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Biochemical and Genetic Analyses of a Catalase from the Anaerobic Bacterium *Bacteroides fragilis*

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A single catalase enzyme was produced by the anaerobic bacterium *Bacteroides fragilis* when cultures at late log phase were shifted to aerobic conditions. In anaerobic conditions, catalase activity was detected in stationary-phase cultures, indicating that not only oxygen exposure but also starvation may affect the production of this antioxidant enzyme. The purified enzyme showed a peroxidatic activity when pyrogallol was used as an electron donor. It is a hemoprotein containing one heme molecule per holomer and has an estimated molecular weight of 124,000 to 130,000. The catalase gene was cloned by screening a *B. fragilis* library for complementation of catalase activity in an *Escherichia coli* catalase mutant (*katE katG*) strain. The cloned gene, designated *katB*, encoded a catalase enzyme with electrophoretic mobility identical to that of the purified protein from the *B. fragilis* parental strain. The nucleotide sequence of *katB* revealed a 1,461-bp open reading frame for a protein with 486 amino acids and a predicted molecular weight of 55,905. This result was very close to the 60,000 Da determined by denaturing sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the purified catalase and indicates that the native enzyme is composed of two identical subunits. The N-terminal amino acid sequence of the purified catalase obtained by Edman degradation confirmed that it is a product of *katB*. The amino acid sequence of KatB showed high similarity to *Haemophilus influenzae* HktE (71.6% identity, 66% nucleotide identity), as well as to gram-positive bacterial and mammalian catalases. No similarities to bacterial catalase-peroxidase-type enzymes were found. The active-site residues, proximal and distal heme-binding ligands, and NADPH-binding residues of the bovine liver catalase-type enzyme were highly conserved in *B. fragilis* KatB.

In aerobic conditions, aerobic and facultative organisms preferentially utilize oxygen as a terminal electron acceptor (45). As a consequence of the partial reduction of oxygen to water during microbial respiration, the reactive oxygen species superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical ($OH^\cdot$) are formed as intermediate compounds (20, 28). In addition, superoxide anion and hydrogen peroxide may also be generated by autoxidation of dehydrogenases, catechols, thiols, flavins, and oxidases and by UV radiation (14). Moreover, in the Fenton reaction, free hydrogen peroxide readily reacts with available metal transitions such as ferrous iron to form a more powerful and highly reactive oxidant, hydroxyl radical (28, 57). Oxygen radicals are implicated in damage to membrane lipids, proteins, and DNA (33, 66).

Microorganisms have developed efficient enzymatic and nonenzymatic mechanisms to eliminate the oxygen by-products along with synthesis of DNA repair enzymes, metabolic enzymes, and oxidative defense regulators (66). Toxic O_2^- is eliminated by dismutation to H_2O_2 by superoxide dismutase (20), and accumulation of toxic H_2O_2 is prevented by the action of catalases and peroxidases (29).

On the other hand, in anaerobic conditions, generation of reactive oxygen species is thought not to be a problem because of the absence of oxygen, and the lack of protective mechanisms against oxygen toxicity in anaerobic bacteria was seen as an explanation for their sensitivity upon oxygen exposure (45). Many studies have shown that anaerobic bacteria are not uniformly sensitive to oxygen, and there is a broad range of oxygen tolerance from species extremely sensitive to oxygen to those that are able to remain viable in the presence of oxygen for extended periods of time (16, 45). It is believed that in some anaerobic bacteria, as in aerobic organisms, the presence of superoxide dismutase and catalase plays a role in the detoxification of oxygen by-products (45).

Among the anaerobic bacteria, the opportunistic pathogen *Bacteroides fragilis* is one of the most aerotolerant species, and this aerotolerance may prove to be an important virulence factor. This is supported by the fact that clinical isolates of *B. fragilis* are more resistant to oxygen exposure than are fecal strains (56). The former strains were able to survive in the presence of oxygen for 48 (56) to 72 (67) h, while the latter totally lost viability after 4 h of oxygen exposure (56). These results suggest that a system directly related to oxygen tolerance may exist, and in fact, both superoxide dismutase and catalase have been found in *B. fragilis* (22, 74).

Catalases are among the oxidative stress-starvation response proteins that have been extensively studied in aerobic bacteria, but much less is known about the enzymes in anaerobic organisms (39). Catalase has been used as a biochemical tool for differential identification of *Bacteroides* species, and some researchers have suggested exposure of the bacterial cells to air before performance of the test (32), indicating that activity is induced upon contact with oxygen. In contrast, Wilkins et al. (74) detected catalase activity in anaerobic growth without oxygen exposure.

In this report, we describe the isolation, cloning, and nucleotide sequence of *B. fragilis* catalase. Thus, this work is the first in a series of molecular studies aimed towards understanding the regulatory mechanism(s) that controls the enzymes responsible for aerotolerance in anaerobic bacteria.

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MATERIALS AND METHODS

Strains and growth conditions. *B. fragilis* 638 (54) was grown routinely in supplemented brain heart infusion broth (Difco Laboratories, Detroit, Mich.) (63) in an anaerobic chamber at 37°C. Menadione was omitted from the medium. The catalase-deficient mutant *Escherichia coli* UM255 (*pro leu rpsL hsdM hsdR endI lacY katG2 katE12*::Tn*10 recA*), kindly provided by P. C. Loewen, University of Manitoba, Winnipeg, Manitoba, Canada (47), and *E. coli* DH5α were grown aerobically in Luria-Bertani medium with 50 μg of ampicillin per ml when required.

Isolation and purification of catalase. *B. fragilis* was grown anaerobically in supplemented brain heart infusion broth (63) to the late log growth phase (optical density at 550 nm, approximately 0.7 to 0.8) and then aerated by shaking in air at 250 rpm for 1 h at 37°C. A cell pellet was obtained by centrifugation at 6,000 × *g* for 15 min at 4°C and washed twice with TSD buffer, composed of 50 mM Tris-HCl (pH 8.0), 150 mM NaCl, and 100 μM dithiothreitol. The following procedures were carried out at 4°C. The cell pellet was suspended in TSD buffer containing 40 μg of RNase A per ml, 50 μg of DNase I per ml, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, and 1 μg of pepstatin A per ml. The cells were disrupted by two passages through a French pressure cell (American Instrument Co., Silver Spring, Md.) at 16,000 lb/in^2. After centrifugation at 34,000 × *g* for 30 min, the clarified supernatant was dialyzed against 50 mM Tris-HCl–100 μM dithiothreitol (pH 8.0) and applied to a DEAE-agarose (Bio-Rad, Hercules, Calif.) column equilibrated with the same buffer. Proteins were eluted with a 0 to 0.5 M NaCl gradient in the equilibrium buffer, and fractions containing catalase activity were pooled and concentrated by membrane filtration. The concentrated sample was subjected to 7.5% nondenaturing polyacrylamide gel electrophoresis (PAGE) and electroeluted at 40 mA of constant current with a Prep-Cell 491 (Bio-Rad Laboratories, Inc., Melville, N.Y.). Fractions containing catalase activity were pooled and concentrated. This sample was electroeluted a second time as described above, except that an 8.5% polyacrylamide gel was used. Fractions containing catalase activity and a single protein band following PAGE were pooled and concentrated. Protein concentration was determined by the Bradford method (7) using lysozyme as the standard.

Catalase determination. Catalase activity was measured spectrophotometrically as described by Aebi (1). Decomposition of H_2O_2 was monitored at 240 nm by using E = 0.04 μmol/cm^2 (51). One unit of catalase is the amount of enzyme which decomposes 1 μmol of H_2O_2 per min at 25°C.

PAGE. Nondenaturing PAGE and denaturing sodium dodecyl sulfate (SDS)-PAGE were performed as described by Laemmli (37). Following electrophoresis, proteins were detected by staining the gel with Coomassie blue R250.

Visualization of catalase activity on nondenaturing PAGE was performed by the ferric chloride-potassium ferricyanide method of Woodbury et al. (75). Peroxidase activity was detected by incubating gels with one of the following solutions: (i) 40 mM pyrogallol and 7.4 mM H_2O_2 in 50 mM sodium-potassium phosphate buffer, pH 6.0 (43); (ii) 2.8 mM diaminobenzidine and 20 mM H_2O_2 in 50 mM sodium-potassium phosphate buffer, pH 7.0 (24); or (iii) 0.1 mM guaiacol and 1 mM H_2O_2 in 15 mM sodium phosphate buffer, pH 6.0 (61). Bovine liver catalase and horseradish peroxidase were used as standards.

