosidase (Béguin, 1990). Cellulolytic bacteria generally produce a variety of endoglucanases, but with few exceptions cellobiohydrolase is not a common feature of bacterial cellulase systems (Béguin, 1990). A distinction between anaerobic and aerobic bacteria is that, in the former, cellulase activity may reside in a large structured complex typified by the cellulosome of *Clostridium thermocellum* (Lamed & Bayer, 1988) while, in the latter, cellulase-related enzymes are secreted free in the culture supernatant and do not appear to aggregate or associate with the cell surface.

The purification and characterization of discrete forms of the different cellulases expressed by single organisms has proved to be very difficult. Cloning and expressing the genes encoding these enzymes in a non-cellulolytic host constitutes an effective alternative strategy for characterizing the individual components of a cellulase system. Such an approach has been applied to elucidate the molecular architecture of cellulases from aerobic microorganisms. In *Cellulomonas fimi* (Ong *et al.*, 1989), *Thermomonospora fusca* (Ghangas & Wilson, 1988), *Trichoderma reesei* (Van Tilbeurgh *et al.*, 1986) and *Pseudomonas fluorescens* subsp. *cellulosa* (Gilbert *et al.*, 1990) these enzymes consist of a non-catalytic cellulose-binding domain (CBD) linked to the catalytic

domain (CD) by sequences rich in hydroxyamino acids. Kellett *et al.* (1990) and Ferreira *et al.* (1990) have shown that a similar pattern of molecular structure exists in xylanases and an arabinofuranosidase from *Ps. fluorescens* subsp. *cellulosa*.

In previous work our group has utilized the cloning approach to determine the structures and properties of endoglucanases and xylanases from *Ps. fluorescens* subsp. *cellulosa* (Gilbert *et al.*, 1987, 1988, 1990; Kellett *et al.*, 1990). Recently, we have focused on another class of enzyme which can be involved in the hydrolysis of cellulose, namely cellodextrinase. Typically, this enzyme hydrolyses soluble cello-oligosaccharides with the production of cellobiose, but exhibits little or no activity against insoluble cellulose or CM-cellulose (Huang & Forsberg, 1987). This paper describes the cloning and sequencing of a gene, from *Ps. fluorescens* subsp. *cellulosa*, which encodes a cellodextrinase. The *N*-terminal region of the enzyme exhibits sequence conservation with other plant cell wall hydrolases from this organism. The conserved region contains a CBD which is discrete from the C-terminally located CD.

MATERIALS AND METHODS

The *Escherichia coli* strains employed in this study were JM83 and JM101 (Norlander *et al.*, 1983). The vectors used were pUC18, pUC19, M13mp18 and M13mp19 (Norlander *et al.*, 1983). *E. coli* strains were cultured in Luria broth (LB) or on LB agar (2%, w/v). Ampicillin (100 μ g/ml) and 5-bromo-4-chloro-3-indolyl- β -D-galactoside (2 μ g/ml) were added to the medium to select for transformants and recombinants respectively.

Abbreviations used: MUC, 4-methylumbelliferyl β -D-cellobioside; MUCase, enzyme that hydrolyses MUC; CELC, cellodextrinase C; CBD, cellulose-binding domain; CD, catalytic domain; LB, Luria broth.

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The nucleotide sequence of the cellodextrinase C gene (*celC*) will appear in the EMBL/GenBank/DBJ Nucleotide Sequence Databases under the accession no. X61299.

General recombinant DNA procedures

Agarose-gel electrophoresis, Southern hybridization, transformation of *E. coli*, preparation and modification of genomic and plasmid DNA using restriction enzymes and T4 DNA ligase were as described by Gilbert *et al.* (1987, 1988). *BAL31* deletions were performed as follows: pFG1 was cleaved with *HindIII*, digested with *BAL31* as described by Hall *et al.* (1988) and restricted with *EcoRI*. The resultant DNA fragments containing truncated *celC* derivatives were purified and cloned into *SmaI/EcoRI*-digested pUC19. The precise extent of the *BAL31* deletions was determined by nucleotide sequencing. Plasmids containing truncated *celC* in-phase with *lacZ'* were retained for further studies.

