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Molecular cloning of *Erwinia chrysanthemi* pectinase and cellulase structural genes

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***Erwinia chrysanthemi* 3937 secretes four major pectate lyase isoenzymes (PL, EC 4.2.2.2) and one endocellulase (Cx, EC 3.2.1.4). A genomic library of this strain was constructed in the Lambda L47-1 vector, and screened for the presence of PL and Cx on pectate and carboxymethylcellulose agar. Among the seven Cx-positive phage clones, three were shown to encode an enzyme of the same mol. wt. as the one found in the culture supernatant of strain 3937. The 34 PL-positive phage clones were analyzed by electrofocusing and could, according to the PL they produced, be arranged in five classes. Phages from three classes produced three different single PL, named PLb, c and d. No common fragment was evidenced between the inserts of the phages of these three classes. This demonstrated that, in strain 3937, PLb, c, and d were encoded by three different genes called *pelB, C,* and *D*. Furthermore, our results suggest the existence of two additional genes encoding PLa and e. In addition, a pectin methylesterase gene was found closely linked to *pelD*.**

Key words: endo-β-1,4 glucanase/isoenzymes/multigene family/pectate lyase/pectin methylesterase

Introduction

Erwinia chrysanthemi is an enterobacterium pathogenic for many plants, monocots as well as dicots (Starr and Chatterjee, 1972). The most usual symptom is a soft rot which seems to be related to the attack of major constituents of the plant cell wall by the pectic, cellulolytic and proteolytic enzymes produced by the infecting *Erwinia*. These enzyme activities can be detected both in the tissues of infected plants and in liquid cultures of the bacterium. The analysis of mutants has shown that these enzymes are exported by *E. chrysanthemi* and recovered in the culture supernatant (Andro *et al.*, 1984). The pectinolytic activity of the strain used in the present study (3937) seems to consist on two types of enzymes: (i) one pectin methylesterase (PME; EC 3.1.1.11) which demethylates the pectin by cleaving the ester-bond located on C6, generating polygalacturonate and methanol; and (ii) several pectate lyases (PL; EC 4.2.2.2) which, without the addition of an H_2O molecule, cleave the alpha 1–4 bond between galacturonate residues, generating unsaturated galacturonate oligomers, i.e., oligomers presenting a double bond between carbons 4 and 5, at the C-4 extremity of the molecule (Preiss and Ashwell, 1963). By electrofocusing on polyacrylamide gels, the PL present in the culture supernatant of *E. chrysanthemi* 3937 are separated as 14 bands (with five major bands) which can be identified by their activity (Bertheau *et al.*, 1984).

Some *E. chrysanthemi* strains can also digest crystalline cellulose by a mechanism which has not yet been elucidated. At present, one endo-β-1,4-glucanase (Cx; EC 3.2.1.4) is purified and partially characterized (Boyer *et al.*, 1985). Moreover, *E. chrysanthemi* produces at least one exocellular protease (C. Wandersman, Y. Bertheau and T. Andro, in preparation).

Because it is an enterobacterium, *E. chrysanthemi* is perfectly suitable for genetic analysis: chromosome mobilization has been obtained by conjugation, leading to the construction of genetic maps (Chatterjee and Starr, 1977; Kotoujansky *et al.*, 1982; Schoonejans and Toussaint, 1983). Genetic transfer has also been obtained by generalized transduction (Chatterjee and Brown, 1980; Résibois *et al.*, 1984) and by transformation (S. Reverchon and T. Andro, unpublished data). Furthermore, several strains of *E. chrysanthemi* proved to be sensitive to mutator phage Mu (Faelen *et al.*, 1981) and Mu derivatives have been used to isolate *E. chrysanthemi* mutants. However, despite the screening of ~10 000 independently isolated mutants, it has not been possible to find mutants defective in the structural gene of a pectinase (unpublished data). Such a negative result could be explained by the existence of more than one such gene.