Molecular weight determination. The molecular weight of the native protein was estimated by two methods. One was the PAGE method of Hedrick and Smith (30). Briefly, the 100 × log (R_f × 100) values of the standard proteins' relative migration were determined for several different acrylamyde gel concentrations. The negative slope value obtained for each protein was plotted against its respective molecular weight. The second method was high-performance liquid chromatography (HPLC; Beckman Instruments Inc.) with a Hydropore-5-SEC (Rainin Instrument Co., Inc.) size exclusion column equilibrated with 0.1 M potassium phosphate, pH 7.0. Subunit molecular weight was obtained following denaturing SDS-PAGE.

Spectrum absorption of the catalase. Determination of the UV and visible spectra of the native enzyme in 50 mM Tris-HCl–150 mM NaCl (pH 8.0) was carried out with a DU-65 spectrophotometer. The pyridine hemochrome spectra of the catalase were measured in aqueous alkaline pyridine solution (13), and the reduced form of the protein was obtained by addition of 2 mM sodium dithionite. Heme content of the protein was determined by pyridine-NaOH (13). Bovine hemin (Sigma) was used as the standard.

N-terminal amino acid sequence. The diisothiocyanate-coupled polypeptide was subjected to fully automated solid-phase Edman degradation on a Milligen-Biosearch 6625 Prosequencer (Millipore Corp., Marlborough, Mass.) (2).

DNA preparation and analysis. Routine DNA manipulations were carried out in accordance with standard procedures (3, 58). Small-scale DNA plasmid preparations for screening were carried out by the alkaline lysis method (4). Plasmid deletion clones for nucleotide sequencing were purified with a Qiagen Plasmid Mini kit (Qiagen Inc., Chatsworth, Calif.).

Southern hybridization analysis was performed with a nylon membrane (Hybond-N; Amersham Corp., Arlington Heights, Ill.), and the DNA probe was labelled with ^{32}P by random primer reaction with a commercial kit (Pharmacia LKB, Inc., Piscataway, N.J.). Hybridization was performed overnight at 65°C with 5× SSC (1× SSC is 0.015 M NaCl plus 0.015 M sodium citrate)–5× Denhardt's solution–1% SDS–20 mM sodium PP_i–100 μg of fragmented salmon sperm DNA per ml. Probed nylon membranes were washed twice in 2× SSC plus 0.1% SDS for 1 h each time and twice in 0.1× SSC plus 0.1% SDS for 1 h each time at 52°C.

TABLE 1. Determination of catalase activity in *B. fragilis* and transformed *E. coli*[a]

Bacterial strain	Catalase activity of (U/mg of protein)	
	With O_2	Without O_2
B. fragilis 638	35.6[b]	<0.02[c]
E. coli UM-255(pFD567)	494.0	NT[d]
E. coli UM-255(pUC19)	<0.08	NT

[a] Chloramphenicol (100 μg/ml) was added to the cultures just before the cells were harvested.
[b] Bacterial cells were exposed to oxygen for 1 h at 37°C.
[c] Cells were not exposed to oxygen prior to harvesting.
[d] NT, not tested.

Cloning of catalase. Chromosomal DNA from *B. fragilis* 638 was partially restricted with *Sau*3AI, and fragments between 5 and 15 kb were collected by size fractionation on neutral sucrose gradients (3). A genomic library was constructed by ligating the *Sau*3AI fragments into the unique *Bgl*II site of a positive selector, pEcoR251 (65). *E. coli* UM255 was transformed by electroporation (12), and transformants were selected on Luria-Bertani agar containing 50 μg of ampicillin per ml. Colonies were screened for catalase activity by applying a 3% H_2O_2 solution to each agar plate and looking for the appearance of bubbles. Potential catalase-positive colonies were immediately streaked onto a new plate.

Production of nested deletions and DNA sequencing. Two catalase subclones, pFD567 and pFD568, bearing a 2.7-kb *Eco*RI-*Eco*RI fragment in both orientations were purified by CsCl density gradient centrifugation and restricted with *Sal*I. The ends of the DNA were filled in with α-phosphorothioate deoxynucleotides by using the Klenow fragment (55). The thio-protected plasmid DNAs were subsequently restricted with *Sma*I, treated with exonuclease III and S1 nuclease to obtain unidiretional deletions, ligated, and transformed into *E. coli* UM255 (31). Transformants containing suitable-size deletions were identified and then sequenced with the reverse sequencing primer of pUC19. Sequencing was performed on double-stranded DNA templates by the dideoxy-chain termination reaction (59) with modified T7 polymerase (Sequenase 2.0; U.S. Biochemical Corp., Cleveland, Ohio). Five additional primers based on available sequence information were used to fill gaps in the existing sequence.

DNA sequence and database comparison. Computer analysis of nucleotide and amino acid sequence data was performed with the University of Wisconsin Genetics Computer Group DNA sequence analysis software (10). Phylogenetic relationships were inferred by the parsimony method from a multiple amino acid sequence alignment generated by Pileup with the PHYLIP phylogeny inference package (version 3.5), and a consensus tree was constructed from 100 bootstrap replications.

Nucleotide sequence accession numbers. The nucleotide sequence of *B. fragilis katB* has been submitted to the GenBank database and assigned accession number U18676. Other sequences used for the analysis (together with their designations) and their GenBank accession numbers are as follows: *B. firmus katA* (Bacfi), M74194; *B. stearothermophilus perA* (Bacst), M29876 (44); *B. subtilis* vegetative catalase *kat-19*$^+$ (Bacsu), M80796 (6); *E. coli* hydroperoxidase I (Ecolihpi), M21516 (68); *E. coli* hydroperoxidase II (Ecolihpii), M55161 (70); *Haemophilus influenzae* catalase *hktE* (Hiu), U02682, (5); *Lactobacillus sake katA* (Lacsk), M84015 (35); *Listeria seeligeri* (Lisee), M75944 (27); *Micrococcus luteus* (Miclu), P29422 (48); *Mycobacterium intracellulare* catalase-peroxidase (Mycit), Q04657 (46); *M. tuberculosis* catalase-peroxidase (Myctb), S42739 (76); *Pseudomonas seringae catF* catalase isozyme (Psesyr), U03465; *Rhodobacter capsulatus cpeA* catalase-peroxidase (Rpsecap), X71420 (19); *Salmonella typhimurium* hydroperoxidase I (saltyhpi), P17750 (40); *Streptomyces venezuelae* bromoperoxidase-catalase (Strve), P33569; *Penicillium vitale* catalase (Pencil), P11934 (69); bovine liver catalase (BLC), P00432 (60).

RESULTS

Catalase activity in crude extracts of late-log-phase *B. fragilis* exposed to oxygen was significantly higher than in cells maintained in anaerobic conditions (Table 1). The anaerobic activity was less than 0.02 U/mg of protein when chloramphenicol was added to the culture prior to harvesting. Without the addition of chloramphenicol, anaerobic catalase values ranged up to 1.4 U/mg of protein (data not shown). In contrast, after aeration for 1 h, catalase activity increased to 35.6 U/mg of

TABLE 2. Purification of *B. fragilis* catalase from 46.1 g of wet cells exposed to oxygen for 1 h

Fraction	Vol (ml)	Protein concn (mg/ml)	Total protein (mg)	Activity (U/ml)	Total U	Sp act (U/mg)	Fold purification	Enzyme yield (%)
Crude extract	90	35.6	3,200	725	65,200	20.4	1	100
DEAE-agarose	4	51.6	206	2,900	11,600	56	2.7	17.8
Electroelution (7%)	0.87	2.4	2.1	12,900	11,200	5,400	264	17.2
Electroelution (8.5%)	0.4	1.2	0.48	9,400	3,800	8,000	392	5.8

protein. Late-stationary-phase cells also had an increased catalase activity of up to 6 U/mg of protein.

To investigate whether exogenous peroxide could induce catalase activity, 20, 50, 100, 200, 500, and 1,000 μM hydrogen peroxide was added to late-log-phase cells at 1-h intervals. Samples analyzed during the course of hydrogen peroxide addition showed no significant increase in catalase activity (data not shown).

Purification of catalase. A rapid purification strategy using ion-exchange chromatography and electroelution from polyacrylamide gels provided a satisfactory yield of catalase and avoided longer enzyme manipulations. The results of this purification procedure are summarized in Table 2. The purified native catalase from the bacterial crude extract on nondenaturing PAGE is shown in Fig. 1A.

Following PAGE, a single catalase activity component was detected in crude extracts of *B. fragilis* cells exposed to oxygen but not in cells maintained anaerobically (Fig. 1B). Moreover, the purified catalase exhibited an electrophoretic mobility identical to that of the catalase from crude extracts. In addition, stationary-phase cell extract also showed the same electrophoretic pattern (data not shown), indicating that in both conditions, the catalase activity is probably due to a single enzyme.