Nucleotide sequencing

The 3.2 kb *PstI* restriction fragment of pFG1, containing *celC*, was circularized by self-ligation. The generation of random DNA fragments by sonication and cloning into the *SmaI* site of M13mp18 was as described by Minton *et al.* (1983). Recombinant clones were sequenced essentially by the dideoxy chain-termination method of Sanger *et al.* (1980) except that T7 DNA polymerase (Tabor & Richardson, 1987) was used instead of the Klenow fragment of DNA polymerase. Sequences were compiled and ordered using the computer programs of Staden (1980). Both strands of *celC* were completely sequenced. For direct plasmid sequencing, DNA, prepared by the method of Birnboim & Doly (1979), was denatured in 1 M-NaOH for 5 min, the alkali was removed by passing the nucleic acid through a Sepharose 6B spin column and the DNA was annealed with reverse primer at 42 °C for 15 min; sequencing reactions were performed as above.

Construction and screening of plasmid library of *Ps. fluorescens* subsp. *cellulosa* DNA

Ps. fluorescens subsp. *cellulosa* DNA was partially digested with *Sau3a* and restriction fragments 5–10 kb in size were purified by agarose-gel electrophoresis and electroelution. The DNA was ligated to dephosphorylated *SmaI*-cleaved pUC18 and transformed into *E. coli* JM83. Recombinants were selected by plating the transformed bacteria on medium containing ampicillin and 5-bromo-4-chloro-3-indolyl β -D-galactoside. The gene library was screened for clones expressing cellulodextrinase activity as follows: recombinants were grown overnight at 37 °C with shaking in 1.5 ml microcentrifuge tubes containing LB and ampicillin. The cells were subjected to osmotic shock (Hsiung *et al.*, 1986) and were incubated with 1 mM- (final concentration) 4-methylumbelliferyl β -D-cellobioside (MUC) in 50 mM-potassium phosphate/12 mM-citric acid, pH 6.5, at 37 °C for 10 min. Those clones that expressed cellulodextrinase activity fluoresced brightly under long-wavelength u.v. light.

Assays

The periplasmic fractions from MUCase-positive clones were assayed for xylanase and cellulase-related activities exactly as described by Hazlewood *et al.* (1990). The products generated by the action of the MUCase on cello-oligosaccharides (G2–G6) were identified by h.p.l.c. Periplasmic proteins (in a final volume of 5 ml) were prepared from 100 ml cultures of *E. coli* harbouring pFG1. Reaction mixtures (1 ml final volume) contained periplasmic proteins (0.1 ml) and 0.1 mg of the appropriate cello-oligosaccharide/ml in 50 mM-potassium phosphate/12 mM-citric acid, pH 6.5, and were incubated for up to 1 h at 37 °C. Insoluble material was removed by centrifugation at 13000 *g* for 5 min, and portions (20 μ l) of supernatant were applied to a Dionex CarboPac PA1 column (4 mm $\times$ 250 mm). The detection system

was a Dionex pulsed amperometric detector using a 10 K detector range with applied potentials of E1, +0.05 V; E2, +0.6 V; E3, –0.6 V. The detection cell used a gold working electrode. Material was eluted from the column with a 5–25 mM-sodium acetate gradient in 150 mM-NaOH. Protein was determined by the method of Lowry *et al.* (1951).

Cellulose-binding studies

Cell-free extracts (2 ml) or periplasmic proteins (2 ml) prepared from *E. coli* cells harbouring full-length or truncated derivatives of *celC* were mixed with 2 ml of crystalline cellulose (Avicel, pH 10.5; 5%, w/v) suspended in 100 mM-Tris/HCl buffer, pH 7.5, as described by Gilbert *et al.* (1990). Cellulose-bound and unbound protein were analysed by SDS/PAGE (Laemmli, 1970).

