CLONING AND EXPRESSION OF CELLULASE XF-818 OF *Xylella fastidiosa* IN *Escherichia Coli*

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ABSTRACT: *Xylella fastidiosa*'s genome was the first of a plant pathogen to be completely sequenced. Through comparative sequence analysis many genes were identified and, among them, several potentially involved in plant-pathogen interaction. However, the biological role of each gene should be assigned experimentally. On this regard, heterologous protein expression is a powerful tool to produce proteins from such genes, allowing their characterization. *X. fastidiosa* lives inside xylem vessels and eventually would degrade pit membranes from xylem cells to move radialy into the host. The identification of several putative plant cell wall degrading enzymes on *X. fastidiosa* genome prompted the assession of the function of such proteins. The open reading frame (ORF) Xf-818 was cloned into expression vector pET20b and *E. coli* cells harboring such plasmid exhibited cellulase activity. Using IPTG at 0.4 mmol L^{1} with a 12 h incubation at 32°C are the best conditions to produce higher amounts of heterologous protein. The enzyme degrades cellulose confirming the endoglucanase activity of Xf-818.
Key words: citrus variegated chlorosis, cellulases, cloning and expresssion

CLONAGEM E EXPRESSÃO DA CELULASE XF-818 DE *Xylella Fastidiosa* EM *Escherichia Coli*

RESUMO: *Xylella fastidiosa* foi a primeira bactéria fitopatogênica que teve seu genoma completamente seqüenciado. A identificação de diversos genes, através de similaridade de seqüências, indicou os possíveis mecanismos de patogenicidade da bactéria. Entretanto, a determinação da função de um gene requer a confirmação experimental e, neste aspecto, a expressão heteróloga é uma poderosa ferramenta. *X. fastidiosa* coloniza somente o xilema das plantas hospedeiras e a identificação putativa de diversos genes semelhantes a enzimas que degradam a parede celular vegetal, estimularam o presente estudo de catacterização destas enzimas. A clonagem da ORF Xf-818 de *X. fastidiosa* no vetor de expressão pET20b possibilitou a produção da proteína heterologamente em *E. coli*. O emprego de IPTG a 0,4 mmol L^{-1} com 12 h a 32°C, possibilitou as melhores condições para *E. coli* produzir a proteína heteróloga. Clones de *E. coli* que expressam Xf-818, apresentam atividade celulásica, degradando eficientemente a celulose. A identificação de Xf-818 como uma endoglicanase foi assim confirmada.
Palavras-chave: clorose variegada dos citros, celulases, clonagem e expressão

INTRODUCTION

Xylella fastidiosa Wells is a xylem restricted pathogen of several plants and important crops, causing different symptoms according to the host (Hopkins, 1989). Citrus variegated chlorosis (CVC) was first observed in the states of São Paulo and Minas Gerais (Brazil) in 1987 (Rossetti & De Negri, 1990), and *X. fastidiosa* was identified as the etiological agent of CVC (Rossetti et al., 1990; Chang et al., 1993). The bacterium spreads among citrus trees by means of leafhopper sharpshooter vectors and infected budwoods (Rossetti et al., 1995; Lopes, 1996). CVC spread fast from 1987 to 1992 on citrus growing areas of the São Paulo State, and the main cause was the use of graft-infected seedlings with *X. fastidiosa* (Tubelis et al., 1993). In grapevines, this patogen causes the Pierce's Disease (PD), and most of the knowledge about the bacterium is derived from studies on this host.

X. fastidiosa is a major concern regarding losses on citrus plantations. The Fundecitrus estimates that in 2001 about 36 % of the orange trees in São Paulo were infected, and that the highest incidence was in the most important region for citrus production, Bebedouro (20º56'58"S, 48º28'45"W) and Barretos (20º33'26"S, 48º34'04"W) (www.fundecitrus.com.br). Currently, producers deal with CVC through cultural practices.