To determine the number and the functional organization of the genes encoding this set of pectinases and cellulase, we constructed a gene library from one *E. chrysanthemi* strain (3937), using the Lambda vector L47-1 (Loenen and Brammar, 1980). Here we report the isolation and characterization of several hybrid phages carrying the regions coding for PL, PME and Cx.

Results

Isolation of λL47-1 clones carrying pel and cel genes

Phage clones of our library were screened for production of PL and Cx. Special plates were designed to reveal either PL or Cx activities around individual phage plaques, without killing the phages (Figure 1). Among 5000 phage clones screened for the presence of PL, 34 turned out to be positive (Pel^+), while seven clones out of 5000 other phages were found to be Cx producers (Cel^+). All these positive phages were purified twice in the same way. Then, their purity was checked by plating them on the same type of plates, except for the nylon membrane which was omitted (Figure 2).

Characterization of the Pel^+ clones

To determine the number and the pI of the PL isoenzymes produced by each Pel^+ clone, the phage lysates were analysed by electrofocusing, PL activities being revealed directly in the polyacrylamide gel (Figure 3). While no activity was detected in the lysate of λL47-1, at least one of the five PL major isoenzymes was always found in the lysates of the Pel^+ clones. The 34 Pel^+ clones could be arranged in five classes according to their pattern of PL (Table I). Each of the five major isoenzymes was represented in at least one class.

These data suggested that: (i) all the *pel* genes encoding the five major isoenzymes were cloned, (ii) these genes were expressed in *Escherichia coli*, and (iii) regarding their electric

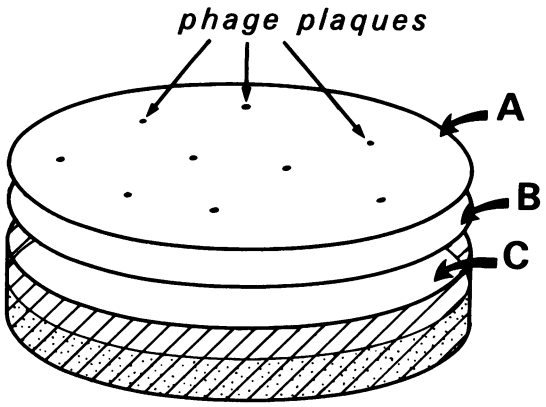

Fig. 1. Screening for phage clones carrying *pel* or *cel* genes. The plates were made of two layers (**A** and **C**) separated by a nylon membrane (**B**; Biodyne™ **A**, Pall Ultrafine Filtration Corp.). The top layer **(A)** consisted of 3 ml of L top agar with 10 mM $MgSO_4$ (Miller, 1972) containing the phages together with the indicator bacteria [strain C600(P2)]. The bottom layer **(C)** consisted of M9 medium (Miller, 1972) with 0.5% (w/v) glycerol, 0.1% (w/v) yeast extract (Difco), 1 mM $MgSO_4$, 1 mM $CaCl_2$, 1.5% (w/v) Bacto-agar (Difco), and 0.5% (w/v) either sodium polygalacturonate (Sigma) or carboxymethylcellulose (CMC, Serva Co.) to screen for presence of PL or Cx, respectively. After 24 h of incubation at 37°C, the top layer was removed with the nylon membrane, and halos of the enzymic activities were revealed: PL by flooding the polygalacturonate-containing plates with a saturated solution of copper acetate, under gentle stirring, and Cx by flooding the CMC-containing plates with 0.1% (w/v) Congo red in water, for 15 min, followed by bleaching with 1 M NaCl (Wood, 1981). Then, the membranes with their top layer were put back on the plates and each phage plaque located right above a halo was picked up.

