Identification and physical characterization of a Col E1 hybrid plasmid containing a catalase gene of *Escherichia coli*

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A hybrid *Escherichia coli*: Col E1 plasmid, pLC36-19, containing a catalase gene has been identified in the Clarke and Carbon colony bank. Catalase activity was amplified two- to three-fold in the pLC36-19-containing strain relative to other hybrid-plasmid-containing strains and this activity could be induced three- or four-fold by hydrogen peroxide or ascorbic acid. The plasmid was transferred to a strain chromosomally deficient in catalase synthesis, resulting in a strain with high and inducible levels of catalase. The plasmid was also transferred to a minicell-producing strain and minicells harbouring the plasmid were found to synthesize a labelled protein with a molecular weight of 84 000 characteristic of catalase from *E. coli*. A catalase activity was also synthesized by the plasmid-containing minicells. Two catalase activities with associated peroxidase activities coded for by the plasmid were separable by polyacrylamide gel electrophoresis and migrated coincident with chromosomally encoded catalase–peroxidase activities. A third catalase activity which did not have an associated peroxidase activity was not coded for by the plasmid. A physical map of the 25.5-kilobase pair plasmid was constructed by restriction nuclease analysis and the relative positions of 38 restriction sites were defined.

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Dans la banque de colonies Clarke et Carbon, nous avons identifié un plasmide hybride d'*Escherichia coli*:ColEl, le pLC36-19, contenant un gène de la catalase. L'activité catalasique est amplifiée de deux à trois fois dans la souche contenant le pLC36-19 (porteur du gène de la catalase) par rapport aux autres souches contenant le plasmide hybride (sans gène de la catalase) et cette activité peut être induite de trois à quatre fois par le peroxyde d'hydrogène ou l'acide ascorbique. Le transfert du plasmide dans une souche chromosomiquement déficiente pour la synthèse de la catalase produit une souche contenant des taux élevés et inductibles de catalase. Nous avons aussi transféré le plasmide dans une souche productrice de minicellules et les minicellules hébergeant le plasmide synthétisaient une protéine marquée ayant un poids moléculaire de 84 000; ce poids moléculaire est caractéristique de celui de la catalase de *E. coli*. Une activité catalasique est aussi synthétisée par les minicellules contenant le plasmide. Deux activités catalasiques associées à des activités peroxydasiques codées par le plasmide sont séparables par électrophorèse sur gel de polyacrylamide et leur migration coïncide avec les activités catalase–peroxydase codées par les chromosomes. Une troisième activité catalasique, non associée à une activité peroxydasique, n'est pas codée par le plasmide. L'analyse avec des nucléases de restriction nous a permis de construire une carte physique des 25,5 paires de kilobases du plasmide et nous avons déterminé les positions relatives des 38 sites de restriction.

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Introduction

Catalase was one of the first bacterial enzymes described and it has been the object of research for nearly a century. Sometimes referred to as a hydroperoxidase, catalase employs a two electron transfer in the dismutation of hydrogen peroxide to oxygen and water. Peroxidases are also referred to as hydroperoxidases, but they employ a one electron transfer using a hydroperoxide as electron acceptor. In *Escherichia coli* two electrophoretically distinct catalase activities with associated peroxidase activities have been identified and labelled HPI and HPII (1). Following its purification, the main activity, HPI, was characterized as a tetramer with a molecular weight of 337 000 (1). In addition, a third catalase activity without an associated peroxidase activity has been reported (2). Unfortunately very little genetic data concerning catalase has accumulated. A number of loci involved in catalase synthesis in *Salmonella typhimurium* have been reported, but only one locus was mapped by phage transduction (3). In *E. coli* the genetic relationship of the three catalase activities remains obscure.

Somewhat more information has accumulated regarding the regulation of catalase gene expression. A link

ABBREVIATIONS: SDS, sodium dodecyl sulphate; kb, kilobase(s); bp, base pair(s).