Protein sequencing

The MUCase was purified from total periplasmic proteins of *E. coli* harbouring pFG1 by affinity chromatography with Avicel as described for endoglucanase B of *Ps. fluorescens* subsp. *cellulosa* (Gilbert *et al.*, 1990). The N-terminal sequence of the protein was determined by automated Edman sequencing using a 470A gas-phase sequencer equipped with a 120A on-line phenylthiohydantoin analyser (Applied Biosystems; Hunkapillar *et al.*, 1983).

RESULTS

Isolation of a *Ps. fluorescens* subsp. *cellulosa* MUCase gene

A gene library of *Ps. fluorescens* subsp. *cellulosa* chromosomal DNA was constructed in pUC18 and a total of approximately 5000 recombinants were isolated. Two thousand bacteria were screened, as described in the Materials and methods section, for the capacity to synthesize an enzyme(s) that hydrolysed MUC (MUCase); two recombinants were shown to hydrolyse the aryl glycoside. A restriction map of the recombinant plasmids, pFG1 and pFG2, harboured by the MUCase-positive clones was constructed. Results (Fig. 1) showed that the physical maps of the cloned inserts between map positions 1–2.3 in pFG1 and 3.2–4.5 in pFG2 were identical, suggesting that the homologous sequence in the two plasmids contained the MUCase gene

Fig. 1. Restriction maps of recombinant pUC plasmids containing *celC*

The positions of the cleavage sites *Bam*HI (B), *Bgl*II (Bg), *Eco*RV (E), *Pst*I (P), *Pvu*II (Pv), *Sst*I (St), *Sac*I (S) and *Xho*I (X) are shown. Pseudomonad inserts were not cleaved by *Cla*I, *Eco*RI, *Hind*III, *Xba*I, *Nde*I, *Sma*I, *Kpn*I, *Sph*I and *Sal*I. The solid arrow shows the extent and orientation of *celC*. Plasmids in which the 5' region of *celC* was deleted with *BAL31* were designated pFG1–pFG8. The position of the 5' nucleotide of the truncated *celC* genes is shown in the nucleotide sequence depicted in Fig. 4. The phenotypes of *E. coli* strains harbouring the recombinant plasmids are shown.

Fig. 2. Hybridization of pFG1 with *Ps. fluorescens* subsp. *cellulosa* genomic DNA

The DNA probe was hybridized with *Ps. fluorescens* subsp. *cellulosa* DNA which had been restricted with *Eco*RI (1), *Eco*RV (2), *Hind*III (3) and *Pvu*II (4). The conditions used for radiolabelling the probe and subsequent Southern hybridization were as described by Gilbert *et al.* (1988). The filters were autoradiographed on X-ray film at -70°C for 3 h.

which is designated *celC*. This was confirmed by cloning the 3.2 kb *Pst*I restriction fragment from pFG1, containing the homologous sequence, into pUC18 in both orientations to generate pFG10 and pFG11. Both plasmids directed the synthesis of a functional MUCase.

To investigate whether *celC* was present as a single copy, genomic DNA from *Ps. fluorescens* subsp. *cellulosa* was subjected to Southern hybridization using pFG1 as the probe. The data (Fig. 2) were consistent with the insert in pFG1 being a continuous sequence of *Ps. fluorescens* subsp. *cellulosa* DNA, which was not reiterated elsewhere in the organism's genome. Thus *celC* cloned in pFG1 is a single-copy gene.

Substrate specificity

Substrate specificity of the MUCase expressed by *E. coli* JM83 harbouring pFG1 was determined. The data revealed that the enzyme hydrolysed MUC (3.1 units/mg of protein), but did not hydrolyse xylan, CM-cellulose, insoluble celluloses or substrates normally cleaved by β -glucosidase. These results indicate that the enzyme differs from xylanases A and B of *Ps. fluorescens* subsp. *cellulosa* which act on MUC, in addition to xylan (Gilbert *et al.*, 1988). To investigate whether the MUCase cleaves cello-oligosaccharides, the enzyme was incubated with cello-oligosaccharides and the products generated were analysed by h.p.l.c. The results (Fig. 3) showed that cellohexaose and

Fig. 3. H.p.l.c. analysis of sugars released after incubating periplasmic proteins of *E. coli* cells harbouring pFG1 with cello-oligosaccharides