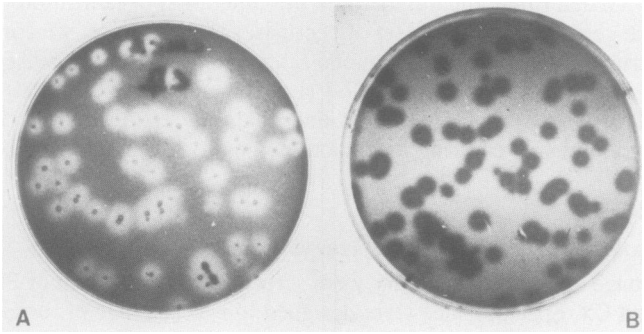

Fig. 2. Purification of Pel^+ and Cel^+ phages. Purity of the stocks of the Pel^+ and Cel^+ phages was controlled on the same type of plates as described in Figure 1, but without the nylon membrane. **A:** halos surrounding plaques of a Cel^+ phage, λC7, after revelation with Congo red. **B:** halos surrounding plaques of a Pel^+ phage, λ41, after revelation with copper acetate.

properties, these enzymes were not modified.

To ascertain that the isoenzymes identified by electrofocusing were PL, this activity was assayed in the lysate of one Pel^+ phage from each class (Table II). The data demonstrated that the five clones produced a PL activity which was fully inhibited in the presence of 10 mM EDTA. Since PLb, PLc and PLd were found alone in the lysate of λ11, λ122 and λ131, respectively, we concluded that these three isoenzymes were indeed PL. Moreover, activity per plaque forming unit (p.f.u.) in the lysates and activity per bacterial cell in the culture supernatant of *E. chrysanthemi* were of the same order of magnitude. This gave an indication that the level of expression of the corresponding genes (tentatively called *pelA* to *pelE*) was high in this system. As expected, no activity was detected in the lysate of λL47-1.

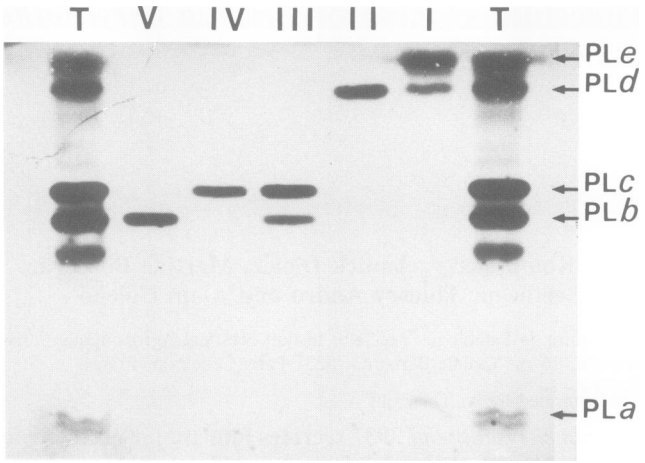

Fig. 3. Electrofocusing of the Pel^+ phage lysates in thin polyacrylamide gel. The visible bands on the gel are due to the activity of the PL isoenzymes, revealed by the 'sandwich technique' (Bertheau *et al.*, 1984). **Tracks T:** 14 μl of 3937 culture supernatant; **I, II, III, IV, V:** 20 μl of λ81, λ131, λ41, λ122, λ11 phage lysates, respectively, prepared as described in Materials and methods.

Table I. PL isoenzymes produced by the Pec^+ phages

Classes of Pec^+ phages	Names of the PL[a]	pI of the PL[b]
class I (11 phages)	PLa	4.7
	PLd	>9.5
	PLe	>9.5
class II (4 phages)	PLd	>9.5
class III (16 phages)	PLb	7.7
	PLc	8.3
class IV (2 phages)	PLc	8.3
class V (1 phage)	PLb	7.7

[a]The PL present in each phage lysate were characterized by electrofocusing in thin polyacrylamide gels as described in Materials and methods.
[b]pI were determined on the gels with a surface electrode (LKB 2117-111).