A recent scientific advance was achieved by unravelling the genome of *X. fastidiosa* from citrus (Simpson et al., 2000). This genome has several poten-

tially pathogenic or virulence associated factors and, among them, there is a streaking similarity with virulence mechanisms in *Xanthomonas campestris* (Simpson et al., 2000; Lambais et al., 2000; Dow & Daniels, 2000). A search on candidate genes revealed an almost complete similar xantham gum operon (Silva et al., 2001), several putative plant cell wall degrading enzymes, regulatory components such as *rpf* gene family members (Dow & Daniels, 2000), genes potentially involved in antibiotic, siderophores, and even toxin synthesis. Leite et al. (2002) described a potential mechanism driving adhesion to plant cells and aggregation, a hypothesis supported by several adhesion and pili genes putatively found in the *X. fastidiosa* genome (Simpson et al., 2000).

Among such diverse, but complementary, putative factors, the plant cell wall degrading enzymes deserve especial attention. The presence of a cellobiohydrolase, three endoglucanases, three xylanases, one polygalacturonase, one pectate lyase and the β-glucosidase and β-xylosidase (Table 1) provides the bacterium with a plant-cell-degrading enzyme arsenal.

Hopkins (1985) did not detect cellulolytic and pectinolytic activity on culture medium of *X. fastidiosa* from grapevine. Fry et al. (1994) detected proteases produced by *X. fastidiosa*-PD but there was no correlation between enzyme production and virulence. Purcell & Hopkins (1996) report that the movement of xylem limited bacteria between vessels would suggest the production of enzymes capable to degrade pit membranes. Adjacent middle layer and primary cell walls form the pit membrane, which are composed by pectic components such as pectin and pectate, cellulose and hemicelluloses. As cellulose and hemicellulose are structural components of primary walls, the degradation of such polymers would enable *X. fastidiosa* to move through pit membranes, moving from one cell to another, on radial spread into the host.

As cellulases and xylanases were found in the *X. fastidiosa* genome, this study worked on the hypothesis that these enzymes posses such hydrolytic activity. To test this hypothesis, a putative cellulase gene Xf-818 was cloned, introduced and expressed into *E. coli* to produce and purify the protein, allowing enzyme characterization.

E. coli expression system has several advantages: *E. coli* grows faster than *X. fastidiosa* and in a cheaper and simpler medium. Also, as *E. coli* does not have endoglucanase, all endoglucanase activity is derived from the cloned gene. As *X. fastidiosa* has three putative cellulases, expressing each one separately in *E. coli* make possible to circumvent co-elution of cellulases during purification steps. Cloned genes can also be engineered to produce proteins containing fusion tags designed to help in purification steps, such as hexahistidine tag, maltose binding protein and protein A (Nilsson et al., 1997). The optimization of the expression of the *X. fastidiosa* gene Xf-818 in *E. coli*, and the cellulase activity (EC 3.2.1.4 b-1,4 endoglucanase) of its protein product is herein described.

MATERIAL AND METHODS

Similarity searches between the translated open reading frame (ORF) sequence from Xf-818 and previously described proteins were made with Blast*p* (Altschul et al., 1990) using the swissprot protein database at NCBI (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi?). Prediction of localization of the signal peptide was achieved with SignalP V1.1 (Nielsen et al., 1997; http://www.cbs.dtu.dk/services/SignalP/).

Reagents and culture medium

Oligonucleotides for both PCR and DNA sequencing were purchased from Operon and Life Technologies. Restriction enzymes and amplification reagents were from Gibco BRL. Sequencing reagents were purchased from Perkin Elmer. *E. coli* was grown on LB medium (Sambrock et al., 1989); solid medium contained agar at 1.8% (w/v) and appropriate antibiotics were supplemented to this medium (50 μg kanamycin mL^{-1} and 100 μg ampicillin mL^{-1}).

Plasmid, cosmid and *E. coli* strains

Expression vector pET20b(+) (Novagen), Cosmid 07H04 containing ORF Xf-818 from *X. fastidiosa* 9a5c employed during sequencing project (ONSA/FAPESP), the *E. coli* DH5α cloning strain and strain BL21(λDE3) (Studier & Moffatt, 1986; Novagen) were obtained from other institutes. Plasmids and cosmid were extracted through alkaline lysis (Sambrock et al., 1989) and were quantified on 0.8% agarose gels (w/v).

