NOTES

Homology among bacterial catalase genes

JACEK SWITALA, BARBARA L. TRIGGS-RAINE, AND PETER C. LOEWEN[1]
Department of Microbiology, University of Manitoba, Winnipeg, Man., Canada R3T 2N2

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Catalase activities in crude extracts of exponential and stationary phase cultures of various bacteria were visualized following gel electrophoresis for comparison with the enzymes from *Escherichia coli*. *Citrobacter freundii, Edwardsiella tarda, Enterobacter aerogenes, Klebsiella pneumoniae*, and *Salmonella typhimurium* exhibited patterns of catalase activity similar to *E. coli*, including bifunctional HPI-like bands and a monofunctional HPII-like band. *Proteus mirabilis, Erwinia carotovora*, and *Serratia marcescens* contained a single band of monofunctional catalase with a mobility intermediate between the HPI-like and HPII-like bands. The cloned genes for catalases HPI (*katG*) and HPII (*katE*) from *E. coli* were used as probes in Southern hybridization analyses for homologous sequences in genomic DNA of the same bacteria. *katG* was found to hybridize with fragments from *C. freundii, Ent. aerogenes, Sal. typhimurium*, and *K. pneumoniae* but not at all with *Ed. tarda, P. mirabilis, S. marcesens*, or *Er. carotovora*. *katE* hydridized with *C. freundii* and *K. pneumoniae* DNAs and not with the other bacterial DNAs.

Key words: catalase genes, bacteria, homology.

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Les activités de la catalase dans des extraits bruts de cultures en phases exponentielle et stationnaire de diverses bactéries ont fait l'objet d'examens sur gels électrophorétiques, pour les comparer avec celles des enzymes d'*Escherichia coli*. Les profils d'activités de la catalase chez les bactéries *Citrobacter freundii, Edwardsiella tarda, Enterobacter aerogenes, Klebsiella pneumoniae* et *Salmonella typhimurium* ont été similaires à ceux d'*E. coli*, incluant les bandes bifonctionnelles semblables à HPI et la bande monofonctionnelle ressemblant à HPII. Le *Proteus mirabilis*, l'*Erwinia carotovora* et le *Serratia marcescens* n'ont fourni qu'une bande monofonctionnelle, avec une mobilité intermédiaire entre les bandes ressamblant à HPI et HPII. Des clones des gènes pour les catalases HPI (*katG*) et HPII (*katE*) d'*E. coli* ont été utilisés comme sondes dans des analyses d'hybridation Southern pour les séquences homologues de l'ADN génomique des mêmes bactéries. Le gène *katG* s'est hybridé avec des fragments provenant des bactéries *C. freundii, Ent. aerogenes, Sal typhimurim* et *K. pneumoniae*, mais non avec ceux de l'*Ed. tarda*, le *P. mirabilis*, le *S. marcescens* ou l'*Er carotovora*. Le *katE* s'est hybridé avec les fragments dérivés de *C. freundii* et de *K. peneumoniae*, mais non avec ceux de l'ADN des autres bactéries.

Mots clés : gènes de la catalase, bactéries, homologies.

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Escherichia coli produces two catalases or hydroperoxidases named HPI and HPII (Claiborne and Fridovich 1979; Claiborne *et al.* 1979; Loewen and Switala 1986). In comparison with the typical tetrameric catalases isolated from eucaryotic sources (Deisseroth and Dounce 1970; Jacob and Orme-Johnson 1979; Seah and Kaplan 1973) and some bacteria (Clayton 1959; Herbert and Pinsent 1948; Jouve et al. 1983), HPI and HPII have some unusual properties that make them unique. Whereas the typical catalase is a tetramer of 65-kDa subunits with one protoheme IX group per subunit. HPI is a tetramer of 81-kDa subunits and two protoheme IX groups per tetramer. Furthermore, it is bifunctional, exhibiting both catalase and peroxidase activities (Claiborne and Fridovich 1979). HPII is active as a hexamer of 93-kDa subunits with one group resembling heme *d* per subunit (Loewen and Switala 1986), giving it a green rather than brown color.