Table II. PL activity in *E. chrysanthemi* and in the lysates of Pel^+ phages of the five classes[a]

Strain or phages	PL produced[b]	PL activity (U/p.f.u.[c], or U/c.f.u[c])10^{-11}	
		standard conditions[d]	standard conditions + 10 mM EDTA
Pel^+ phages			
λ11	PLb	1.50	<0.05
λ41	PLb,c	7.22	<0.05
λ122	PLc	3.55	<0.05
λ81	PLa,d,e	2.46	<0.05
λ131	PLd	1.95	<0.05
E. chrysanthemi			
3937	PLa,b,c,d,e	6.47	<0.05

[a]The classes are defined in Table I.
[b]PL were characterized by electrofocusing (Figure 3).
[c]Abbreviations: p.f.u., plaque forming unit; c.f.u., colony forming unit.
[d]Standard conditions for PL assay at 235 nm are described in Materials and methods.

While the five PL were never simultaneously found in the lysate of any Pel^+ phage, class I phages produced PLa,d,e, and class III PLb,c (Table I). This suggested that the *pel* genes were distributed in two distinct areas on the chromosome of strain 3937. To verify this, DNA from phages representing the five

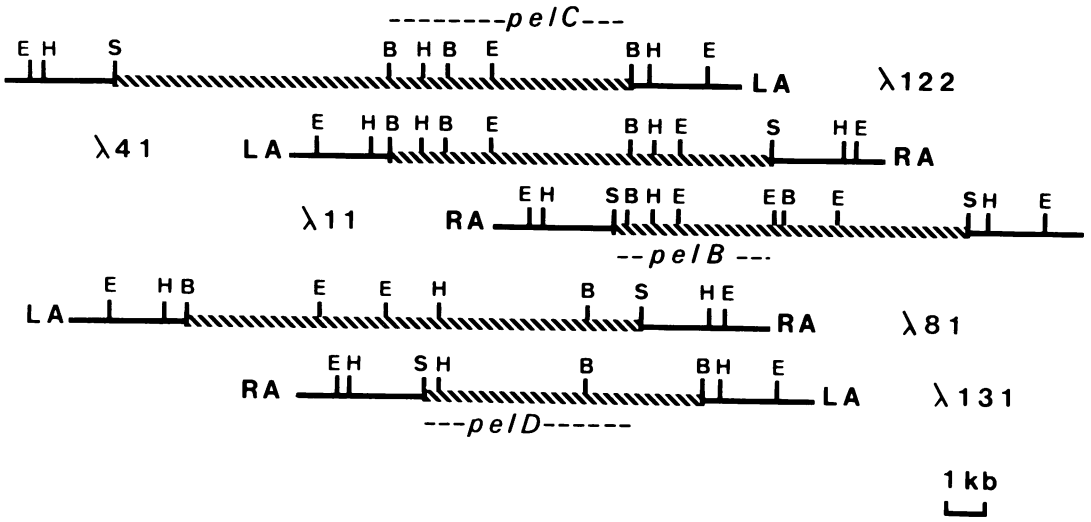

Fig. 4. Restriction maps of five Pel$^+$ phages. Single lines represent Lambda DNA, and the hatched lines are the inserted DNA segments. The location of *pelB,C* and *D* genes is indicated by dotted lines. Abbreviations for endonuclease restriction sites: B, *Bam*HI; *E*, *Eco*RI; H, *Hind*III; S, *Sau*3A. LA and RA indicate the position of the left and right Lambda L47 arms, respectively.

classes of Pel$^+$ clones was purified and digested by the endonucleases *Bam*HI, *Eco*RI and *Hind*III, and by combinations of these enzymes. This provided the data to construct restriction maps of the inserts carried by these phages (Figure 4).

As expected, inserts of λ81 and λ131 shared common sequences and it was also the case for inserts of λ11, λ41 and λ122. On the other hand, there was no overlapping between the two sets of clones. This confirmed that the *pel* genes were located in two clusters on the chromosome of 3937: one cluster included the genes for the two basic and the acidic isoenzymes, PLa,d,e, and the other the genes for the two neutral isoenzymes, PLb,c. Moreover, the overlap between the insert of λ11 (encoding PLb), and the insert of λ122 (encoding PLc) was only 300 bp long, which is not sufficient to carry a PL structural gene. This fact showed that two different genes, *pelB* and *pelC*, encode the two neutral PL, PLb and PLc, respectively, these two genes being closely linked on the 3937 chromosome.