To investigate if the purified catalase also has peroxidatic activity, native polyacrylamide gels of the purified protein were soaked in the presence of organic electron donor substrates and examined for peroxidase activity (Fig. 2). A strong peroxidase activity towards pyrogallol was detected, as shown in Fig. 2A. A very faint band of activity also was observed when diaminobenzidine was used (Fig. 2B). However, no activity was detected towards guaiacol (Fig. 2C) or when a mixture of 4-aminoantipyrine and 2,4-dichlorophenol was used as the substrate (data not shown).

Following denaturing SDS-PAGE of the purified enzyme, a major polypeptide band with an estimated molecular weight of 60,000 was observed. The purity of the isolated protein was determined by scanning the stained gel, and results showed that the catalase polypeptide was approximately 96% pure. This preparation was subjected to N-terminal amino acid analysis by Edman degradation. The first 20 amino acids were found to be MENKTLTAANGRPIADNQNS.

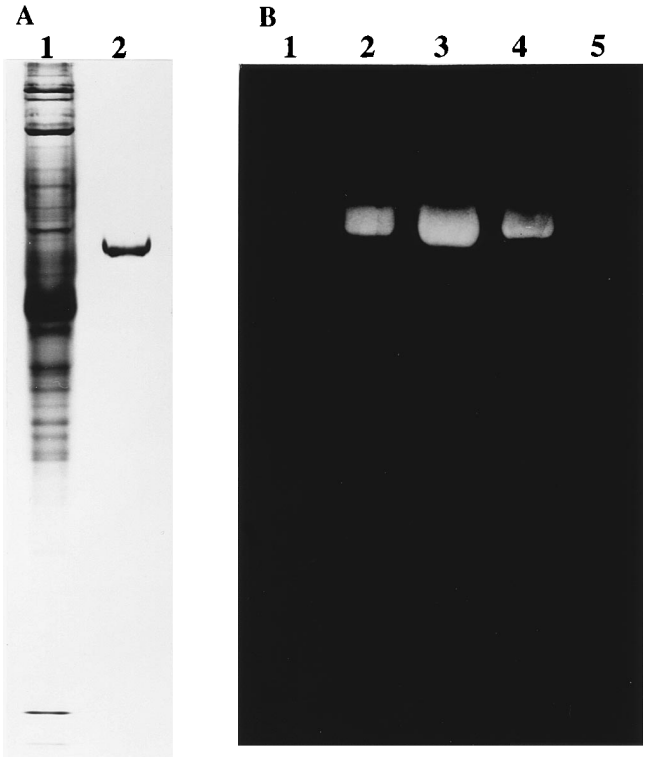

FIG. 1. Nondenaturing PAGE (7.5 to 20% acrylamide exponential gradient). (A) Gel stained for proteins. Lanes: 1, 100 μg of proteins from a crude extract of *B. fragilis* exposed to oxygen for 1 h; 2, 15 μg of purified *B. fragilis* catalase. (B) Gel stained for catalase activity. Lanes: 1, 56 μg of proteins from a crude extract of *B. fragilis* not exposed to O_2; 2, 56 μg of proteins from a crude extract of *B. fragilis* exposed to O_2 for 1 h; 3, 1.8 μg of purified *B. fragilis* catalase; 4, 5 μg of proteins from a crude extract of *E. coli* UM255 transformed with pFD567 containing the *B. fragilis* catalase gene; 5, 5 μg of proteins from a crude extract of *E. coli* UM255 transformed with pUC19.

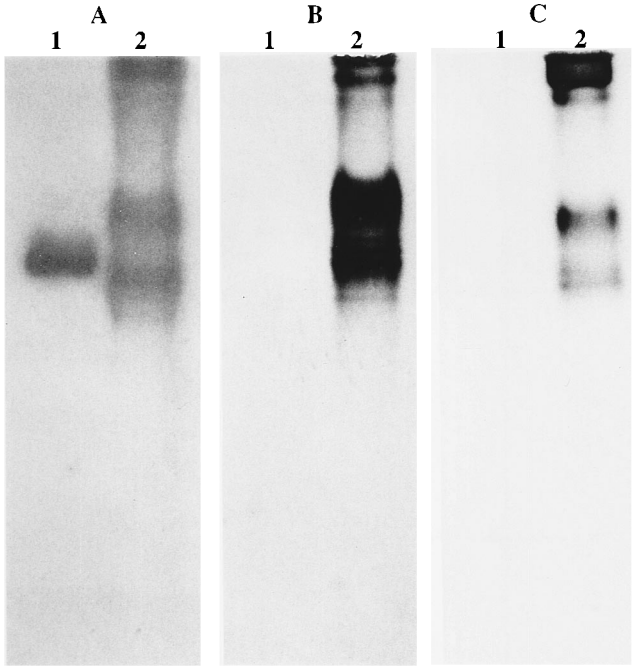

FIG. 2. Nondenaturing PAGE (7.5 to 20% acrylamide exponential gradient). The gel was stained for peroxidase activity as follows: A, pyrogallol; B, diaminobenzidine; C, guaiacol. Lanes: 1, 2 U of *B. fragilis* catalase; 2, 2 U of horseradish peroxidase isoenzymes.

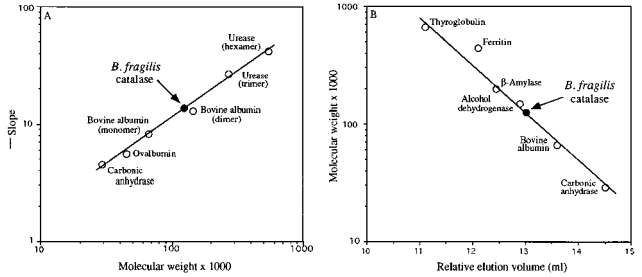

FIG. 3. Determination of native molecular weight. (A) Nondenaturing PAGE. The $100 \times \log (R_f \times 100)$ value of each protein was plotted against the percent gel concentration, and the negative slope was obtained for each protein. (B) Gel exclusion HPLC.

The native protein molecular weight was estimated to be approximately 124,000 by nondenaturing PAGE (Fig. 3A). A similar molecular weight of approximately 130,000 was determined by size exclusion HPLC (Fig. 3B). Thus, the enzyme holomer is composed of two identical subunits.

The spectrum of the native catalase from *B. fragilis* is shown in Fig. 4. A Soret band at 404 nm and minor peaks at 498, 552, 575, and 623 nm were present. Addition of 2 mM sodium dithionite caused a decrease but not a shift in the Soret band and disappearance of the bands in the visible region but the appearance of a peak at 635 nm. The reduced pyridine hemochrome exhibited a shift in the Soret band from 410 to 417 nm (Fig. 4, insert). The spectrum in the visible region of the protoheme also shows two peaks at 521 and 554 nm, indicating that the hemochrome complex has an absorption spectrum similar to that of protoheme IX (13). However, it yielded two minor peaks at 503 and 579 nm which are present in both the oxidized and reduced forms. On the basis of the heme content determined as pyridine hemochrome, it was estimated to be one protoheme IX molecule per dimer of the catalase. This low heme content correlates with the relatively low A_{404}/A_{280} ratio of 0.7.

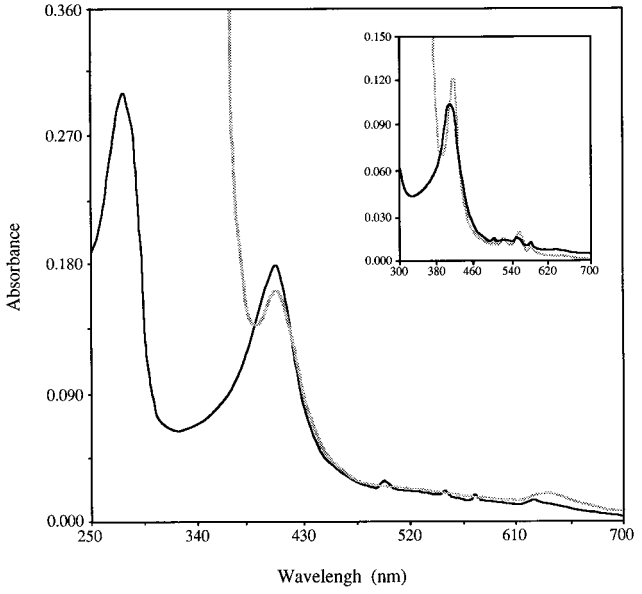

FIG. 4. Absorption spectra of 1.2 μM *B. fragilis* catalase in 50 mM Tris-HCl (pH 8.0)–150 mM NaCl. The insert shows the pyridine hemochromogen complex absorption. The oxidized (——) and reduced (· · · · · ·) forms of the enzyme with 2 mM sodium dithionite were used.