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between the synthesis of components of the respiratory chain and the synthesis of catalase has been suggested (4). Catabolite repression was implicated in the regulation of catalase synthesis in yeast (5) and bacteria (4, 6, 7), but evidence to the contrary obtained in bacteria has been presented (8, 9). The basal level of catalase in *E. coli* ranges from 3 to 15 units/mg dry cell weight depending upon the growth medium and aeration state (2, 4), and the addition of H_2O_2 or ascorbate (2, 7) to cultures of *E. coli* caused a rapid sixfold increase in the catalase–peroxidase activities. This suggested that H_2O_2 or a reaction product was acting as an inducer of catalase synthesis, but the mechanism of this induction remains undefined.

The cloning of a catalase gene on a plasmid would facilitate an expansion of this limited body of information relating to catalase gene expression. This paper describes the identification of a strain in the Clarke and Carbon colony bank (10) which contains a hybrid Col E1 – *E. coli* plasmid DNA harboring a gene or genes coding for the two catalase–peroxidase activities present in *E. coli*.

Materials and methods

Materials

Restriction endonucleases *Pst*I, *Kpn*I, *Cla*I, and *Sph*I were from Boerhinger-Mannheim, while *Hae*III, *Hind*III, *Hinc*II, *Pvu*II, *Bam*HI, *Sal*I, *Hpa*I, *Eco*RI, *Sma*I, *Ba*I, *Bgl*I, *Xba*I, and *Ava*I were from Bethesda Research Laboratories. Agarose and all other chemicals were from Sigma.

Strains

The Clarke and Carbon colony bank (10) is derived from *E. coli* C600 (JA200) which is F^+ *rec*A *trp*Δ *thr leu* and each strain carries a hybrid Col E1 plasmid. The strain JA200/pLC36-19 carries the plasmid pLC36-19 located on plate 36 patch 19 counting from the upper left. *Escherichia coli* strain CSH57a (*ara leu lacY proC purE gal trp his agr*G *mal*A *rps*L *xyl mtl ilv met thi*) was mutagenized with nitrosoguanidine and a catalase-deficient strain UM2 was isolated. The characterization of this strain and other catalase-deficient strains will be described elsewhere but no assayable catalase, representing an activity of less than 0.01 units/mg dry cell weight, was evident in either midlog or stationary phase cells. The minicell producing strain was P678-54 (CGSC 4928 F^- *thr leu*B *min*A *min*B *thi ara lac*Y *gal mal*A *xyl mtl azi rps*L *ton*A *sup*E).

Media

Cultures were grown in liquid medium using LB medium (11) containing 10 g bactotryptone (Difco), 5 g yeast extract (Difco), and 10 g NaCl per litre. LB broth plates containing 12 g agar/L (Difco) were used for screening and selection. Where necessary, 1 unit/mL Colicin E1 (12) was spread on the plates. Minimal agar plates (11) contained 15 g agar, 10.5 g K_2HPO_4, 4.5 g KH_2PO_4, 1 g $(NH_4)_2SO_4$, and 0.5 g sodium citrate per litre of water which was supplemented with 1 m*M* $MgSO_4$ and 0.5 mL of 1% thiamine hydrochloride after autoclaving. Required carbon sources and antibiotics were added as necessary.

Screening of the Clarke and Carbon colony bank

Fresh patches of the 2000 strains in the collection were grown on plates spread with Colicin E1. A drop of 30% hydrogen peroxide was applied to the corner of each patch and the rate of oxygen evolution was monitored visually. Any colonies exhibiting above-average oxygen evolution in comparison with other patches on the same plate were picked for further study. Each of the 88 strains chosen was grown in liquid medium and 2-mL aliquots were assayed for catalase activity in the Gilson oxygraph using a Clark electrode (13). One unit of catalase is defined as the amount of enzyme that breaks down 1 μmol H_2O_2/min.