Cloning of PME gene

The lysates of the Pel$^+$ phages were screened for the presence of a PME activity, in cup-plates revealed by the method of Cruickshank and Wade (1980). This activity was actually detected in the majority of the lysates belonging to class I and II, and thus seemed to be closely linked to PLd. However, one clone, λ61, generated only the PLd without PME, indicating that these activities could be separated and might not be due to the same polypeptide.

Characterization of the Cel$^+$ clones

The lysates of the seven Cel$^+$ phages detected in the library were submitted to electrophoresis in a gradient of polyacrylamide (Figure 5). Under these conditions, the Cx in the lysates of λC1, λC2, λC3, and the Cx found in the culture supernatant of 3937, had the same migration, i.e., the same size. In some repetitions, a second band of activity appeared in the gel, below the first one, in 3937 supernatant and in the lysates as well. We do not know whether it was corresponding to a second isoenzyme or to a smaller form of the protein. The activity in the remaining clones was too low to be characterized, and no activity was detected in the lysate of λL47-1.

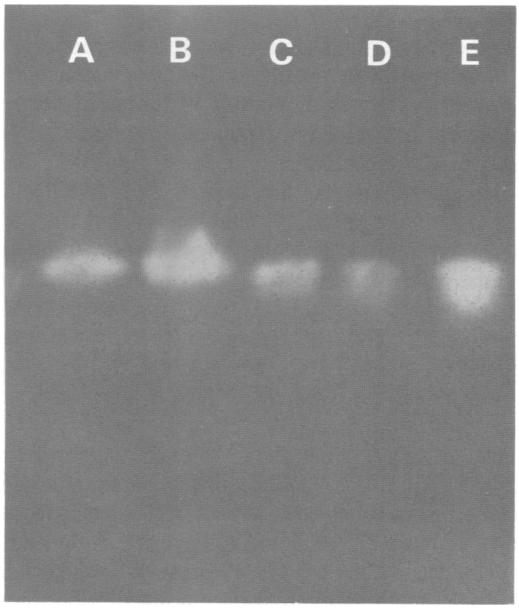

Fig. 5. Electrophoresis of Cel$^+$ phage lysates. The lysates were lyophilized and resuspended in 1/10 of the initial volume. The equivalent of 0.25 ml of lysate was electrophoresed as described in Materials and methods. The Cx activities in the gel were revealed by the 'sandwich technique' (Bertheau *et al.*, 1984), using an agar gel containing 0.5% carboxymethylcellulose in Tris-maleate 0.1 M pH 5. **Tracks A, B** and **C:** lysate of λC1, λC2 and λC3; **tracks D** and **E:** 15 and 30 μl of culture supernatant of 3937, respectively.

Discussion

Since it was *a priori* unlikely that pectinase and cellulase would be secreted by *E. coli*, and since our detection test required the enzymes to diffuse in the testing agar medium, we decided to use a phage cloning vector rather than a plasmid, so that the cells would be lysed at the end of the lytic cycle, releasing the enzymes into the medium. A similar strategy had previously been used for the cloning of the α-amylase gene of *Bacillus licheniformis* (Joyet *et al.*, 1984). Since then, the cloning of *pel* and *cel*

genes in a RP4 plasmid (Van Gijsegem *et al.*, accompanying paper), and in cosmids (Keen *et al.*, 1984; Barras *et al.*, 1984), has shown that, although not secreted, a small proportion of these enzymes were released by the colonies of *E. coli*, allowing the positive clones to be detected. Another advantage of using λL47-1 was the fact that the cloning between its *Bam*HI sites gave an opportunity to force the expression of a cloned gene by getting its transcription from the phage pL promotor.

The Clarke-Carbon formula (Clarke and Carbon, 1976) allows us to calculate the number of clones required to cover the totality of the *E. chrysanthemi* chromosome. Assuming that this chromosome is 3000 kb long (this by analogy with the *E. coli* chromosome) and that the cloned fragments had an average size of 15 kb, 918 clones should have been sufficient to cover the whole genome with a probability of 99%. Our library consisted of 12 600 clones and therefore had to be representative.