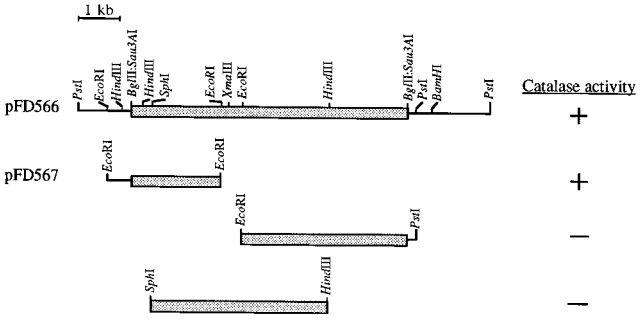

FIG. 5. Restriction endonuclease map of the *B. fragilis* 638 chromosomal DNA fragment, subclones of this fragment, and their ability to complement catalase activity in *E. coli* UM255. pFD566 is the original 6.6-kb fragment cloned into the *Bgl*II site of pEcoR251. All subclones were cloned into the multiple polycloning site of pUC19.

Cloning of catalase. The gene library of *B. fragilis* 638 was screened in *E. coli* UM255, and several transformants able to decompose hydrogen peroxide were observed. Plasmid DNA from one of these clones, pFD566, restored catalase activity when transformed into naive *E. coli* UM255, confirming that expression of catalase was due to the presence of the plasmid. Restriction analysis of pFD566 showed that it contained a 6.6-kb insert DNA fragment (Fig. 5).

Restriction fragments of pFD566 were subcloned into pUC19, and each construct was tested for the ability to restore catalase activity in *E. coli* UM255 (Fig. 5). The construct pFD567 conferred catalase activity, suggesting that the catalase gene was located within a 2.7-kb *Eco*RI fragment. The cloned catalase gene, containing the λ P_R promoter, was highly expressed in the host strain *E. coli* UM255, producing 494 U/mg of protein, while the strains tranformed with the host vector pUC19 alone showed a much lower activity of <0.08 U/mg of protein (Table 1). Furthermore, the cloned gene expressed in *E. coli* UM255 also showed electrophoretic mobility identical to that of purified catalase. This strongly indicates that the cloned catalase gene from *B. fragilis* corresponds to the same enzyme detected and purified from the parental strain (Fig. 1B). The catalase gene located on pFD567 is designated *katB*.

Nucleotide sequence of *katB*. The strategy for sequencing the 2.1-kb *katB* region is shown in Fig. 6. The 1,956 bp of the *B. fragilis* nucleotide sequence extended from the *Bgl*II-*Sau*3AI site to the *Eco*RI site and revealed a single open reading frame of 1,461 bp (nucleotides 217 to 1677; Fig. 7). This open reading frame encodes a protein of 486 amino acids with a predicted molecular mass of 55,905 Da. Comparison of the N-terminal sequence of purified KatB to the *katB* open reading frame revealed a 19-of-120 match. Thus, the identities of the gene and gene product were confirmed.

The deletion clones used for sequencing were examined for catalase activity in *E. coli*, and it was found that removal of nucleotides 1 to 64 had no effect on activity (clone R3-11), but when nucleotides 1 to 281 were deleted, removing 22 codons from the N terminus of *katB* (clone R5-24), catalase activity was no longer detected (Fig. 6 and 7). Deletion of nucleotides 1668 to 1956, containing three codons, from the C-terminal region did not affect activity (clone D1-1). However, catalase activity was lost when the last 21 codons for the C terminus (nucleotides 1612 to 1956) were deleted (clone D2-7).

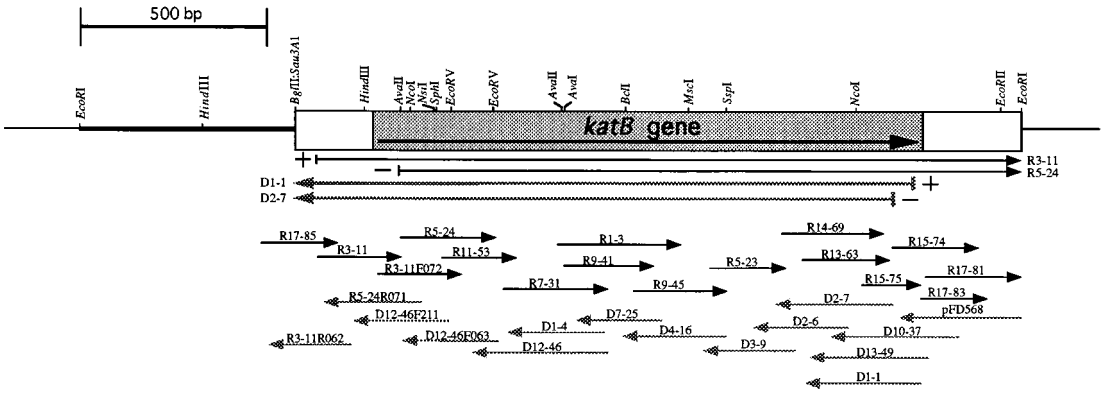

FIG. 6. DNA sequencing strategy used for the *B. fragilis katB* region. A partial restriction endonuclease map of the sequenced gene is indicated. The *katB* open reading frame is depicted as a shaded box. The arrow in the box indicates the direction of transcription. A series of R and D clones were used to sequence both strands, and the directions and extents of sequencing reactions are shown as solid and dotted arrows. The long arrows indicate the fragment sizes of the deleted clones that were able (+) or unable (−) to complement catalase activity in *E. coli* UM255.

Presence of the *katB*-specific sequence among *Bacteroides* species. Southern blot hybridization analysis was carried out to investigate whether the *katB* gene is homogeneously distributed among the members of the *B. fragilis* group. Chromosomal DNAs of several *Bacteroides* spp. were restricted with *Eco*RI, blotted onto nylon membranes, and then hybridized with the 0.79-kb *Sph*I-*Ssp*I fragment of *katB* (Fig. 8). The probe strongly hybridized to all chromosomal DNAs from *B. fragilis. B. fragilis* ATCC 25285 and 638 seem to have in common the *katB* gene region of an approximately 2-kb *Eco*RI-*Eco*RI fragment. However, some variations may exist, as the restriction fragment containing *katB* in *B. fragilis* VPI 2393 was detected in a larger fragment of approximately 5.1 kb. We observed weak similarities to *B. ovatus* ATCC 8483, *B. thetaio-*

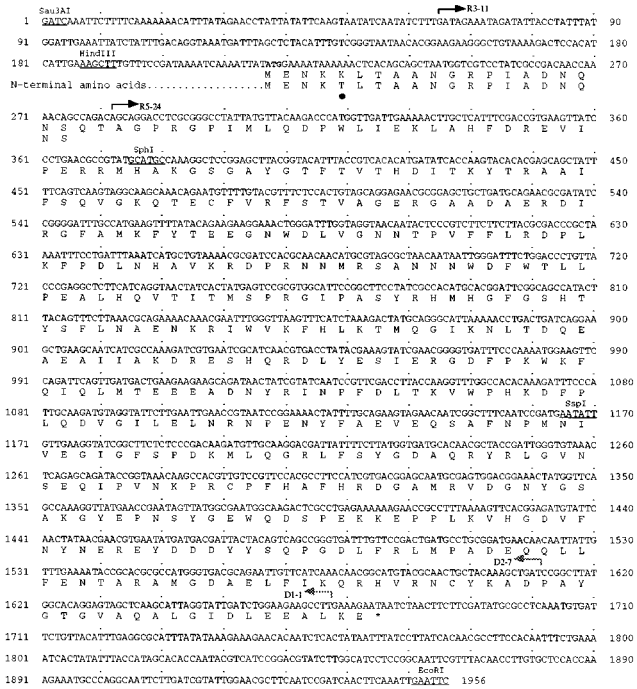

FIG. 7. Nucleotide sequence of the *katB* region and the deduced amino acid sequence. The start and stop codons of the open reading frame are indicated by bold letters and an asterisk, respectively. The partial N-terminal amino acid sequence obtained by Edman degradation is aligned with the N-terminal region of the translated peptide, and the mismatch at position 5 is indicated by a solid circle. Restriction endonuclease sites useful for isolation of the DNA probe are listed. The arrows indicate the initial nucleotide sequences of deleted DNA clones.