Transfer of pLC36-19 to P678-54 and catalase-deficient UM2

Minicell-producing strain P678-54 or catalase-deficient UM2 and donor JA200/pLC36-19 were each grown to a cell density of 50 Klett units in LB medium. One-millilitre aliquots of both cultures were mixed and aerated by gentle shaking at 37°C for 1 h. Aliquots of 0.1 mL were plated directly on minimal agar plates supplemented with glucose, streptomycin, Colicin E1, and other nutrients required by P678-54 or UM2. Of 100 streptomycin and Colicin E1 resistant colonies tested for catalase, 53% exhibited catalase activity. One colony from each cross was chosen and labelled P678-54/pLC36-19 or UM2/pLC36-19. The oxygraph assay of extracts for catalase and peroxidase is described in the text.

Visualization of catalase and peroxidase activities on polyacrylamide gels

Catalase and peroxidase activities were visualized according to Gregory and Fridovich (14) on 9% polyacrylamide gels run as described by Davis (15). Staining was as described (14) except that 3 m*M* H_2O_2 (2) was used for catalase. Protein concentrations were determined by the Lowry method (16).

Catalase synthesis in minicells

Minicells were prepared from P678-54 and P678-54/pLC36-19 and the protein synthesized in the minicells was labelled using ^{14}C-labelled amino acids as described by Weiner et al. (17). Catalase synthesis in minicells was determined using the oxygraph following incubation of 0.07–0.09 mg dry cell weight in 1.8 mL of LB medium for various times. As determined by weighing aliquots of minicells dried at 105°C for 48–72 h, 1.0 A_{550} unit of minicells weighed 0.77 mg.

Restriction analysis

Restriction endonuclease reactions were carried out in a final volume of 10 μL. To an appropriate volume of buffer, as specified on the data sheets supplied with the enzymes, was added 0.5 μg of DNA isolated from JA200/pLC36-19 by the method of Birnboim and Doly (18) following chloramphenicol enhancement (19) and 0.5 units of restriction enzyme after which the mixtures were incubated at room temperature for 18 h. For double digestions, both enzymes were present in the incubation. To stop the reaction, 1 μL of 10% Ficoll 400 and 1 μL of a solution containing 0.05% bromphenol blue and 10% sodium dodecyl sulphate was added. DNA fragments were analyzed by electrophoresis on vertical 0.8% agarose slab gels (16 × 16 × 0.15 cm) in a buffer containing 0.1 *M* Tris–borate (pH 7.8) and 2 m*M* sodium – EDTA at a potential of 5 V/cm for 4 h and vertical 8% polyacrylamide gels (16 × 16 × 0.15 cm) run in the same buffer at a potential of 6 V/cm for

TABLE 1. Catalase activity in various cultures before and 30 min after the addition of 0.5 m*M* H_2O_2

Strain	Basal activity (units/mg dry cell weight)	Induced activity (units/mg dry cell weight)
JA200/pLC36-19	8.75	28.68
JA200/pLC37-21	3.73	21.30
JA200/pLC39-13	3.82	17.30
CSH57a	3.42	19.04
UM2	<0.01	<0.01
UM2/pLC36-19	9.80	28.75

6 h. Gels were stained for 30 min in a 5 μg/mL solution of ethidium bromide and photographed under near ultraviolet light. Fragment sizes larger than 1000 base pairs were taken from the agarose gels using *Bam*HI, *Eco*RI, and *Hind*III digests of λ bacteriophage DNA for size standards. Fragment sizes smaller than 1000 base pairs were taken from the polyacrylamide gels using a *Hae*III digest of pBR322 DNA for size standards (20).