In this library, we have been able to isolate clones carrying the structural genes of PL, PME and Cx. Regarding the Cx activity, the situation looked simple: one gene should exist encoding one enzyme. A similar conclusion has been stated for *E. chrysanthemi* 3665: only one endo-β-1,4 glucanase has been purified from the culture supernatant of that strain (Boyer *et al.*, 1985), and the corresponding gene has been cloned in a cosmid, in *E. coli* (Barras *et al.*, 1984).

On the other hand, the situation was more complicated regarding the PL activity. Our results showed the existence of at least three different *pel* genes, encoding PLb,c,d. Moreover, Van Gijsegem *et al.* (accompanying paper) state that PLd and PLe are not encoded by the same gene in the strain B374. As the pattern of PL isoenzymes was the same in both 3937 and B374, this result could most probably be extrapolated to 3937.

The only remaining ambiguity concerned PLa and PLe. However, the wide difference in pI between these two isoenzymes (>5 units) made it very unlikely that they could stem from a unique polypeptide. Thus, by taking into account our results with those of Van Gijsegem *et al.*, we conclude to the existence of five genes encoding the five major PL of *E. chrysanthemi* 3937: *pelA* to *pelE*.

The same set of *pel* genes seems to exist in a third strain, *E. chrysanthemi* CUCPB1237 (A. Collmer, personal communication). Furthermore, it has been shown in this strain that the two isoenzymes of basic pI, PLd and e, have the same mol. wt. of 45 000. Likewise, the neutral PL, PLb and c, have the same mol. wt. of 39 000. From an evolutionary point of view, these data might mean that *pelB,C,D,E* stem from the duplication of two original genes, one generating *pelB,C*, the other *pelD,E*. On the other hand, Keen *et al.* (1984) recently reported the cloning from *E. chrysanthemi* EC16 in *E. coli* of two genes encoding two PL of pI 8.8 and 9.8. Thus, the strain EC16 might present a simpler PL pattern than the one observed in the other strains. An alternative explanation is that the electrofocusing technique used by these authors might not have allowed them to separate and detect the five PL isoenzymes.

The originality of our work lies in the separation of the two genes encoding the neutral PL, *pelB* and *pelC*. Our attempts to subclone *pelB* in a high copy number plasmid have been so far unsuccessful. This could indicate that, when overproduced, the PLb protein is toxic for the *E. coli* host. The lack of clones carrying the *pelB,C* region in a gene library of B374, constructed in a cosmid vector (N. Reverchon, personal communication) support this idea. Such a toxic effect would not have impaired the cloning efficiency in a phage vector.

Nevertheless, the origin of the nine minor bands of PL activity, detected by electrofocusing in culture supernatants of *E. chrysanthemi* 3937, and in other strains as well (our unpublished results), remains to be explained. We observed that some of these bands appeared in the Pel$^+$ phage lysates after a certain time of storage at 4°C; they could thus be generated by modifications of major PL during storage. The other minor bands might correspond to multimers built by association of different major PL. Less likely, other *pel* genes might exist which have not been detected in the different cloning experiments.

The question raised by our results is why does *E. chrysanthemi* possess five genes to generate its PL activity? The simultaneous production of several isoenzymes is a rather unusual phenomenon among prokaryotes. What is the exact role played by each of these isoenzymes in the pectinolysis and the phytopathogenicity of *E. chrysanthemi*? To answer these questions, the subcloning of *pel* and *cel* genes is underway and should help the purification and characterization of the different isoenzymes. Furthermore, experiments of directed mutagenesis have been undertaken to generate mutants lacking one or another enzyme from this set of pectinases and cellulase.

Materials and methods

Bacterial strains and phages

Bacterial strains are listed in Table III. The Lambda vector L47-1 (Loenen and Brammar, 1980) is △(srlλ1-2), imm434, cI$^-$, NIN5, *chiA131*, Red$^+$ and Gam$^+$.