The structural genes encoding the subunits of two *E. coli* K12 catalases have been genetically mapped, cloned, and physically characterized. HPI is encoded by *katG*, which maps at 89.2 min on the *E. coli* chromosome (Loewen *et al.* 1985*b*). It has been cloned (Triggs-Raine and Loewen 1987) and sequenced (Triggs-Raine *et al.* 1988), revealing an open reading frame of 2180 bp predicting the sequence of a 726 amino acid protein. *katE* has also been cloned (Mulvey *et al.* 1988) and sequenced (M. Mulvey, I. von Ossowski, and P. Loewen, unpublished results). Both genes are, therefore, available for use as probes in Southern blot analyses of other DNAs for the presence of homologous sequences.

The isolation of catalase genes from other organisms has been the object of work in several laboratories (Okada *et al.* 1987; Bethards *et al.* 1987) and the availability of the *E. coli* catalase genes for use as probes suggested that they might be useful in the search for homologous genes in other organisms. However, the unusual properties of the *E. coli* enzymes indicated that their structures were probably quite different from typical catalases and, as a consequence, the nucleotide sequences of the genes may differ significantly from the sequences of other catalase genes. Preliminary attempts in this laboratory to identify *katG*-homologous DNA in *Bacillus subtilis* and *Pseudomonas aeruginosa* were unsuccessful, suggesting that there was in fact significant divergence in the catalase sequences even among bacteria. The object of this work was to expand these preliminary results and determine whether the *E. coli* catalase genes would be useful for screening other organisms. Bacteria in the

[1]Author to whom all correspondence should be addressed.