Results

Identification of a plasmid carrying the catalase locus

The Clarke and Carbon colony bank (10) was plated and an initial screening for catalase activity was carried out by spotting 30% H_2O_2 on the edge of each colony. Because such things as glucose and metal ions in the medium affect catalase levels (9) such that the same culture streaked on different plates can exhibit widely varying catalase levels, all colonies which exhibited above normal rates of oxygen evolution relative to other colonies on the same plate were chosen. Eighty-eight strains were picked from this initial screening making a second screening necessary. This was carried out by measuring the rate of oxygen evolution from cultures to which hydrogen peroxide was added (Table 1). Only one strain JA200/pLC36-19 exhibited above normal catalase levels, 8–9 units/mg dry cell weight or about two times more than the basal level of 3–4 units/mg dry cell weight observed in other strains in LB medium. In other respects such as the growth rate and cell yield, this strain was similar to other randomly chosen plasmid-carrying strains.

Response of catalase in JA200/pLC36-19 to H_2O_2

Either H_2O_2 added directly or ascorbate, which gives rise to H_2O_2, will cause an induction of catalase synthesis to a level five- to seven-fold over the basal level (2). As presented in Table 1, the basal level of 8.75 units/mg dry cell weight in JA200/pLC36-19 was increased to 28.68 units/mg dry cell weight by the addition of 0.5 m*M* H_2O_2. Ascorbate at 5 m*M* had a similar effect (data not shown). This final, induced level was only threefold higher than the uninduced state, but still higher than the induced levels observed in other plasmid and nonplasmid strains assayed at the same time. This suggested that the catalase gene contained on the plasmid was subject to induction in a manner similar to the chromosomal genes, although some undetermined factor limited the extent of induction.

Visualization of catalase and peroxidase activities on polyacrylamide gels

Crude extracts from JA200/pLC36-19 and JA200/pLC37-21 were electrophoresed on 9% polyacrylamide gels and the catalase and peroxidase activities were visualized. As shown in Fig. 1, the predominant pair of faster-moving catalase activities was present in both strains in approximately the same relative amounts and migrated coincident with peroxidase activities. Because the purified catalase has been shown to possess a peroxidase activity (1), this method of analysis clearly distinguishes and identifies the main catalase activity bands. In addition, there was a third slower moving band of catalase with which there was no associated peroxidase activity. Because of the weak nature of this slower band and the qualitative nature of data from the gels, it was not possible to determine its response to H_2O_2 induction. H_2O_2 did cause a significant increase in the intensity of both the main catalase and peroxidase activities. As shown in Fig. 1, electrophoresis of 260 μg of protein from the uninduced culture of JA200/pLC36-19 yielded much weaker catalase and peroxidase bands than did 243 μg of protein from an H_2O_2-induced culture. This was also true for the culture of JA200/pLC37-21, but the intensities of the bands were lower as would be expected.

Expression of the plasmid-encoded catalase gene in catalase-deficient strains and minicells

To confirm that the plasmid pLC36-19 did in fact harbour a structural gene for catalase and not for a protein which caused an enhancement of chromosomal catalase expression, the plasmid was transferred into a catalase-deficient strain. The isolation and characterization of this strain UM2, derived by nitrosoguanidine mutagenesis of CSH57a, will be described elsewhere, but there was no catalase or peroxidase evident