Subcloning of Xf-818 into expression vector pET20b(+)

The coding sequence of Xf-818 (Simpson et al., 2000) was amplified from cosmid 07H04 by PCR. Forward primer containing an engineered *Nde*I restriction site (underlined) at the beginning of the ORF (F592Sl 5'CCGGTCGACATATGTCGTTTTCCAAACAC) and reverse primer containing a engineered *Hind*III restriction site spanning the stop codon (R592Hd 5'GGAAAATAAGCTTCAATAGTTTGAAC) were employed. Around 50 ng of cosmidial DNA, 0.2 pmoles of each primer, 20 mmol L^{-1} Tris-HCl pH 8.4, 50 mmol L^{-1} KCl, 1.5 mmol L^{-1} magnesium chloride, 200 μM of each dNTP and 1.5 U of Taq DNA polymerase were employed on PCR amplification in a 40 μL volume. Amplification was preceeded by 3 min at 94°C, followed by 35 cycles of 30 s at 94°C, 60 s at 60°C, and 120 s at 72°C. At the end of 35th cycle, the reaction was kept for 10 min at 72°C.

PCR-amplified DNA was cut with *Nde*I and *Hind*III and linked to pET20b cut with the same enzymes. Electroporated *E. coli* DH5α clones containing the plasmids were screened by restriction digestion. The coding region of Xf-818 was sequenced to assure that no mutation was introduced during PCR amplification. The primers described above, primers T7 promoter (5'TAATACGACTCACTATAGGG) and T7 terminator (5'TGCTAGTTATTGCTCAGCGGT), and two internal primers that anneal inside Xf-818 (818intN – 5'GCGTCATCGGCTTGG and 818intC 5'CGCGCACGTGATTCC) were used. Sequence reads were analyzed on Phred + Phrap + Consed package (Gordon et al., 1998). Plasmid pNAW3, containing Xf-818 cloned into pET20b, was electroporated into competent *E. coli* BL21(λDE3).

Expression of Xf-818 protein in *E. coli*

Seven single colonies resulting from transformation of pNAW3 into BL21(λDE3) were selected and grown on liquid LB medium. After overnight growth (37°C at 220 rpm), an aliquot of 1 mL was centrifuged (1 min; 10,000 *g*), the pellet was suspended in the same medium and a 100 μL aliquot was stored at -20°C for electrophoresis. The remaining culture was induced with IPTG at 0.4 mmol L^{-1}. Cells were kept at 37°C, 200 rpm for 3.5 h. A drop of 50 μL of each culture medium with cells of *E. co*li was deposited on the surface of a plate containing solid LB with carboxymethyl cellulose (CMC) at 0.1% (w/v). Plates were stored overnight at 37°C and revealed for cellulase as described below. From the culture supernants, endoglucanase was evaluated through spectrophotometric assay and cell pellet was used to SDS-PAGE.

Another assay was performed to determine the better temperature and time of harvesting after induction. An overnight culture of the single clone selected on the previous assay (electrophoresis, plate and spectrophotometric assay) was inoculated on 1.5 L of LB medium and grown for 3 h at 37°C/220 rpm. The medium was induced with 0.4 mmol L^{-1} IPTG, divided into portions and stored at 28, 31 or 37°C under agitation. One mL aliquots were withdrawn at 0, 2, 4, 8 and 19 h after induction. Each sample was centrifuged (2 min; 12,000 *g*) and the pellet was used on electrophoresis as described above. The supernatant was used to evaluate endoglucanase activity.