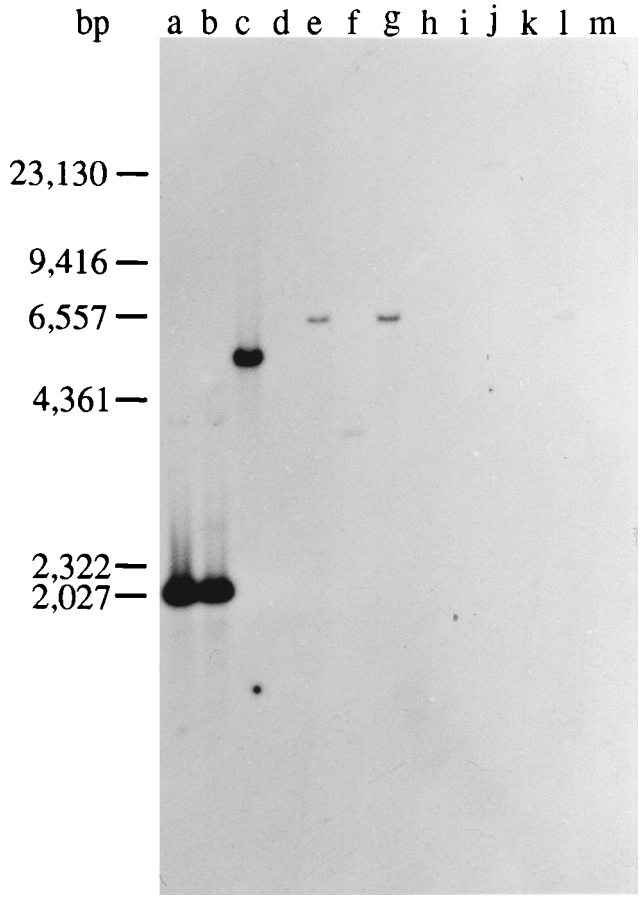

FIG. 8. Autoradiograph of chromosomal DNAs probed with the ^{32}P-labelled *Sph*I-*Ssp*I fragment of *B. fragilis katB*. Lanes: a, *B. fragilis* 638; b, *B. fragilis* ATCC 25285; c, *B. fragilis* VPI 2393; d, *B. vulgatus* 341; e, *B. thetaiotaomicron* VPI 5482; f, *B. ovatus* V211; g, *B. thetaiotaomicron* VPI 2302; h, *B. uniformis* V528; i, *B. stercoris* ATCC 43183; j, *B. distasonis* CLA 348; k, *B. merdae* ATCC 43185; l, *B. eggerthi* ATCC 27754; m, *H. influenzae* (clinical strain).

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            ...60                                                    119...
BLC         ...MAHFDRERIP ERVVHAKGAG AFGYFEVTHD ITrYsKAkVF ehIGKrTpIa VRFSTVAGEs...
Bfrag       ...LAHFDREvIP ERrnHAKGsG AYGtFtVTHD ITKYTrAAIF SqVGKqTEcF VRFSTVAGEr...
Hiu         ...LAdFvREvIP ERrnHAKGsG AFGtFtVTHD ITKYTrAkIF SEVGKkTEmF aRFtTVAGEr...
Bacsu       ...LAHFnRERVP ERVVHAKGAG AhGYFEVTnD VTKYTKAAfL SEVGKrTplF IRFSTVAGEl...
Lacsk       ...LAHFnRERIP ERVVHAKGAG rkGYFkVTkD msaYTKAAVF SgVGKkTpli tRFSqVAGEa...
Strve       ...LAHFnRERIP ERVVHArGAG AYGtFtlTrD VsrWTrAAfL SEVGKrTEtF lRFSTVAGsl...
Lisee       ...LAHFDRERVP ERVVHArGAG AhGkFvtkks mkKYTKAqfL qEeGteTEVF aRFSTVihgq...
Miclu       ...hqHFDRmnIP ERrpHAKGsG AFGvFEVTeD VssYTKAlVF .EpGveTEVL lRFSTVAGEn...
Consensus   ...LAHFDRERIP ERVVHAKGAG AFGYFEVTHD ITKYTKAAVF SEVGK-TE-F VRFSTVAGE-...

            ...130                 *    *       *      169...
BLC         ...GFAVKFYTED GNWDLVGNNT PIFFIRDall FPsFIHSQKR...
Bfrag       ...GFAmKFYTEE GNWDLVGNNT PVFFlRDPlK FPDLnHavKR...
Hiu         ...GFAlKFYTEE GNWDLVGNNT PVFFlRDPrK FPDLnkavKR...
Bacsu       ...GFAVKFYTEE GNYDiVGNNT PVFFIRDaiK FPDFIHtQKR...
Lacsk       ...GFAVKFYTEE GNYDiVGNNT PVFFVnDPlK FPDFIHSQKR...
Strve       ...GWAlKFYTEE GNYDLVGNNT PVFFIkDaiK FPDFIHtQKR...
Lisee       ...GFsVKFYTEE GNYDFVGNNl PVFFIRDaiK FPDvIHSlKp...
Miclu       ...GFAlrFYTsE GNYDLVGNNT PIFFlRDPmK FthFIrSQKR...
Consensus   ...GFAVKFYTEE GNYDLVGNNT PVFFIRDP-K FPDFIHSQKR...