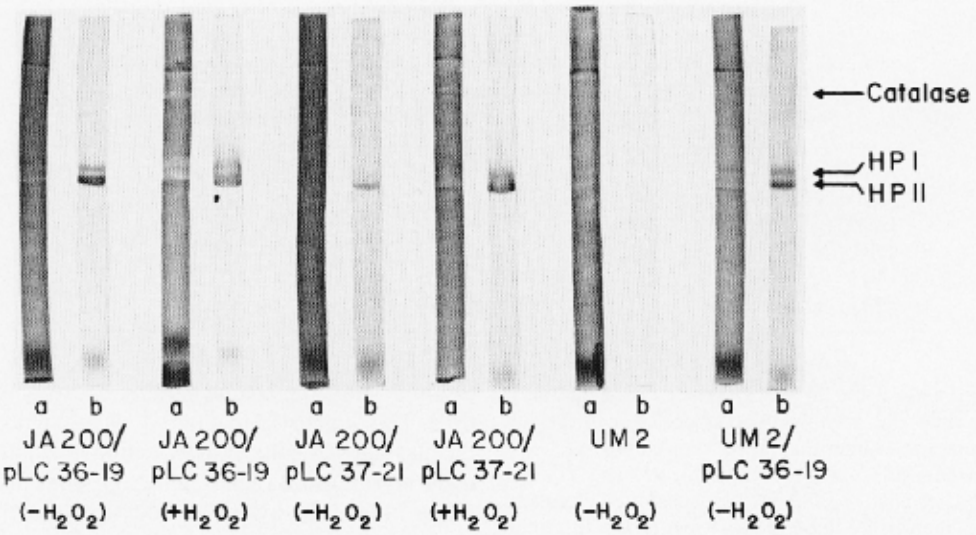

FIG. 1. Visualization of catalase and peroxidase activities on polyacrylamide gels. Crude extracts prepared by sonication were electrophoresed on 9% polyacrylamide gels and stained for either catalase (*a*) or peroxidase (*b*). Samples were taken before H_2O_2 addition ($-H_2O_2$) and 30 min after the addition of 0.5 m*M* H_2O_2 ($+H_2O_2$). The following amounts of protein were electrophoresed: from JA200/pLC36-19 ($-H_2O_2$), 260 μg; from JA200/pLC36-19 ($+H_2O_2$), 243 μg; from JA200/pLC37-21 ($-H_2O_2$), 274 μg; from JA200/pLC37-21 ($+H_2O_2$), 210 μg; from UM2 ($-H_2O_2$), 1760 μg; from UM2/pLC36-19 ($-H_2O_2$), 270 μg.

TABLE 2. Synthesis of catalase in minicells from P678-54 and P678-54/pLC36-19

Time of incubation (min)	Catalase (units/mg dry cell weight)	
	P678-54	P678-54/pLC36-19
0	0.53	1.53
10	0.53	2.02
20	0.57	2.55

following electrophoresis of 1700 μg of protein (Fig. 1) nor was there any assayable catalase in extracts (Table 1). Following the mating, several Colicin E1 and streptomycin resistant colonies were isolated, one of which was characterized as UM2/pLC36-19. The basal level of catalase was similar to that observed in JA200/pLC36-19 and the addition of H_2O_2 caused a similar increase in catalase activity (Table 1). Visualization of the catalase and peroxidase activities following electrophoresis on polyacrylamide gels revealed that the two main catalase–peroxidase activities HPI and HPII were present, but that the slower moving catalase band was absent.

The plasmid pLC36-19 was also transferred to the minicell-producing strain P678-54 and minicells harboring pLC36-19 were assayed for catalase synthesis and the incorporation of ^{14}C-labelled amino acids. A 32.0% increase in the basal level of catalase was observed in pLC36-19-harboring minicells during a 10-min incubation, whereas no increase was observed in minicells without the plasmid (Table 2). Proteins synthesized by minicells in the presence of ^{14}C-labelled amino acids were analyzed on SDS–polyacrylamide gels. As shown in Fig. 2, a new protein band with a molecular weight of 84 000 appeared in the extracts from pLC36-19-containing minicells and this corresponded very well with the published molecular weight of a catalase monomer (1). Therefore, the gene (or genes) coding for the catalase–peroxidase activities is present on pLC36-19 and is expressed normally, whereas the gene for the single catalase activity is not present.