For time course endoglucanase activity, 300 mL of LB medium were inoculated with 5 mL of an overnight culture of BL21(DE3) harboring pNAW3 clone previously selected. The culture was grown for 3 h, IPTG was added at 0.4 mmol L^{-1} final concentration and after 8 h (37°C /280 rpm) cells were pelleted by centrifugation (10 min; 5,000 *g*). The pellet was suspended into buffer (50 mmol L^{-1} Tris-HCl pH 7.5, 300 mmol L^{-1} NaCl, 10% glycerol and 1 mmol L^{-1} PMSF). Triton X-100 was added to a final concentration of 0.1%. After three cycles of freezing and thawing, $MgCl_2$ (10 mmol L^{-1}) and DNaseI (10 μg mL^{-1}) were added. All reagents were homogenized and standed at room temperature for 10 min. This lysate was centrifuged to 5,000 *g* for 25 min at 4°C. The resulting supernatant was centrifuged again at 40,000 *g* for 30 min at 4°C. We used ammonium sulfate at 20 and 80 % saturation and EDTA was added to 1 mmol L^{-1} before protein precipitation. Each fraction was dialyzed against distilled water and the desalted solution was centrifuged (20,000 *g*; 10 min; 4°C). Proteins pelleted at 80 % saturation were used on endoglucanase assays to confirm the cellulase activity of heterologous protein.

SDS-PAGE and SDS-PAGE zymograms

The same cell cultures employed on the plate test were used for electrophoresis to visualize the amount of recombinant protein. A 100-μL aliquot of each culture, both before and after induction with 0.4 mmol L^{-1} IPTG, were centrifuged (2 min; 12,000 *g*) and the pellet was dissolved with 100 μL buffer (1 volume of TE buffer and 1 volume of electrophoresis sample buffer). Lysozyme was added (10 mg μL^{-1}) and kept for 15 min at 30°C. This lysate was heated in a water bath at 100°C for 5 min, cooled and centrifuged (2 min; 12,000 *g*). A 10-μL aliquot of each sample was applied on the top of a 4.5% stacking gel. Resolving gel was at 10%. SDS-PAGE was run according to Laemmli (1970). After electrophoresis run at 4°C, the gel was stained with Coomassie blue.

To detect CMCase activity after SDS-PAGE, 0.1% CMC was included in the polyacrylamide solution before gel was cast. After electrophoresis, the gel was washed and stored at 37°C into 20 mmol L^{-1} Tris-HCl buffer, pH 7.2. After two buffer exchanges (1.5 h each wash) the gel was immersed into the same buffer with 0.1% (v/v) Triton X-100. This buffer was exchanged twice and the gel was stored overnight at 37°C in buffer without Triton X-100. The gel was stained with Congo red and destained with 1 M NaCl washes (Park et al., 1998; Wood et al., 1988).

Endoglucanase assay

Plates with cultures had the cells washed off with water and killed with 2% sodium hipochloride. The medium was covered with Congo Red 0.1% (w/v) for 1 h, washed with water and several times with NaCl 1 M. Cellulase positive clones give a yellow halo against a red background on this cellulase plate assay (Wood et al., 1988).

An indirect assay for endoglucanase was made by incubation of a 50-μL aliquot of supernatant (from expression assay above) with 200 μL of 1% CMC (w/v) into 50 mmol L^{-1} sodium acetate buffer, pH 5.2. After 2 h at 40°C, reducing sugars were determined according to the

Lever procedure (Lever, 1972), having glucose as a standard. The amount of protein present in the supernatant was measured by the Bradford procedure (Bradford, 1976), having BSA as standard. One unit of endoglucanase (EC 3.2.1.4 cellulase, CMCase or β-1,4-endoglucanase) is the amount of protein in milligrams enough to produce 1 µmol of reducing sugars (an amount of 0.18 µg of glucose as determined according to Lever, 1972) from the substrate by min (Foong & Doi, 1992).

RESULTS AND DISCUSSION

On recent years, high-throughput genome analysis enabled several complete genomes to be unravelled. Although such information is invaluable, genomic data needs functional confirmation in order to allow biological roles of genes to be confirmed. Although a putative function can be assigned to an open reading frame (ORF) by sequence comparison, such information needs to be confirmed through analysis of the protein product of the putative gene. Also, characterization of the product is essential in case of enzymes to determine the specificity towards different substrates, stability and other properties (Hough & Danson, 1999).

The coding sequence of 1779 bp from Xf-818 was sucessfully cloned into pET20b, creating pNAW3 (Figure 1). The Xf-818 gene is under the control of the inducible promoter T7, and remains translatable from its original start codon.