            ...320              o                  o  o          379...
BLC         ...NPvNYFAEVE QlAFdPSNMp PGIepSPDKM LQGRLFAYnD thRhRLGpNY lQiPVNcPYR...
Bfrag       ...NPENYFAEVE QSAFnPmNiV eGIgfSPDKM LQGRLFsYqD AqRYRLGvNs eQiPVNkP.R...
Hiu         ...NPENFFADVE QSAFaPSNLV PGIgaSPDrM LQaRLFnYaD AqRYRLGvcY rQiPVNRP.R...
Bacsu       ...NPENYFAEVE QatFSPgtLV PGIdvSPDKM LQGRLFAYhD AhRYRvGaNh qaLPINRa.R...
Lacsk       ...NPtNnFeDIq elAFSPaNLV PGIeaSPDKL LQGRLFqYkD AeRYRLGaNY eQLPVNRP.k...
Strve       ...NPENiFAEVE QSiFSPahFV PGIgpSPDKM LQGRLFAYqD AhRYRvGiNa dhLPVNRP.h...
Lisee       ...NPDNiFAEtE svgFnPgvLV PGmlpSeDrv LQGRLFsYsD tqRhRvGpNY lQLPINsP.k...
Miclu       ...NPkNFFAEIE saAFSPSNtV PGIglSPDrM LlGRaFAYhD AqlYRvGahv nQLPVNsPdd...
Consensus   ...NPENYFAEVE QSAFSPSNLV PGI--SPDKM LQGRLFAY-D A-RYRLG-NY -QLPVNRPYR...
```

FIG. 9. Multiple alignment of the deduced amino acid sequence of *B. fragilis* KatB with sequences of mammalian and bacterial catalases. Conserved amino acid residues are capitalized. The active-site residues of BLC (His-74, Ser-113, and Asp-147) are indicated by asterisks. The proximal (Pro-335, Arg-353, and Tyr-357) and distal (Val-73, His-74, Asn-147, Phe-152, and Phe-160) heme site ligands are indicated by open and solid circles, respectively. The consensus patterns of His-74 active site F-x-[RH]-x(4)-[EQ]-R-x(2)-H-x(2)-[GAS] and Tyr-357 proximal site R(L,I,V,M,F,A)F(G,A,S,T)YxD(A,S,T)(Q,E,H) are underlined. See Materials and Methods for species designations.

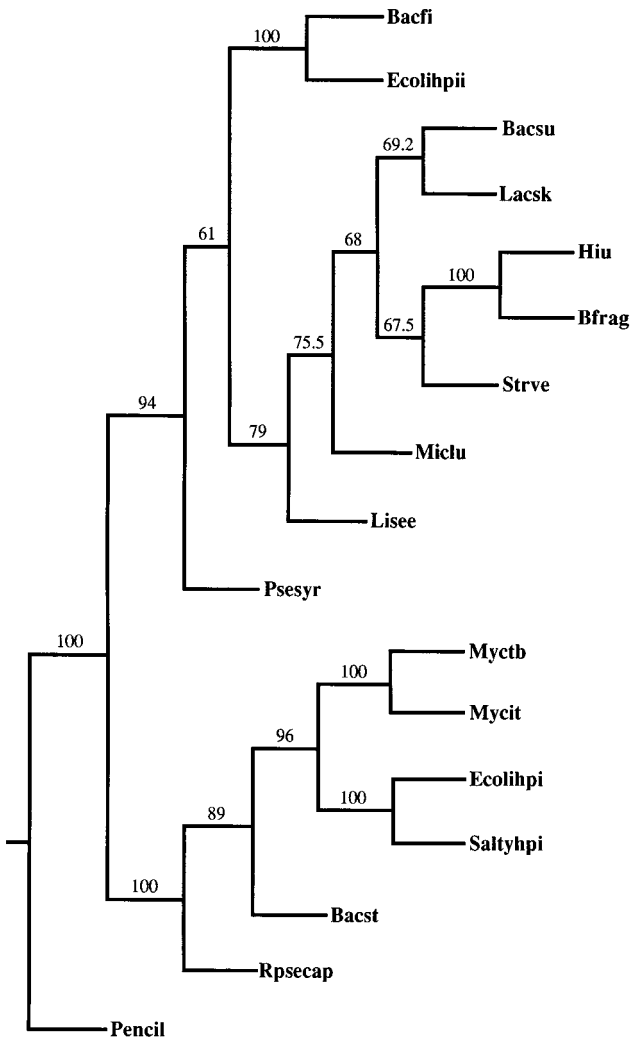

FIG. 10. Phylogenetic relationship of *B. fragilis* KatB and representative microbial catalases. This unrooted tree was constructed from a multiple alignment similar to that shown in Fig. 9, except that additional catalases listed in Materials and Methods were included. The tree topology was obtained by use of the parsimony program (Protpars) of Felsenstein (15), and the numbers represent how often the groups of species to the right of a fork occurred in 100 trees tested. See Materials and Methods for species designations.

taomicron VPI 2302 and VPI 5482, *B. distasonis* CLA 348, and *B. eggerthi* ATCC 27754.

Comparison of amino acid sequences. The predicted amino acid sequence obtained for KatB was compared to other catalase sequences in the GenBank-EMBL/Swiss-Prot databases. When KatB was individually compared with other catalases, it showed the highest amino acid identity with *H. influenzae* catalase HktE (71.6% identity and 81.9% similarity). KatB also showed high similarity to catalases from the gram-positive bacteria *S. venezuelae* (56.1% identity), *B. subtilis* (55.6% identity), and *L. sake* (52.7% identity), as well as to the bovine (49.9% identity) and human (49.5% identity) catalases. No similarity to bacterial catalase-peroxidase-type enzymes such as *E. coli* hydroperoxidase I, *S. typhimurium* hydroperoxidase I, and *R. capsulatus* CpeA was found.

The identity between KatB and other catalases centered around important structural motifs. For example, comparison of bovine liver-type catalase revealed that residues involved in the active site of the bovine enzyme, His-74, Ser-113, and Asn-147 (17, 49), were conserved in KatB at the His-54, Ser-93, and Asn-127 positions. Moreover, the proximal heme site which interacts with Pro-335, Arg-353, and Tyr-357 as ligands in bovine catalase was localized as Pro-315, Arg-333, and Tyr-337 in KatB. The five distal heme site ligands in bovine catalase, Val-73, His-74, Asn-147, Phe-152, and Phe-160, also were conserved, except that Val-73 was replaced by Met-53 in KatB (Fig. 9). In addition, the NADPH binding site residues of the bovine catalase, Arg-202, Asp-212, Lys-236, and His-304 (18), seemed to be conserved in KatB as Arg-182, Lys-216, and His-284, but Asp-212 has been replaced by His-192 in KatB (data not shown).

The phylogenetic relationship between *B. fragilis* KatB and 15 bacterial catalases and 1 fungal catalase was determined from a progressive multiple alignment of the amino acid sequences followed by parsimony analysis (Fig. 10). This comparison clearly shows two groups within the bacterial catalases. The first major group consists of catalases from both gram-negative and gram-positive bacteria which contain the consensus pattern residues of the active site and proximal heme ligand residues of typical catalases (17, 49). A second major group is composed of the catalase-peroxidase-type enzymes of *M. tuberculosis*, *M. intracellulare*, *S. typhimurium* hydroperoxidase I, *E. coli* hydroperoxidase I, *R. capsulatus*, and *B. stearothermophilus.*

The strong homology between *B. fragilis* KatB and *H. influenzae* HktE was clearly seen in this analysis. These catalases were grouped together in an important gram-positive cluster including *S. venezuelae*, *B. subtilis*, and *L. sake.*

DISCUSSION

The human intestinal tract provides an anaerobic environment where both obligate and facultative anaerobic bacteria compete and survive (14, 16, 66). Outside of the intestine, facultative bacteria such as *E. coli* and *S. typhimurium* are readily able to adapt to the reactive oxygen species that are formed during metabolism in the presence of oxygen or released from phagocytes as part of their oxygen-dependent killing defense mechanism (14, 66). However, it is not clear how anaerobes such as *B. fragilis* adapt to oxygenated environments. *B. fragilis* is one of the most oxygen-tolerant anaerobes, and there is some suggestion that strains found in human infections are more aerotolerant than nonclinical strains (56, 67). This suggests that there is an adaptive mechanism that protects them during an oxidative stress. Previous work (23) suggested that catalase is one of the important enzymes involved in the aerotolerance of some *Bacteroides* species. Thus, the studies described in this report were initiated to pursue a better understanding of the role catalase plays in the aerotolerance and oxygen response of the opportunistic pathogen *B. fragilis.*

The results presented here show that the native KatB protein has an estimated molecular weight of 124,000 to 130,000 as determined by two methods, electrophoretic mobility and size exclusion HPLC. The subunit molecular weight determined by SDS gel analysis was 60,000, and this agreed closely with the 55,905 obtained from the predicted amino acid sequence. These results indicate that the native enzyme is composed of two identical subunits. The *B. fragilis* enzyme is similar in size to other dimeric catalases with molecular masses of 127,000 to 160,000 Da (11, 21, 36, 52). Catalases containing two subunits have been found in *S. venezuelae* (36), *Comamonas compransoris* (52), *Klebsiella pneumoniae* kpA (21), and *M. tuberculosis* (11). The spectrum of the hemochrome complex of the enzyme indicates that it is a hemoprotein containing a protoheme IX. Although the heme/subunit ratio of 1:2 is also found in other bacterial catalases (8, 9), the low heme content may be due to the loss of heme during purification procedures (8).

Even though our results showed that native KatB had strong peroxidatic activity with pyrogallol as the electron donor, the *B. fragilis* enzyme is more characteristic of typical catalases rather than the catalase-peroxidase enzymes for two reasons. The first is the lack of reduction by dithionite as seen in the native form of the protein (50, 62). This may be due to the steric hindrance that does not provide access to the heme prosthetic group of the enzyme (62). Secondly, comparison of the conserved motifs in the KatB amino acid sequence revealed that the heme-binding ligands and active-site residues are highly conserved with the true catalases, whereas the conserved active site and domains of peroxidases that are present in bacterial catalase-peroxidases (19, 73) were not identified in the *B. fragilis* catalase. The restricted peroxidatic activity associated with *B. fragilis* KatB is probably due to the nonspecific catalysis that occurs in typical catalases toward certain types of organic electron donor compounds (62).