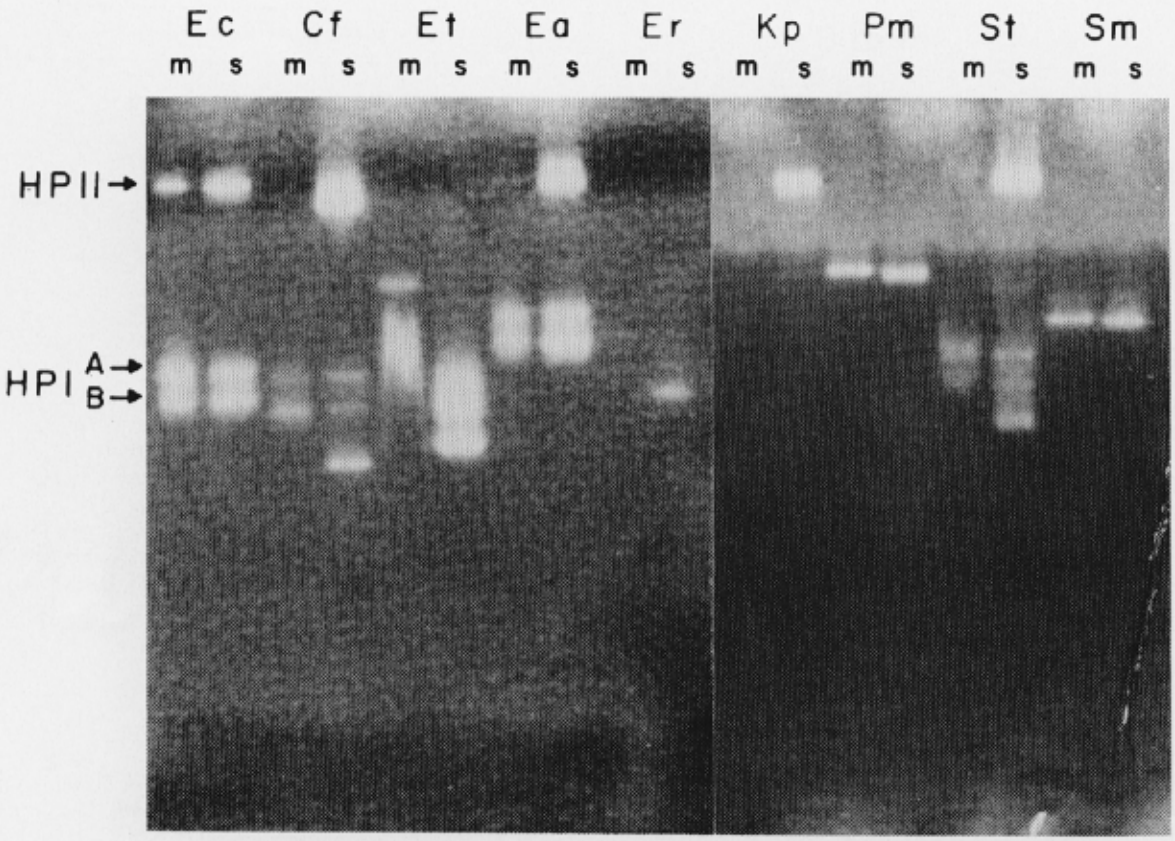

FIG. 1. Visualization of catalase activities in crude extracts of mid log (*m*) and stationary (*s*) phase cultures. The cultures were grown in LB medium (Miller 1972) and extracts containing 1–2 units of catalase (Rorth and Jensen 1967) were separated on 9% nondenaturing gels (Davis 1964) with pH 8.1 Tris-HCl. The gels were stained for catalase (Clare *et al.* 1984), using 20 mM H_2O_2. The extracts of *Er. carotovora* (Er) and mid log phase *K. pneumoniae* (Kp) contained very little catalase, resulting in less than 1 unit being loaded. The locations of HPI A and B and HPII from *E. coli* (Ec) are indicated. The remaining lanes contained extracts from the following bacteria: Cf, *C. freundii*; Et, *Ed. tarda;* Ea, *Ent. aerogenes*; Pm, *P. mirabilis*; St, *Sal. typhimurium;* Sm, *S. marcescens*.

Enterobacteriaceae family were chosen because of the close evolutionary relationship to *E. coli*, which would enhance the possiblity of sequence homology. These included *Klebsiella pneumoniae, Edwardsiella tarda, Salmonella typhimurium* LT2, *Citrobacter freundii* M8B, *Serratia marcescens, Enterobacter aerogenes* ATCC 13048, *Proteus mirabilis*, and *Erwinia carotovora* ATCC 495.

The first step in this study was to identify the types of catalases produced by the various bacteria. This was accomplished by visualization of the catalase activity in crude extracts of the selected strains following electrophoresis on nondenaturing polyacrylamide gels. As shown in Fig 1, *C. freundii, Ent. aerogenes, Ed. tarda*, and *Sal. typhimurium* contained double bands of catalase migrating in close proximity to the double bands of HPI from *E. coli*. These HPI-like bands also exhibited peroxidase activity (stained using the procedure of Gregory and Fridovich (1974); data not shown), confirming their close relationship to HPI. Of the other strains, *Er. carotovora, P. mirabilis*, and *S. marcescens* contained only a single band of monofunctional catalase in both mid log and stationary phase cultures. In the case of *P. mirabilis*, this was consistent with published work (Jouve *et al.* 1983). The extracts of *K. pneumoniae* also contained just one predominant catalase band, which differed from other work showing there to be three different species of catalase, one of which had properties similar to HPI (Goldberg and Hochman 1989*a*). If more than the 1–2 units of enzyme were run on the gel, a double band of bifunctional catalase-peroxidase became evident migrating slightly faster than the *E. coli* HPI. The differences between this and the earlier work of Goldberg and Hochman (1989*a*) may have arisen from the different conditions used for electrophoresis, which can affect the electrophoretic mobility of the enzymes (Loewen *et al.* 1985*b*), and growth, which can affect the amounts of each type of catalase present in extracts (Loewen *et al.* 1985*a*).

The stationary phase extracts of three strains, *C. freundii, Er. tarda*, and *Sal. typhimurium*, also contained a third band of bifunctional catalase–peroxidase migrating slightly faster than the HPI-like bands. Whether this was simply another charge-variant form of the HPI-like protein or a third unique catalase with properties similar to HPI could not be determined from these data. This question will be addressed in the case of *Sal. typhimurium* by studying the proteins encoded by a clone of *katG*.