Simpson et al. (2000) have assigned putative functions to around 50 % of ORFs from *X. fastidiosa* 9a5c, and among the genes described there are several potentially involved in plant-pathogen interaction, specially in plant cell wall degradation (Table 1). Using Blast (Altschul et al., 1990) we have analyzed the best matches of protein Xf-818 (Table 2) and the highest similarities were among bacterial endoglucanases. It has a better homology with EngXCA from *Xanthomonas campestris* pv. *campestris,* an endoglucanase with minor role on initial stages of pathogenic process in radish and turnip (Gough et al., 1988; 1990). All matches of endoglucanases Xf-818 belong to the family 5 of glycoside hydrolases (Table 2) (Henrissat, 1991; Henrissat & Bairoch, 1993). In fact, all three endoglucanases putatively found on *X. fastidiosa* (Xf-810, Xf-818 and Xf-2708) belong to family 5 (Simpson et al., 2000).

Protein Xf-818 has two domain structures, with a N-terminal domain composed of a catalytic domain of family 5, interspersed by a lynker sequence to a type II cellulose binding domain (CBD) (Figure 2B). Cellulase activity was confirmed on CMC plates, a diagnostic test that shows the catalytic behavior of cellulases over cellulose chains (Figure 3). Congo red dye complexes with cellulose polymer but not with oligomers formed after its cleavage. Therefore, alterations associated with dye binding can be used to monitor hydrolytic activity on endoglucanases, but not cellobiohydrolases, another kind of cellulases acting over cellulose (Wood et al., 1988). Cellulase activity was also assessed by the formation of reducing sugars during hydrolysis of CMC (Figure 4). Confirmation of Xf-818 protein as a endoglucanase, sup-

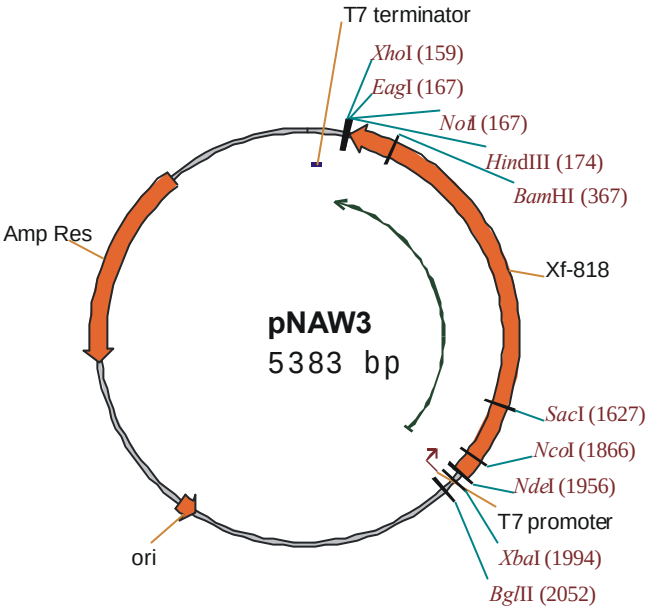

Figure 1 - Restriction map of the plasmid pNAW3. Gene Xf-818 was cloned between *Nde*I and *Hind*III sites, under control of the T7 promoter. It has T7 terminator at its end, after the *Hind*III site. Important features of the plasmid are shown.