Culture media

Unless otherwise stated, *E. coli* strains were grown in L medium (Miller, 1972), at 37°C. *E. chrysanthemi* was grown at 30°C in L medium. When PL induction was required, 0.5% sodium polygalacturonate (Sigma Chemical Co.) were added.

Preparation of E. chrysanthemi DNA

DNA from Hfrq was prepared as follows: a 20 ml culture of the strain in L broth was centrifuged and the pellet was washed with TE_{50-50} buffer (50 mM Tris, 50 mM Na_2EDTA, 16 mM NaOH, pH 8.0). Spheroplasts were prepared by resuspending the pellet in 1 ml of a lysis mixture composed of 2 ml of 50% (w/v) sucrose in TE_{50-50}, 1 ml of a 1 mg/ml solution of RNase A (type II-A, Sigma) in water, 1 ml of a 10 mg/ml solution of lysozyme (Sigma) in TE_{50-50}, and 6 ml of TE_{50-50}. After 10 min in ice the spheroplasts were lysed by addition of 0.5 ml of 2% (w/v) lauroyl sarcosine (Sigma) in TE_{50-50}, drop by drop with gentle mixing. After 15 min in ice, the lysate was treated with 0.2 ml of 5 mg/ml pro-

Table III. Bacterial strains and phages

Bacterial strians	Characteristics[a]	Origin
E. chrysanthemi		
Hfrq	Hfr, Nal^R, Tet^R, Lac$^+$ Hfr derivative of the wild-type strain 3937	Kotoujansky *et al.*, 1982
E. coli		
C600	F$^-$, *thi, thr, leuB, lac1, tonA21, supE44, hsdR*, λ$^-$, Str^R	P. Boistard
C600(P2)	C600, lysogenic for P2	Loenen and Brammar, 1980
LE392	F$^-$, *hsdR514, supE44, supF58, lac1 or* △(*lacIZY*)*6, galK2, galT22, metB1, trpR55*, λ$^-$	Enquist and Sternberg, 1979
BHB2688	N205, RecA$^-$ (λimm434, cI(ts), b2, *red3, Eam4, Sam7*)λ	Hohn, 1979
BHB2690	N205, RecA$^-$ (λimm434, cI(ts), b2, *red3, Dam15, Sam7*)λ	Hohn, 1979

[a]Abbreviations: Nal^R, nalidixic acid resistant; Str^R, streptomycin resistant.

teinase K (Boehringer Mannheim Co.) in TE_{50-50}, followed by incubation overnight (i.e., ~14 h) at 37°C. The mixture was extracted twice with phenol (prepared according to Maniatis *et al.*, 1982) and equilibrated with TE_{50-50}, followed by two extractions with chloroform:isoamyl lcohol (24:1) and one extraction with water-saturated ether. The DNA was precipitated with ethanol at −20°C, washed with ethanol:water (70:30), then centrifuged 15 min at 12 000 *g*, and resuspended in TE_{10-1} (10mM Tris-HCl, 1 mM Na_2EDTA, pH 7.9) After this, the DNA was treated with phenol and chloroform, precipitated by ethanol, washed with ethanol:water, and resuspended in TE_{10-1}. This procedure gave ~500 μg of DNA whose size was longer than 50 kb, as shown by electrophoresis in a 0.3% agarose gel, with DNA of phage Lambda as size marker. 100 μg of that DNA was digested with *Sau*3A (Bethesda Research Laboratories Inc.) in limiting condition to obtain about one cut every 100 recognition sites. The mixture of fragments generated by the digestion was run on a 10−40% sucrose gradient (16 h at 30 000 r.p.m. in a Kontron TST41.14 rotor) to separate the fragments with an average size of ~15 kb. A little less than 20 μg of DNA were recoverd after this last step.