The KatB structural gene is 1,461 nucleotides long and encodes a protein of 486 amino acids. Multiple amino acid sequence alignments and parsimony analysis showed a strong relationship between KatB and a group of catalases containing mostly gram-positive bacterial and mammalian-type catalases (Fig. 10). The relationship between *Bacteroides* genes (proteins) and those of gram-positive bacteria is not new (discussed in reference 64); however, a surprising observation was the 71% amino acid identity (66% nucleotide identity) with the *H. influenzae* HktE enzyme. Generally, *Bacteroides* spp. which are not closely related to other gram-negative eubacteria (72) share little homology with other species, with identities at the amino acid level of <40% most common. There is no obvious explanation for the high degree of homology between KatB and HktE, and a codon usage comparison of these two genes revealed that, while the codon usage patterns are similar, they are also closely aligned to representative genes from their respective species (data not shown). These results indicate that it is likely that there was a horizontal transfer of the catalase gene from a common gram-positive ancestor into both the anaerobe *B. fragilis* and *H. influenzae.*

Catalase activity is not universal among *Bacteroides* spp.; *B. fragilis* and *B. distasonis* are catalase positive, while *B. thetaiotaomicron*, *B. ovatus*, and *B. eggerthi* possess variable catalase activity (74). *B. vulgatus* and *B. uniformis* are catalase negative. Results presented here show that *B. fragilis* produces a single catalase enzyme similar to that of *B. distasonis*, but in contrast, the *B. distasonis* enzyme is twice the size, with a molecular weight of 250,000 (25). Thus, there clearly are differences in catalase structure among *Bacteroides* spp. This is supported by hybridization analysis showing that the 0.79-kb *Sph*I-*Ssp*I fragment of *B. fragilis katB* has strong homology with *B. fragilis* chromosomal DNA but not with that of other *Bacteroides* spp. (Fig. 8). Some variation in the catalase gene region may exist within the species as observed between *B. fragilis* VPI 2393 (homology group II; 34) and the two homology group I strains ATCC 25285 and 638. Unfortunately, there are not many studies on the structure and composition of catalases from *Bacteroides* spp. that can be used for a comparison with *B. fragilis* KatB.

As mentioned above, catalase is likely to be involved in the aerotolerance of *B. fragilis*. Thus, it is not surprising that we observed a significant increase in catalase specific activity when cells were exposed to oxygen. On the other hand, addition of 20 to 1,000 μM hydrogen peroxide to late-log-phase cells had no effect on catalase activity (data not shown). Although the peroxide may not have reached an intracellular concentration sufficient to induce a detectable response, our results suggest that an increase in the E_h of the culture due to the peroxide is not sufficient for high catalase activities. These findings are in agreement with previous work showing that aerotolerance in anaerobic cultures is observed upon aeration (71) and molecular oxygen, but not an adverse oxidation-reduction potential, influences *B. fragilis* aerotolerance (53, 71). We also observed increased catalase activity in stationary-phase cells, suggesting that catalase is synthesized as part of a strategy for surviving in starvation conditions. Consistent with this observation was the finding of Gregory et al. (26) that showed inhibition of catalase formation with addition of glucose to *B. fragilis* cultures growing in a peptone medium. The *B. fragilis* catalase response is, therefore, similar to *E. coli* hydroperoxidase II (encoded by *katE*) in the way that it is upregulated by oxygenation and stationary phase but not peroxides (39, 41, 70). In *E. coli*, *katE* is regulated by the starvation-stationary phase stress regulatory system *rpoS* (*katF*) (38, 42). In contrast, *B. fragilis* KatB has a strong identity (71%) with *H. influenzae* HktE. The latter, although it has 50% identity with *E. coli* KatE (5), is reported to be downregulated in stationary phase and upregulated by exposure to hydrogen peroxide under the *oxyR*-like regulatory system, similar to peroxide-inducible *E. coli katG* (5). The regulatory system that might be involved in the regulation of *B. fragilis katB* should prove quite interesting and is presently under investigation. Further studies of catalase and its regulation will help us to understand, at the molecular level, the mechanisms by which aerotolerant pathogenic *B. fragilis* strains

respond to the anaerobic-aerobic-anaerobic shifts that may occur during the establishment of an infection.

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REFERENCES

1. **Aebi, H.** 1984. Catalase *in vitro*. Methods Enzymol. **105:**121–126.
2. **Andrews, D. W., M. Girardi, and J. Mark.** 1990. Chemistry and sequencing of covalently immobilized proteins and peptides. Technical literature MG 345. Millipore Corporation, Marlborough, Mass.
3. **Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl.** 1987. Current protocols in molecular biology. John Wiley & Sons, Inc., New York.
4. **Birnboim, H. C., and J. Doly.** 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res. **7:**1513–1523.
5. **Bishai, W. R., H. O. Smith, and G. J. Barcak.** 1994. A peroxide/ascorbate-inducible catalase from *Haemophilus influenzae* is homologous to the *Escherichia coli katE* gene product. J. Bacteriol. **176:**2914–2921.
6. **Bol, D. K., and R. E. Yasbin.** 1991. The isolation, cloning and identification of a vegetative catalase gene from *Bacillus subtilis*. Gene **109:**31–37.
7. **Bradford, M. M.** 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72:**248–254.
8. **Brown-Peterson, N. J., and M. L. Salin.** 1993. Purification of a catalase-peroxidase from *Halobacterium halobium*: characterization of some unique properties of the halophilic enzyme. J. Bacteriol. **175:**4197–4202.
9. **Clairborne, A., and I. Fridovich.** 1979. Purification of the o-dianisidine peroxidase from *Escherichia coli* B. J. Biol. Chem. **254:**4245–4252.
10. **Devereux, J., P. Haeberli, and O. Smithies.** 1984. A comprehensive set of sequence analysis programs for the VAX. Nucleic Acids Res. **12:**387–395.
11. **Dias, G. A., and L. G. Wayne.** 1974. Isolation and characterization of catalase produced by *M. tuberculosis*. Am. Rev. Respir. Dis. **110:**312–319.
12. **Dower, W. J., J. F. Miller, and C. W. Ragsdale.** 1988. High efficiency transformation of *E. coli* by high voltage electroporation. Nucleic Acids Res. **16:**6127–6145.
13. **Falk, J. E.** 1964. Porphyrins and metalloporphyrins. Their general, physical and coordination chemistry, and laboratory methods, p. 181–182. Elsevier Publishing Co., Amsterdam.
14. **Farr, S. B., and T. Kogoma.** 1991. Oxidative stress responses in *Escherichia coli* and *Salmonella typhimurium*. Microbiol. Rev. **55:**561–585.
15. **Felsenstein, J.** 1989. PHYLIP-phylogeny inference package (version 3.2). Cladistics **5:**164–166.
16. **Finegold, S. M., and W. L. George.** 1989. Anaerobic infections in humans. Academic Press, San Diego, Calif.
17. **Fita, I., and M. G. Rossmann.** 1985. The active center of catalase. J. Mol. Biol. **185:**21–37.
18. **Fita, I., and M. G. Rossmann.** 1985. The NADPH binding site on beef liver catalase. Proc. Natl. Acad. Sci. USA **82:**1604–1608.
19. **Forkl, H., J. Vandekerckhove, G. Drews, and M. H. Tadros.** 1993. Molecular cloning, sequence analysis and expression of the gene for catalase-peroxidase (*cpeA*) from the photosynthetic bacterium *Rhodobacter capsulatus* B10. Eur. J. Biochem. **214:**251–258.
20. **Fridovich, I.** 1978. The biology of oxygen radicals. Science **201:**875–880.
21. **Goldberg, I., and A. Hochman.** 1989. Purification and characterization of a novel type of catalase from the bacterium *Klebsiella pneumoniae*. Biochim. Biophys. Acta **991:**330–336.
22. **Gregory, E. M., and C. H. Dapper.** 1983. Isolation of iron-containing superoxide dismutase from *Bacteroides fragilis*: reconstitution as a Mn-containing enzyme. Arch. Biochem. Biophys. **220:**293–300.
23. **Gregory, E. M., and D. D. Fanning.** 1983. Effect of heme on *Bacteroides distasonis* catalase and aerotolerance. J. Bacteriol. **156:**1012–1018.
24. **Gregory, E. M., and I. Fridovich.** 1974. Visualization of catalase on acrylamide gels. Anal. Biochem. **58:**57–62.
25. **Gregory, E. M., J. B. Kowalski, and L. V. Holdeman.** 1977. Production and some properties of catalase and superoxide dismutase from the anaerobe *Bacteroides distasonis*. J. Bacteriol. **129:**1298–1302.
26. **Gregory, E. M., B. J. Veltri, D. L. Wagner, and T. D. Wilkins.** 1977. Carbohydrate repression of catalase synthesis in *Bacteroides fragilis*. J. Bacteriol. **129:**534–535.
27. **Haas, A., K. Brehm, J. Kreft, and W. Goebel.** 1991. Cloning, characterization, and expression in *Escherichia coli* of a gene encoding *Listeria seeligeri* catalase, a bacterial enzyme highly homologous to mammalian catalases. J. Bacteriol. **173:**5159–5167.