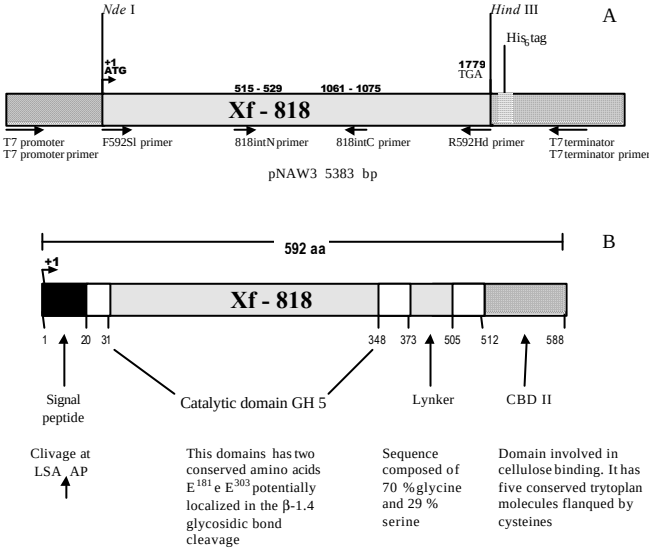

Figure 2 - (A) Annealing positions of primers on the Xf-818 gene cloned into pNAW3. *Nde*I and *Hind*III sites are shown as well as flanking structures of the pET20b plasmid; (B) Structure of Xf-818 protein (NCBI AAF83628/ SwissProt Q9PF60), as determined from the sequence derived from ORF identified on *X. fastidiosa* 9a5c (Simpson et al., 2000). Relevant features of Xf-818 protein domains are shown, with numbered positions referring to beginning and end of domains or specific structures.

Table 1 - Putative genes of *Xylella fastidiosa* related to plant cell wall degrading enzymes (PCWDE).

Gene[1]	Putative function[2]	Size (bp)*	Protein (aa)*	kDa*	e-value*
Xf-1267	Cellobiohydrolase	2052	683	70.9	e^{-142}
Xf-810	Endoglucanase	1698	565	61.6	$3e^{-50}$
Xf-818	Endoglucanase	1779	592	60	$1e^{-145}$
Xf-2708	Endoglucanase	1071	356	39.3	$2e^{-85}$
Xf-878	Endoxylanase	774	257	28.6	$9e^{-04}$
Xf-2395	Acetylxylan esterase	861	286	32.2	$1e^{-13}$
Xf-2779	Endoxylanase	2712	903	101.1	$8e^{-15}$
Xf-2466	Polygalacturonase	1636	544	59.8	e^{-112}
Xf-2359	Pectate lyase	711	236	26.1	$1e^{-10}$
Xf-439	β-glucosidase	2304	767	83.2	e^{-137}
Xf-845	β-xylosidase	2649	882	95.0	$2e^{-80}$

*bp=base pairs, aa=amino acids, kDa=kilodalton, e-value= expect value

[1]Gene number, size in bp and amino acid number are from Simpson et al. (2000) found at http://aeg.lbi.ic.unicamp.br/xf/.

[2]Gene function was assigned according to the similarity found with other genes deposited at GenBank and the highest similarity is shown on the last column.

Table 2 - Homologs of Xf-818 protein as found on protein-protein Blast search at NCBI (http://www.ncbi.nlm.nih.gov/BLAST/)[1].

Acession Number	Cellulase	Organism	Probability	Score
P19487	Endo-1,4-β-glucanase [engXCA]	*X. c.* pv. *campestris*	e^{-137}	487
P23548	Endo-1,4-β-glucanase	*Bacillus polymyxa*	$4e^{-77}$	287
P54583	Endo-1,4-β-glucanase E1	*Acidothermus cellulolyticus*	$4e^{-64}$	244
P10474	Endo-1,4-β-glucanase / 1,4 -β-cellobiohydrolase	*Caldicellulosiruptor saccharolyticus*	$2e^{-42}$	172
Q05332	Endo-1,4-β-glucanase G	*Clostridium thermocellum*	$1e^{-41}$	169
P50400	Endo-1,4-β-glucanase D	*Celullomonas fimi*	$5e^{-33}$	140
P04956	Endo-1,4-β-glucanase B	*Clostridium thermocellum*	$1e^{-30}$	133
P27033	Endo-1,4-β-glucanase C	*Pseudomonas fluorescens*	$3e^{-18}$	92

[1]swissprot protein database

port annotation that was performed during a sequencing project (Simpson et al., 2000), a fact that elicits assuming that other putative plant cell wall degrading enzymes are in fact true enzymes that can act over the plant cell wall (Table 1).