Physical map of pLC36-19

The length of the pLC36-19 plasmid as determined from the sums of various restriction endonuclease products is 25.5 kb of which 6.4 kb (21) is derived from the Col E1 vector and 19.1 kb is inserted DNA. The physical map shown in Fig. 3 was deduced from analysis of the fragments created in single and double digests with different combinations of nucleases summarized in Table 3. The *Pst*I-*Sma*I(*Ava*I) cluster is characteristic of the Col E1 vector segment (21, 22) allowing ready identification of that portion of the plasmid. The additional *Cla*I, *Bal*I, and *Pvu*II sites on the vector were verified by restriction nuclease digestion of 6.4 kb Col

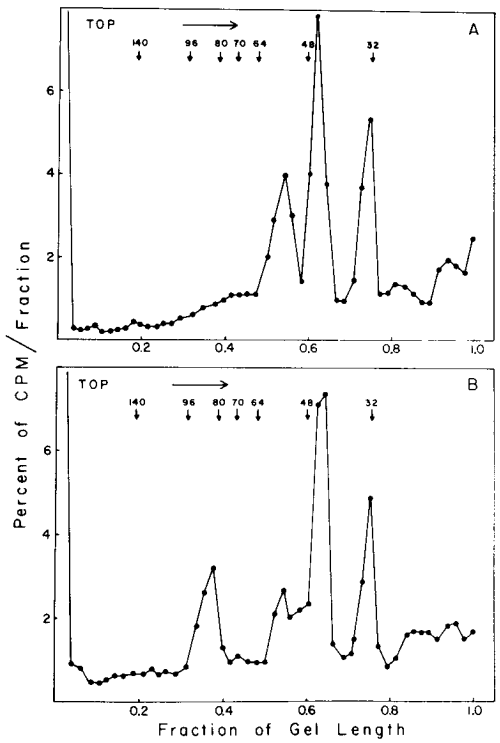

FIG. 2. SDS-polyacrylamide gel electrophoresis of plasmid-coded proteins synthesized in minicells. A 50-μL sample from 200 μL of labelled minicells was applied to the 5% polyacrylamide gel with internal standards of cross-linked hemoglobin (32 000, 48 000, 64 000, 80 000, and 96 000 marked by 32, 48, 64, 80, and 96, respectively, in the figure) or cross-linked hemocyanin (70 000 and 140 000 marked by 70 and 140, respectively, in the figure). The top of the gel is marked by TOP in the figure and migration is in the direction indicated by the large arrow. In A, an extract from minicells without pLC36-19 was run and, in B, an extract from minicells harboring pLC36-19 was run.

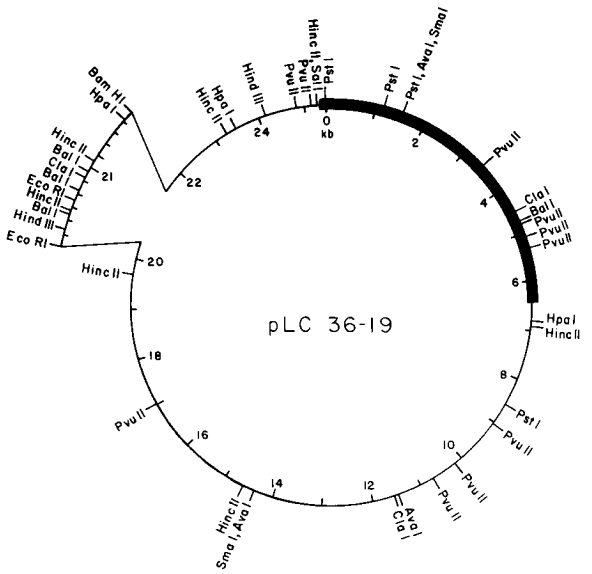

FIG. 3. Restriction endonuclease map of pLC36-19. The map was constructed from fragment sizes determined in single and double digests as tabulated in Tables 3 and 4. The thick line represents the Col E1 cloning vector.

E1 DNA producing the products shown in Table 4. Of the nucleases studied *Bgl*I, *Xba*I, and *Kpn*I did not appear to cleave pLC36-19 and those that cleaved at just one location on the inserted DNA were *Bam*HI, *Sal*I, *Pst*I, and *Sma*I. In all, 38 cleavage sites were oriented on the inserted DNA.