The retention times of the sugar standards glucose (G1), cellobiose (G2), cellotriose (G3), cellotetraose (G4), cellopentaose (G5) and cellohexaose (G6) were 2.25 min, 3.34 min, 4.75 min, 6.4 min, 8.1 min and 9.75 min respectively (f). The incubation conditions for periplasmic proteins and cellobiose (a), cellotriose (b), cellotetraose (c), cellopentaose (d) and cellohexaose (e), and subsequent sugar analyses are described in the Materials and methods section.

cellopentaose were each hydrolysed to a mixture of cellotriose and cellobiose; cellotetraose was cleaved exclusively to cellobiose. The enzyme did not act on cellobiose or cellotriose. These data indicate that the MUCase does not specifically hydrolyse mono- or di-saccharides from the non-reducing end of the glucose polymers, but cleaves internal linkages. Such a substrate specificity suggests that the enzyme is a cellodextrinase (Huang & Forsberg, 1987) and is designated therefore cellodextrinase C (CELCC).

Nucleotide sequence

The 3.2 kb *Pst*I restriction fragment from pFG1 containing *celC* was sequenced in both strands. Translation of the nucleotide sequence (Fig. 4) revealed a single open reading frame encoding a polypeptide of M_r 80189. The first 10 residues of purified CELC, determined by *N*-terminal sequencing, exhibited a perfect match with amino acid residues from 38 to 47 of the translated sequence. It is likely therefore that the residues preceding the mature *N*-terminus constitute a signal peptide. The deduced *N*-terminal region of full-length CELC contained predominantly hydrophobic residues characteristic of bacterial signal peptides, but the basic hydrophilic region, normally present at the *N*-terminus, was less obvious. However, the signal peptide does direct secretion of the cellodextrinase into the *E. coli* periplasm (results not shown), and is efficiently processed. Interestingly, two endoglucanases, a xylanase and an arabinofuranosidase, from *Ps. fluorescens* subsp. *cellulosa* also lacked a basic hydrophilic *N*-terminus, but contained a hydrophobic region within their respective signal peptides (Hall & Gilbert, 1988; Gilbert

Fig. 4. Nucleotide sequence of *celC*

The derived amino acid sequence of CELC is shown. The horizontal arrow shows the determined N-terminal sequence of mature CELC. Serine-rich sequences of CELC are boxed. The 5' nucleotide of truncated derivatives of *celC* are indicated by vertical arrows, and the plasmid encoding the derivative is also shown.

Fig. 5. Alignment of homologous regions of CELC with other cellulases and xylanases

The enzymes compared were as follows: *Ps. fluorescens* subsp. *cellulosa* endoglucanases A (EGA; Hall & Gilbert, 1988) and B (EGB; Gilbert *et al.*, 1990); *Ps. fluorescens* subsp. *cellulosa* xylanases A (XYLA; Hall *et al.*, 1989), B (XYLB; Kellett *et al.*, 1990) and C (XYLC; Kellett *et al.*, 1990); *C. fimi* exoglucanase (CEXA; Ong *et al.*, 1989) and endoglucanase A (CENA; Ong *et al.*, 1989). Residues that show identity or similarity of structure in all primary structures compared are boxed. The positions of the first and last residues of homologous regions, in their respective primary sequence, are shown.

et al., 1990; Kellett *et al.*, 1990). The proposed translation-initiation codon of *celC* was preceded (7 bp) by the sequence GAGGA, which is characteristic of the ribosome-binding site of Gram-negative bacteria (Hall & Gilbert, 1988). Immediately downstream from the translational stop codon of *celC* is a G+C-rich region of dyad symmetry (G = 19 kcal). This palindromic is followed by a succession of thymine nucleotides and is therefore characteristic of *E. coli* Rho-independent transcription terminators. Codon utilization of *celC* was similar to that observed in *celA*, *celB*, *xynA*, *xynB* and *xynC* from the same organism (Hall & Gilbert, 1988; Hall *et al.*, 1989; Gilbert *et al.*, 1990; Kellett *et al.*, 1990). In common with other genes encoding plant cell wall hydrolases from *Ps. fluorescens* subsp. *cellulosa*, *celC* lacks a marked bias towards G or C nucleotides in the wobble position (A + T of 46% in the wobble position) compared with other *Pseudomonas* genes (Minton *et al.*, 1983). This argues in favour of the bacterium having been incorrectly classified as a pseudomonad.