Of the five strains that contained HPI-like enzyme, only one, *Er. tarda*, did not have a slower migrating band of monofunctional catalase corresponding in electrophoretic mobility to the band of HPII in *E. coli*. Clearly, the slower electrophoretic mobility alone was not enough evidence to characterize the band as being similar to HPII, but the intensity of the band was significantly higher in stationary phase extracts than in mid log phase extracts of *C. freundii, Ent. aerogenes, K. pneumoniae*, and *Sal. typhimurium*, consistent with the increase of HPII observed in *E. coli* during growth into stationary phase (Loewen *et al.* 1985*a*). In the case of *K. pneumoniae*, the slower catalase has been purified and characterized as an atypical catalase (KpA), active as a dimer of 80-kDa subunits (larger than normal

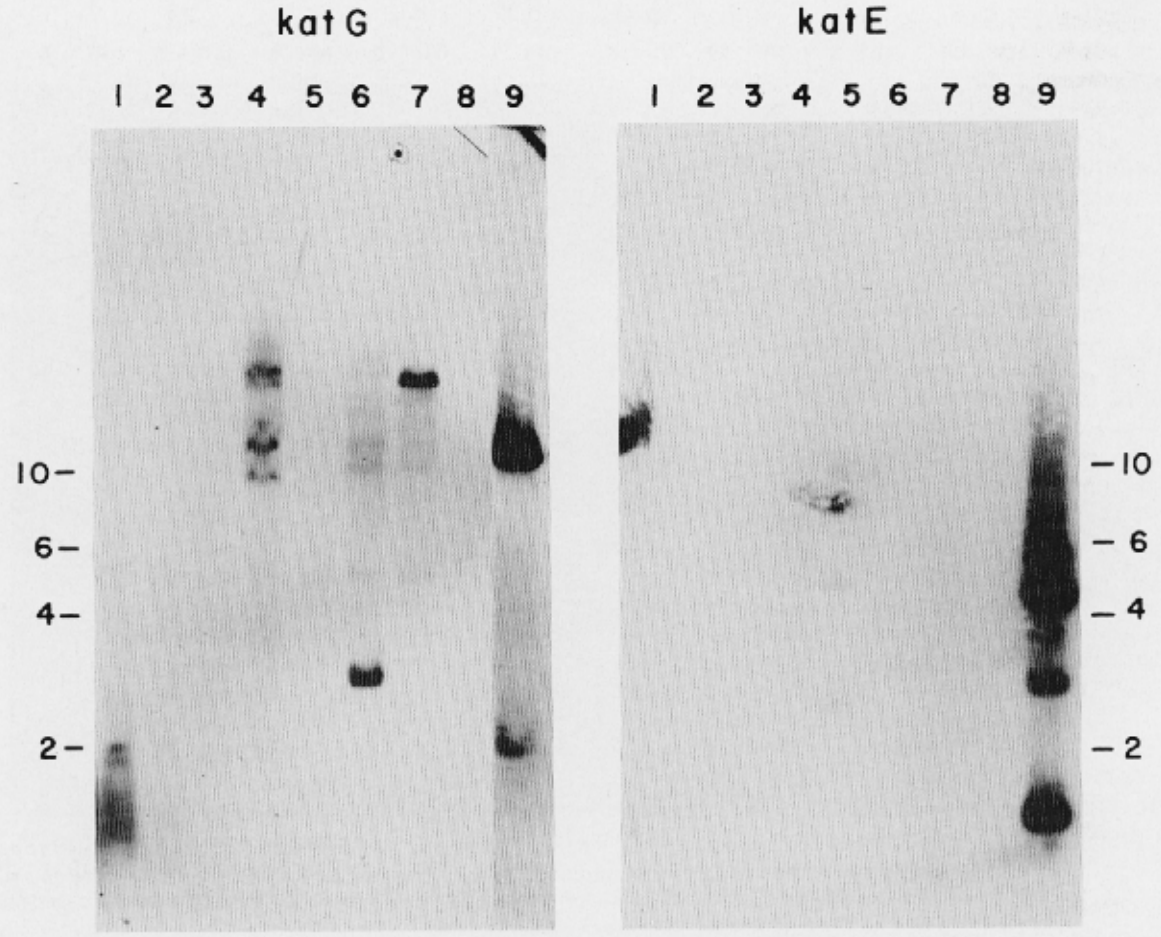

FIG. 2. Southern hybridization analyses of various genomic DNAs using ^{32}P-labelled *katG* (autoradiogram labelled katG) and *katE* (autoradiogram labelled katE) DNAs as probes. DNA from stationary phase cells (Ausubel *et al.* 1989) was digested with *Eco*RV nuclease (Pharmacia), blotted to nylon filters following electrophoresis on agarose gels, and probed at 65°C with nick-translated pBT22 DNA (*katG*) or pAMkatE6 (*katE*) (Maniatis *et al.* 1982). The numbered lanes contained the genomic DNAs from the following bacteria digested with the restriction endonuclease *Eco*RV: 1, *K. pneumoniae*; 2, *S. marcescens*; 3, *P. mirabilis*; 4, *C. freundii*; 5, *Er. carotovora*; 6, *Ent. aerogenes*; 7, *Sal. typhimurium*; 8, *Ed. tarda*; 9, *E. coli*. The locations and sizes (in kilobase pairs) of size markers are indicated along the sides of the autoradiograms.