To optimize protein production, an *E. coli* clone that produces high amount of heterologous protein was selected, and a few parameters that can influence protein production and accumulation were evaluated. The clone of *E. coli* harboring pNAW3 and named clone b is the best Xf-818 protein producer to be employed when scaling up the process of heterologous protein expression (Table 3). Such analysis was confirmed by means of electrophoretic analysis of total protein (Figure 5A).

The next step was to analyze the accumulation of cellulase in the selected clone of *E. coli* after induction. Protein accumulation increased positively a few hours from induction, reaching the highest rate 19 h after induction (Figure 5B). The rate of protein accumulation was apparently dependent of temperature during ex-

Table 3 - Cellulase activity of selected clones of *E. coli* BL21(DE3) harboring pNAW3 after 0.4 mmol L[1] IPTG induction.

Clone[1]	µg glucose produced[2]	SA[3]	Plate assay[4]
pNAW3 a	3.41 ± 0.56	2.65	Positive
pNAW3 b**	7.02 ± 1.48	5.52	Positive
pNAW3 c	1.67 ± 0.27	1.41	Positive
pNAW3 d	3.64 ± 0.17	2.94	Positive
pNAW3 e	3.96 ± 0.94	3.26	Positive
pNAW3 f	3.95 ± 1.2	3.30	Positive
pNAW3 g	1.55 ± 0.53	1.14	Positive
pET20 b	- 1.43 ± 0.64	nd	Negative

[1]Double asterisk indicates selected clone for cellulase production;

[2]Mean of 3 samples ± standard deviation;

[3]SA = Specific activity (µg glucose per mg of protein on sample per min); nd = not determined;

[4]Carboxymethyl cellulose degradation on plate assay (LB medium with 0.1% CMC). Fifty µL of an induced culture was grown overnight, medium was stained with Congo red and destained with NaCl 1 M.

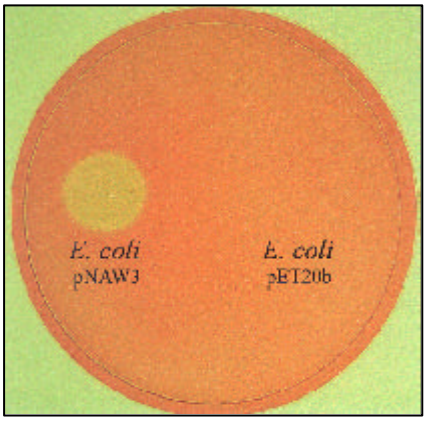

Figure 3 - Cellulase plate assay showing cellulose degradation by the action of Xf-818 expressed in *E. coli* BL21(DE3) by pNAW3. Untransformed *E. coli* was taken as control. Yellow-white halos indicate cellulose degradation.

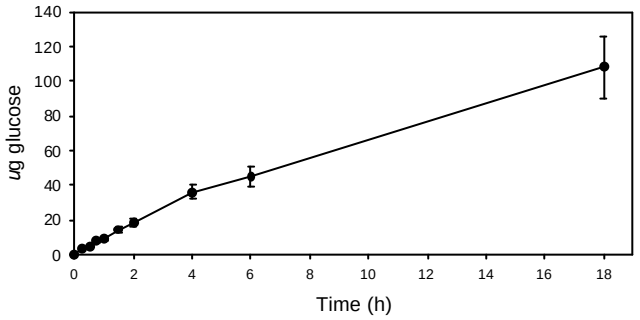

Figure 4 - Reducing sugars produced by Xf-818 during hydrolysis of CMC at 40°C. Measurements of reducing sugars were performed at indicated time intervals. Mean of 3 replicates ± standard deviation.