Within the first 240 N-terminal residues of mature CELC, features common to the primary structure of other cellulases and xylanases from *Ps. fluorescens* subsp. *cellulosa* were discernible (Fig. 5). Two regions rich in serine residues were apparent in all the enzymes. The 100 residues at the N-terminus of mature CELC exhibited a high degree of sequence conservation with the N-terminus of EGB, XYLA, XYLB and XYLC and with the C-terminus of EGA (Hall & Gilbert, 1988; Hall *et al.*, 1989; Gilbert *et al.*, 1990; Kellett *et al.*, 1990). This region also showed homology with the CBD of cellulases from *C. fimi* (Ong *et al.*, 1989); structural identity was most pronounced in sequences encompassing tryptophan residues. Finally, the 44 residues between the serine-rich sequences in CELC showed identity with similarly located regions in EGA, EGB and XYLC of *Ps.*

fluorescens subsp. *cellulosa*. No other homologies between the CD of CELC and microbial plant-cell-wall hydrolases were detected.

Structure and function of CELC

Previous studies have shown that conserved sequences in the N- and C-terminal domains of *Ps. fluorescens* subsp. *cellulosa* cellulases and xylanases are not essential for catalytic activity. To investigate whether the N-terminal region of CELC plays an important role in catalytic activity, BAL31 deletions of the 5' region of *celC* were generated, and the *celC* derivatives were cloned in-phase with *lacZ'* of pUC19 (Figs. 1 and 4). Removal of up to 939 bp encoding the conserved N-terminal domain from the 5' end of *celC* resulted in truncated forms of the gene which directed the synthesis of catalytically active CELC.

To investigate whether the conserved region functions in cellulose binding, the capacity of proteins expressed by *E. coli* cells containing full-length and 5' truncated derivatives of *celC* to bind crystalline cellulose (Avicel) was examined. A periplasmic protein of M_r 77000, synthesized by *E. coli* cells containing full-length *celC*, bound to cellulose and could be eluted with 10% (w/v) SDS (Fig. 6) or 6 M-guanidinium chloride. The protein hydrolysed MUC (when eluted with guanidinium chloride) and its N-terminal 10 residues showed identity with amino acids 38–47 of the translated sequence of *celC*, confirming its identity as CELC. *E. coli* cells containing truncated derivatives of *celC* from which 361 bp had been removed did not express a MUCase which bound to cellulose (Table 1). These data suggest that the region of CELC that exhibits sequence conservation with other *Ps. fluorescens* subsp. *cellulosa* cellulases and xylanases constitutes a CBD.

The 3' 24 bp of *celC* were deleted by cloning the 2.7 kb

Fig. 6. SDS/PAGE of proteins expressed by *E. coli* harbouring pFG1 which bind to cellulose

Periplasmic proteins were incubated with Avicel and the unbound and bound proteins were analysed by SDS/PAGE as described in the Materials and methods section. Lane 1 contained periplasmic proteins; lane 2, filtrate from Avicel/periplasmic protein mixture; lane 3, final Tris/HCl wash of Avicel; lane 4, bound protein eluted from Avicel by boiling for 5 min with 10% (w/v) SDS. A 10% (w/v) polyacrylamide gel was used. The M_r ($\times 10^{-3}$) of the Avicel-bound protein is shown.

Table 1. Binding of truncated forms of CELC to Avicel

The binding of proteins to Avicel was evaluated as described in the Materials and methods section. The percentage of enzyme bound to the polysaccharide was determined by enzyme assay.

Plasmid	N-Terminal residues deleted from full-length CELC	Percentage of CELC bound to Avicel
pFG1	0	100
pFG3	120	1
pFG4	170	0
pFG5	241	0
pFG6	267	0
pFG7	313	0

*Pst*I/*Pvu*II restriction fragment from pFG1 into pUC18. The resultant plasmid, pFG9 (Fig. 1), did not direct the synthesis of a functional cellodextrinase.