but still smaller than the 93-kDa size of HPII subunits), exhibiting an absorbance spectrum similar to that of HPII (Goldberg and Hochman 1989*b*). Consequently, it has only limited structural resemblance to HPII despite the similarity in expression. Nucleic acid hybridization would be useful in determining whether any homology exists among the genes for the electrophoretically slower enzymes.

The second step in this study was to investigate homology between the *E. coli* catalase genes and catalase genes in the other bacteria. The plasmid pBT22 contains *katG* and its regulatory regions as well as 1.2 kb of adjacent DNA, which lacks an open reading frame. It was used as a probe for *katG* homologous DNA in Southern blot analyses of restriction nuclease digests of genomic DNAs from the various strains (Fig. 2). Four strains, *Sal. typhimurium*, *Ent. aerogenes*, *C. freundii*, and *K. pneumoniae*, showed some homology with the *E. coli* gene. Of the strains containing bifunctional catalase– peroxidase bands, only *Ed. tarda* did not hybridize with the *katG* probe. The *Eco*RV digest of *Sal. typhimurium* and *Ent. aerogenes* contained the homologous sequence on one fragment, whereas *C. freundii* and *K. pneumoniae* digests had the homologous sequences on two or more fragments. The exposure time to visualize the bands on X-ray film was 24 h for all of the lanes except the *E. coli* control, run on the same gel, which was exposed for only 4 h, suggesting relatively poor homology. This is consistent with the partial sequence of the *Sal. typhimurium katG* gene (Stauffer and Stauffer 1988), which is 84% homologous with the corresponding part of the *E. coli* gene. The intercistronic regions are significantly different both in size (164 bp for *Sal. typhimurium* and 328 bp for *E. coli*) and sequence (67% homology on 164 bp).

katG contains two *Eco*RV nuclease sites, which should give rise to three bands hybridizing with pBT22, a large one extending beyond the 3′ end of the gene, a 1570-bp fragment from the middle of the gene, and a 750-bp fragment containing just 18 bp from the 5′ end of the *katG*-coding region. Because this smallest band would have hybridized only very weakly with the probe and may have been lost from the bottom of he gel, the presence of just two bands, 1.7 and >10 kb, in the control blot is as expected.

The plasmid pAMkatE6, containing *katE* in addition to approximately 1.6 kb of adjacent DNA, was used as a probe for *katE*-homologous DNA in Southern blot analyses of restriction nuclease digests of genomic DNAs from the various strains. Unlike *katG* there was little hybridization with any of the DNAs except with *C. freundii*, which exhibited two faint bands, and

K. pneumoniae, which exhibited one strong band. An amount of DNA equivalent to that used for the *katG* probes had been run on the gel, indicating that the *katE*-equivalent sequences were not as highly conserved as the *katG* sequences and that HPII from *E. coli* was quite different from most of the monofunctional enzymes observed in Fig. 1. *katE* contains two *Eco*RV nuclease sites, which should give rise to three bands in the control blot, two bands of unknown length extending beyond the ends of the gene and a 2.5-kb band from the middle of the gene. The three predominant bands of 1.5, 2.7, and 4.5 kb in the control blot are consistent with this prediction, while the weaker large bands can be attributed to partial digestion.

In summary, *katG* from *E. coli* will serve as a good probe for the identification and cloning of similar bifunctional catalase–peroxidase genes present in the *Enterobacteriaceae* family and possibly other strains such as *Rhodopseudomonas capsulata*, where a bifunctional enzyme has been found (Hochman and Shemesh 1987). *katE* may also be useful for the identification of genes encoding the monofunctional catalases despite more limited homology among the genes for this type of catalase.

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