Discussion

A hybrid *E. coli* – Col E1 plasmid pLC36-19 which harbors a gene for catalase has been identified in the Clarke and Carbon colony bank. Unlike the 3- to 10-fold amplification of citrate synthase (21) and fumarate reductase (22) activities observed in strains containing plasmids harboring those genes, there was only a 2- or 3-fold amplification of the total cellular catalase activity in the strain carrying the plasmid-encoded catalase gene. This fact is complicated by the presence of an additional nonplasmid-encoded catalase activity and the actual amplification of the specific plasmid-encoded activity may be greater than fourfold. The level of catalase in a pLC36-19-containing strain which was chromosomally deficient in catalase synthesis was as high as the level in the parent strain with both chromosomal and plasmid catalase synthesis, indicating that greater expression of the plasmid genes is possible even when uninduced, but that there may be a mechanism controlling basal enzyme levels in the cell. A further indication that the plasmid-encoded gene was subject to normal control mechanisms was that H_2O_2 caused a normal induction of enzyme synthesis. Subcloning of the catalase gene component of plasmid pLC36-19 into pBR322 will therefore yield the regulatory regions, as well as the structural genes for catalase.

The main catalase–peroxidase activity in *E. coli*, HPI, which is encoded by pLC36-19 exists as a tetramer with a molecular weight of 337 000, two molecules of protoheme IX and a somewhat unusual amino acid composition. The four apparently identical subunits have a molecular weight of 84 000 making a gene length of at least 2250 base pairs necessary. Clearly, the 19.1-kb insert of chromosomal DNA present in pLC36-19 is sufficient to encode the gene and its regulatory regions, leaving a considerable length of extraneous DNA which must be removed by subcloning.

In *Salmonella typhimurium*, a number of loci

TABLE 3. Restriction endonuclease fragments obtained by digestion of pLC36-19

Restriction endonuclease	Fragment sizes (bp)	Sum (bp)
*Cla*I	9870, 8580, 7080	25 530
*Ava*I	12 600, 10 040, 2730	25 370
*Bam*HI	> 23 700	—
*Bal*I	16 010, 9220, 160, 170	25 560
*Eco*RI	> 23 700, 380	—
*Hinc*II	7900, 7050, 5200, 2050, 1800, 1350, 340	25 690
*Hind*III	21 920, 3640	25 560
*Hpa*I	14 500, 8950, 1900	25 350
*Pst*I	17 260, 7010, 1080, 410	25 760
*Pvu*II	7860, 6150, 3600, 3460, 1170, 1150, 650, 480, 380, 370	25 270
*Sma*I	12 700, 12 700	25 400
*Sal*I	> 23 700	—
*Eco*RI-*Pst*I	12 630, 7010, 4530, 1080, 410, 380	25 660
*Eco*RI-*Hind*III	21 920, 3450, 260	25 630
*Eco*RI-*Bam*HI	> 23 700, 760, 380	—
*Eco*RI-*Sma*I	12 700, 6350, 6040, 380	25 090
*Hind*III-*Pst*I	12 210, 7010, 3640, 1080, 1150, 410	25 600
*Hind*III-*Sal*I	21 000, 3640, 1010	25 650
*Hind*III-*Bam*HI	21 920, 2550, 1050	25 520
*Hpa*I-*Pst*I	12 800, 5400, 2100, 1900, 1350, 1080, 410	25 140
*Hpa*I-*Sma*I	7580, 7080, 5370, 3180, 1900	25 110
*Hpa*I-*Hind*III	13 950, 8080, 1900, 950, 750	25 630
*Hinc*II-*Bam*HI	7900, 7050, 5200, 1800, 1650, 1350, 340, 300	25 590
*Hinc*II-*Eco*RI	7900, 7050, 5200, 2050, 1800, 1060, 330, 300	25 670
*Hinc*II-*Pst*I	6210, 5500, 5200, 2050, 1800, 1550, 1350, 1080, 410, 340	25 490
*Hinc*II-*Bal*I	7900, 5200, 4700, 2350, 2050, 1800, 1270, 160	25 490
*Bal*I-*Sma*I	10 100, 6560, 5600, 2910, 180, 170	25 500
*Bal*I-*Pst*I	12 750, 4620, 3650, 3150, 1080, 410, 170, 160	25 990
*Bal*I-*Hind*III	15 900, 6010, 3300, 160, 170	25 540
*Bam*HI-*Pst*I	13 550, 7010, 3750, 1080, 410	25 790
*Ava*I-*Pst*I	11 020, 7010, 3120, 2730, 1080, 410	25 370
*Ava*I-*Bal*I	7320, 6820, 5870, 3120, 2730, 170, 160	25 940
*Pvu*II-*Pst*I	7860, 6150, 3050, 1640, 1610, 1150, 1100, 1080, 650, 480, 410, 380, 370	25 830
*Pvu*II-*Sma*I	7860, 3460, 3380, 2660, 1820, 1760, 1170, 1150, 650, 480, 380, 370	25 140
*Pvu*II-*Bam*HI	6250, 4650, 3600, 3460, 3050, 1170, 1150, 650, 480, 380, 370	25 210
*Cla*I-*Hind*III	9870, 7080, 5040, 3130, 400	25 520
*Cla*I-*Hinc*II	5200, 4860, 4710, 3060, 2460, 2050, 1800, 1350, 242	25 730
*Cla*I-*Sma*I	7080, 6910, 5740, 2680, 2640	25 230
*Cla*I-*Pst*I	9320, 4540, 3830, 2880, 2850, 1080, 410	24 910