DISCUSSION

The main purpose of this study was to define and characterize components of the cellulase system of *Ps. fluorescens* subsp. *cellulosa* which are distinct from endoglucanases. The selection strategy for genes encoding these enzymes was based on the capacity of recombinants to hydrolyse MUC, which is a potential substrate for cellobiohydrolase and cellodextrinase. This approach generated two clones expressing cellodextrinase, but no recombinants expressing cellobiohydrolase activity were detected. Although this indicates that *Ps. fluorescens* subsp. *cellulosa* does not synthesize a true cellobiohydrolase (i.e. one capable of releasing cellobiose from the non-reducing end of insoluble cellulose), other interpretations of the data are possible. (i) The cellobiohydrolase gene may be present in the pseudomonad, but not functional in *E. coli*; (ii) the number of recombinants screened may not have been sufficient to encompass the whole *Ps. fluorescens* subsp. *cellulosa* genome; (iii) cello-

biohydrolase expression may be lethal to *E. coli*. Support for this last notion is provided by the fact that *xynA* (Hall *et al.*, 1989), *xynB*, *celA* and *celB* (unpublished work) all cause cell death when constitutively expressed at high levels in *E. coli*.

The substrate specificity of CELC indicates that the enzyme is an endo-acting cellodextrinase. Interestingly it does not cleave trisaccharides although it hydrolyses MUC. The mode of action of CELC is similar to the cellodextrinase of *Ruminococcus flavefaciens* (Rasmussen *et al.*, 1988), but is distinct from the endo-acting cellodextrinase of *Bacteroides succinogenes* (Huang & Forsberg, 1987).

In common with other plant cell-wall hydrolases from *Ps. fluorescens* subsp. *cellulosa*, CELC contains a CBD discrete from the CD. Identification of the 100-amino acid N-terminal portion of the conserved region of CELC as a CBD is supported by its homology with other bacterial CBDs (Fig. 5). Furthermore, deletion of 82 residues from the mature N-terminus of CELC abolishes cellulose-binding capacity. The serine-rich sequences, present in cellulases and xylanases from diverse micro-organisms, are thought to be spacer sequences that link functional domains within these enzymes. Whether these hydroxyamino acid-rich sequences have an important role in plant cell wall hydrolysis, or whether the DNA encoding this region provides a mechanism for the rapid shuffling of CBDs between enzymes (Ferreira *et al.*, 1990), remains to be established.

The precise role of CBDs in microbial cellulases is an interesting question. Deletion of this domain from *T. reesei* cellulases (Van Tilbeurgh *et al.*, 1986; Tomme *et al.*, 1988) diminishes the capacity of the enzyme to hydrolyse crystalline cellulose. In contrast, removal of the CBD from cellulases of *C. fimi* (Gilkes *et al.*, 1988) and *F. succinogenes* (McGavin & Forsberg, 1989) resulted in very small changes in the catalytic properties of these enzymes. Data presented in this study indicate that deletion of the CBD from CELC did not alter catalytic activity. Furthermore, the presence of a CBD in *Ps. fluorescens* subsp. *cellulosa* xylanases (Kellett *et al.*, 1990; Ferreira *et al.*, 1990), arabinofuranosidase (Kellett *et al.*, 1990) as well as in an endoglucanase (Gilbert *et al.*, 1990) argues against the sequence having a direct role in cellulolysis only. We propose that CBDs in *Ps. fluorescens* subsp. *cellulosa* enable the enzymes that contain them to attach to plant cell walls. As the recalcitrant tissue is degraded different polysaccharides are exposed at the surface and are hydrolysed by the appropriate enzyme(s). Thus CELC is held in close proximity to endoglucanases through its strong attachment to cellulose within the tissue and as the latter enzyme releases cello-oligosaccharides, CELC is available to hydrolyse these substrates further to cellobiose and cellotriose. This view of the role of CBDs may not apply to corresponding regions in the cellulases from other micro-organisms.

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