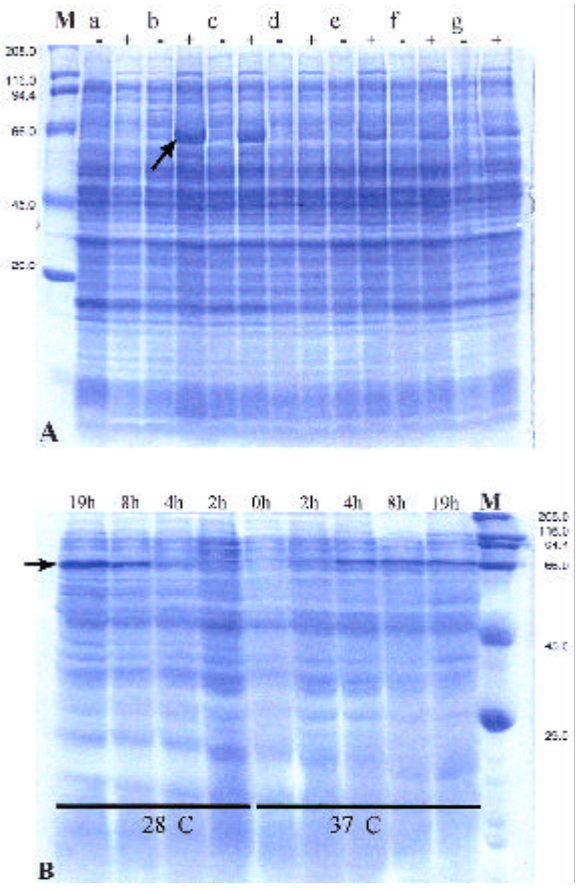

Figure 5 - SDS-PAGE of BL21(DE3) clones expressing gene Xf-818 (pNAW3) on gel at 10 %. **A)** Letters refer to single pNAW3 clones: (–) and (+) are clones either without or with IPTG induction, respectively. **B)** Culture aliquots were taken at indicated times after IPTG induction and electrophoresed. M = high molecular weight protein markers (kDa).

pression. At 37°C protein accumulated faster, but it reached a slightly higher level at the lower temperature. A suitable option is to keep the culture medium at 32°C after IPTG induction and then perform protein extraction after 12 h.

Confirmation that Xf-818 has cellulase activity, considered as an endoglucanase by the Congo red assay is by far the most important fact. Endoglucanase Xf-818 produces increasing amounts of reducing sugars in relation to incubation time (Figure 4), an indication of high activity and stability. An interesting feature of many carbohydrate hydrolysing enzymes is the ability to bind to the substrate, a characteristic also found for cellulases, that is mediated by a domain called cellulose binding domain, which is independent of the catalytic domain (Figure 2B). Although Hopkins (1985) did not detected cellulase activity on the culture medium of *X. fastidiosa* PD, Scarpari (2001) was able to detect the presence of mRNA of Xf-818 on the culture medium, an indicator of cellulase production by *X. fastidiosa* 9a5c.

The ability to knockout *X. fastidiosa* genes (Monteiro et al., 2001; Silva Neto et al., 2002) opens the way to analyze functionally *X. fastidiosa* genes, such as Xf-818 and addresses the role of this protein during plant-pathogen interaction. Cellulases have minor roles on the beginning of symptom development as assessed by cellulase mutants of *E. carotovora* subsp. *carotovora* (Mäe et al., 1995), *Ralstonia solanacearum* (Roberts et al., 1988) and *X. c.* pv. *campestris* (Gough et al., 1990). However, this pattern is different with *Clavibacter michiganensis* ssp. *michiganensis* (Jahr et al., 2000) and *C. m.* ssp. *sepedonicus* (Laine et al., 2000), since endoglucanases are essential for symptom development.

The next step is to produce and purify high amounts of Xf-818 to characterize its substrate specificity, raise antibodies and evaluate its expression during citrus colonization by *X. fastidiosa*. The recent sequencing of other *X. fastiosa* strains, such as Dixon strain from almond, Ann1 from oleander (Bhattacharyya et al., 2002) and a grapevine strain (Van-Sluys et al., 2003), opens the possibility to study the diversity of pathogenicity factor among strains of the same bacterium, but with different host range.

ACKNOWLEDGMENTS

This work was supported by grant from FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo). First author was supported by a scholarship from FAPESP, and the third author was fellow of CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico). Authors also thank Dra. M. Fiore (USP/CENA), Dr. J. Ferro (UNESP/Jaboticabal) and Dra. M.L. Targon (IAC/CCSM) for providing essential material for this research.

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Received October 01, 2002
Accepted August 19, 2003