affecting catalase gene expression have been noted, but their roles have not been identified and only one locus was mapped by phage cotransduction with *pro*AB (3). The inconclusiveness of this data coupled with the complete lack of published genetic information relating to catalase in *E. coli* makes it impossible to precisely locate pLC36-19 on the *E. coli* chromosome. Also unconfirmed by published genetic data is the suggestion that there are at least two loci coding for catalase in *E. coli*. This suggestion was made originally on the basis that there appeared to be constitutive and inducible forms of catalase (4) and was corroborated by the subsequent identification of a catalase which was electrophoretically slower than the main catalase–peroxidase activities (2). The absence of the electrophoretically slower catalase in extracts of UM2/pLC36-19 where all catalase is derived from the plasmid shows that pLC36-19 codes only for the catalase–peroxidase activities and that the gene for the electrophoretically slower catalase is unlinked. Data to be published elsewhere confirms this fact by showing that the gene for the electrophoretically slower catalase is located at 38 min on the *E. coli* chromosome being P1 phage cotransducible in the order *pps-phe-pfk*B-*kat* unlinked to the gene for the catalase–peroxidase

TABLE 4. Restriction endonuclease fragments obtained by digestion of Col E1 DNA

Restriction endonuclease	Fragment sizes (bp)	Sum (bp)
*Pst*I	5010, 1080, 410	6500
*Pvu*II	4570, 1160, 380, 370	6480
*Pvu*II-*Bal*I	4570, 1140, 380, 370	6460
*Pvu*II-*Cla*I	4570, 870, 380, 370, 260	6450
*Pvu*II-*Ava*I	2670, 1720, 1160, 380, 370	6320
*Ava*I-*Bal*I	3260, 3070	6270
*Ava*I-*Cla*I	3390, 2880	6270
*Pst*I-*Cla*I	2830, 2230, 1080, 410	6550

activities. Whether there are one or two genes for the catalase–peroxidase pair remains unanswered genetically, although it has been suggested (1, 4) that HPI is a precursor of HPII.

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