Preparation of Lambda L47-1 phage arms DNA

Restriction and ligation were according to Maniatis *et al.* (1982). Ligation was performed on 100 μg of λL47-1 linear DNA (kindly provided by F. Richaud) to prepare covalently closed DNA, which was subsequently digested with *Bam*HI and *Xho*I to provide three fragments of ~33.5, 5.5 and 1 kb. The longest fragment corresponding to the left and right arms ligated together, was separated from the two smallest fragments by centrifugation in a 10−40% sucrose gradient (16 h at 30 000 r.p.m. in a TST41.14 Kontron rotor).

Construction of E. chrysanthemi gene library in λL47l

0.5 μg of *E. chrysanthemi* DNA digested by *Sau*3A, and 3.5 μg of λL47-1 arms DNA were mixed with T4 DNA ligase (New England Biolabs). 0.5 μg aliquots of this ligation product were *in vitro* packaged into phage particles following protocol II of Maniatis *et al.* (1982). These phages were plated on *E. coli* strain C600 (P2) according to Maniatis *et al.* (1982), to select the hybrid phages by their Spi^- phenotype (Loenen and Brammar, 1980). The *in vitro* encapsidation procedure yielded a total of 12 600 p.f.u. on C600(P2). Controls consisted of packaging extracts supplied with no DNA, or with λ47-1 DNA, or with only phage arms DNA. As expected, these controls gave no phage plaques when plated on C600(P2). This implied that the 12 600 recovered p.f.u. were hybrid phages having their central fragment replaced by *E. chrysanthemi* DNA.

Screening the hybrid phages of the library for PL or Cx production

The phages with the indicator bacteria were plated onto two types of differential media designed to reveal PL or Cx activities around the phage plaques (Figure 1). The method was derived from the 'cup-plate' technique, which is based on radial diffusion of the enzymes into a substrate bearing agar gel slab (Dingle *et al.*, 1953).

PL electrofocusing

The Pel^+ phages were plated on *E. coli* strain C600 (between 10^6 and 10^7 phages per plate), on L medium supplemented with 10 mM $MgSO_4$. The bottom layer contained 12 g/l Bacto-agar (Difco), and the top layer contained 3 g/l agarose standard low mr (Biorad Laboratories). When lysis was achieved, i.e., after 12 h, the plates were flooded with 6 ml of SM buffer (Maniatis *et al.*, 1982) and gently agitated at 4°C for 6 h. 5 ml buffer were recovered: they contained both the phages and the enzymes produced. These so-called 'phage lysates' were used to characterize the enzymes and as a source of phage DNA. 20 μl of each phage lysate were layered on a thin or ultrathin polyacrylamide gel and electrofocusing was performed in a 3−9.5 pH gradient. The PL activities were developed directly on the gel using the method devised in our laboratory (Bertheau *et al.*, 1984).

Electrophoresis of endocellulase

Electrophoresis was performed in a 5−30% polyacrylamide gradient gel for 24−36 h at 500 V, 4°C, using a LKB 2001 Vertical Apparatus. By using this technique, the proteins were not denatured and were stopped by the mesh of the gel, at a position that was a function of their size (Hames and Rickwood, 1981). Endocellulase activity was revealed on an agar gel containing the substrate, carboxymethylcellulose, by the 'sandwich' system previously described (Bertheau *et al.*, 1984).

PL assay

Activity was assayed by recording the kinetics of absorbance at 235 nm of reaction mixture containing sodium polygalacturonate with a Beckman Acta III spectrophotometer, at 37°C (Preiss and Ashwell, 1963). 0.1 ml of sample was added to 1 ml of the reaction mixture containing 2.4 mg/ml of sodium polygalacturonate (Sigma) in 50 mM Tris-HCl pH 8.6. According to Keen *et al.* (1984), 1.73 absorbance units/min was considered to represent the formation of 1 μmol of unsaturated uronide/min, and one unit of activity was defined as the liberation of 1 μmol of product/min under these conditions.

Preparation of DNA from the phage clones

The phages were purified from the phage lysates (see above) as described by Maniatis *et al.* (1982), except the CsCl step gradient which was replaced by a linear gradient contianing 0.75 g CsCl/ml of sample.

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