28. **Halliwell, B., and J. M. C. Gutteridge.** 1984. Oxygen toxicity, oxygen radicals, transition metals and disease. Biochem. J. **219:**1–14.
29. **Hassan, H. M., and I. Fridovich.** 1978. Regulation of the synthesis of catalase and peroxidase in *Escherichia coli*. J. Biol. Chem. **253:**6445–6450.
30. **Hedrick, J. L., and A. J. Smith.** 1968. Size and charge isomer separation and estimation of molecular weights of proteins by disc gel electrophoresis. Arch. Biochem. Biophys. **126:**155–164.
31. **Henikoff, S.** 1984. Unidirectional digestion with exonuclease III creates targeted breakpoints for DNA sequencing. Gene **28:**351–359.
32. **Holdeman, L. V., E. P. Cato, and W. E. C. Moore.** 1977. Anaerobe laboratory manual, 4th ed. Virginia Polytechnic Institute and State University, Blacksburg.
33. **Imlay, J. A., and S. Linn.** 1988. DNA damage and oxygen radical toxicity. Science **240:**1302–1309.
34. **Johnson, J. L.** 1978. Taxonomy of the Bacteroides. I. Deoxyribonucleic acid homologies among *Bacteroides fragilis* and other saccharolytic *Bacteroides* species. Int. J. Syst. Bacteriol. **28:**245–256.
35. **Knauf, H. J., R. F. Vogel, and W. P. Hammes.** 1992. Cloning, sequence, and phenotypic expression of *katA*, which encodes the catalase of *Lactobacillus sake* LTH677. Appl. Environ. Microbiol. **58:**832–839.
36. **Knoch, M., K. H. van Pée, L. C. Vinning, and F. Lingens.** 1989. Purification, properties and immunological detection of a bromoperoxidase-catalase from *Streptomyces venezuelae* and from a chloramphenicol-nonproducing mutant. J. Gen. Microbiol. **135:**2493–2502.
37. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227:**680–685.
38. **Lange, R., and R. Hengge-Aronis.** 1991. Identification of a central regulator of stationary-phase gene expression in *Escherichia coli*. Mol. Microbiol. **5:**49–59.
39. **Loewen, P. C.** 1992. Regulation of bacterial catalase synthesis, p. 97–115. *In* J. G. Scandalios (ed.), Molecular biology of free radical scavenging systems. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
40. **Loewen, P. C., and G. V. Stauffer.** 1990. Nucleotide sequence of *katG* of *Salmonella typhimurium* LT2 and characterization of its product, hydroperoxidase I. Mol. Gen. Genet. **224:**147–151.
41. **Loewen, P. C., J. Switala, and B. L. Triggs-Raine.** 1985. Catalases HPI and HPII in *Escherichia coli* are induced independently. Arch. Biochem. Biophys. **243:**144–149.
42. **Loewen, P. C., and B. L. Triggs.** 1984. Genetic mapping of *katF*, a locus that with *katE* affects the synthesis of a second catalase species in *Escherichia coli*. J. Bacteriol. **160:**668–675.
43. **Loprasert, S., S. Negoro, and H. Okada.** 1988. Thermostable peroxidase from *Bacillus stearothermophilus*. J. Gen. Microbiol. **134:**1971–1976.
44. **Loprasert, S., S. Negoro, and H. Okada.** 1989. Cloning, nucleotide sequence, and expression in *Escherichia coli* of the *Bacillus stearothermophilus* peroxidase gene (*perA*). J. Bacteriol. **171:**4871–4875.
45. **Morris, J. G.** 1980. Oxygen tolerance/intolerance of anaerobic bacteria, p. 7–15. *In* G. Gottschalk, N. Penning, and H. Werner (ed.), Anaerobes and anaerobic infections. Symposia held at the XII International Congress of Microbiology in Munich, 3 to 8 September 1978. Gustav Fisher Verlag, Stuttgart, Germany.
46. **Morris, S. L., J. Nair, and D. A. Rouse.** 1992. The catalase-peroxidase of *Mycobacterium intracellulare*: nucleotide sequence analysis and expression in *Escherichia coli*. J. Gen. Microbiol. **138:**2363–2370.
47. **Mulvey, M. R., P. A. Sorby, B. L. Triggs-Raine, and P. C. Loewen.** 1988. Cloning and physical characterization of *katE* and *katF* required for catalase HPII expression in *Escherichia coli*. Gene **73:**337–345.
48. **Murshudov, G. N., W. R. Melik-Adamyan, A. I. Grebenko, V. V. Barynin, A. A. Vagin, B. K. Vainshtein, Z. Dauter, and K. S. Wilson.** 1992. Three-dimensional structure of catalase from *Micrococcus lysodeikticus* at 1.5 A resolution. FEBS Lett. **312:**127–131.
49. **Murthy, M. R. N., T. J. Reid III, A. Sicignano, N. Tanaka, and M. G. Rossmann.** 1981. Structure of beef liver catalase. J. Mol. Biol. **152:**465–499.
50. **Nadler, V., I. Goldberg, and A. Hochman.** 1986. Comparative study of bacterial catalases. Biochim. Biophys. Acta **882:**234–241.
51. **Nelson, D. P., and L. A. Kiesow.** 1972. Enthalpy of decomposition of hydrogen peroxide by catalase at 25°C (with molar extinction coefficients of H_2O_2 solutions in the UV). Anal. Biochem. **49:**474–478.
52. **Nies, D., and H. G. Schlegel.** 1982. Catalase from *Comamonas compransoris*. J. Gen. Appl. Microbiol. **28:**311–319.
53. **Onderdonk, A. B., J. Johnston, J. W. Mayhew, and S. L. Gorbach.** 1976. Effect of dissolved oxygen and Eh on *Bacteroides fragilis* during continuous culture. Appl. Environ. Microbiol. **31:**168–172.
54. **Pivitera, G., A. Dublanchet, and M. Sebald.** 1979. Transfer of multiple antibiotic resistance between subspecies of *Bacteroides fragilis*. J. Infect. Dis. **139:**97–101.
55. **Putney, S. D., S. J. Benkovic, and P. Schimmel.** 1981. A DNA fragment with an α-phosphorothioate nucleotide at one end is asymmetrically blocked from digestion by exonuclease III and can be replicated *in vivo*. Proc. Natl. Acad. Sci. USA **78:**7350–7354.
56. **Rolfe, R. D., D. J. Hentges, J. T. Barrett, and B. J. Campbell.** 1977. Oxygen tolerance of human intestinal anaerobes. Am. J. Clin. Nutr. **30:**1762–1769.
57. **Ryan, T. P., and S. D. Aust.** 1992. The role of iron in oxygen-mediated toxicities. Crit. Rev. Toxicol. **22:**119–141.
58. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a

laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
59. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:**5463–5467.
60. **Schroeder, W. A., J. R. Shelton, J. B. Shelton, B. Robberson, G. Apell, R. S. Fang, and J. Bona Ventura.** 1982. The complete amino acid sequence of bovine liver catalase and the partial sequence of bovine erythrocyte catalase. Arch. Biochem. Biophys. **214:**397–421.
61. **Shimoni, M., and R. Reuveni.** 1988. A method for staining and stabilizing peroxidase activity in polyacrylamide gel electrophoresis. Anal. Biochem. **175:**35–38.
62. **Sichak, S. P., and A. L. Dounce.** 1986. Analysis of the peroxidatic mode of action of catalase. Arch. Biochem. Biophys. **249:**286–295.
63. **Smith, C. J.** 1985. Characterization of *Bacteroides ovatus* plasmid pBI136 and structure of its clindamycin resistance region. J. Bacteriol. **161:**1069–1073.
64. **Smith, C. J., C. Owen, and L. Kirby.** 1992. Activation of a cryptic streptomycin-resistance gene in the *Bacteroides erm* transposon, Tn*4551*. Mol. Microbiol. **6:**2287–2297.
65. **Southern, J. A., J. R. Parker, and D. R. Woods.** 1986. Expression and purification of glutamine synthetase cloned from *Bacteroides fragilis*. J. Gen. Microbiol. **132:**2827–2835.
66. **Storz, G., L. A. Tartaglia, S. B. Farr, and B. N. Ames.** 1990. Bacterial defenses against oxidative stress. Trends Genet. **6:**363–368.
67. **Tally, F. P., P. R. Stewart, V. L. Sutter, and J. E Rosenblatt.** 1975. Oxygen tolerance of fresh clinical anaerobic bacteria. J. Clin. Microbiol. **1:**161–164.
68. **Triggs-Raine, B. L., B. W. Doble, M. R. Mulvey, P. A. Sorby, and P. C. Loewen.** 1988. Nucleotide sequence of *katG*, encoding catalase HPI of *Escherichia coli*. J. Bacteriol. **170:**4415–4419.
69. **Vainshtein, B. K., W. R. Melik-Adamyan, V. V. Barynin, A. A. Vagin, A. I. Grebenko, and V. V. Borisov.** 1986. Three-dimensional structure of catalase from *Penicillium vitale* at 2.0 A resolution. J. Mol. Biol. **188:**49–61.
70. **von Ossowski, I., M. R. Mulvey, P. A. Leco, A. Borys, and P. C. Loewen.** 1991. Nucleotide sequence of *Escherichia coli katE*, which encodes catalase HPII. J. Bacteriol. **173:**514–520.
71. **Walden, W. C., and D. J. Hentges.** 1975. Differential effects of oxygen and oxidation-reduction potential on the multiplication of three species of anaerobic intestinal bacteria. Appl. Microbiol. **30:**781–785.
72. **Weisburg, W. G., Y. Oyaizu, H. Oyaizu, and C. R. Woese.** 1985. Natural relationship between *Bacteroides* and flavobacteria. J. Bacteriol. **164:**230–236.
73. **Welinder, K. G.** 1991. Bacterial catalase-peroxidases are gene duplicated members of the plant peroxidase superfamily. Biochim. Biophys. Acta **1080:** 215–220.
74. **Wilkins, T. D., D. L. Wagner, B. J. Veltri, Jr., and E. M. Gregory.** 1978. Factors affecting production of catalase by *Bacteroides*. J. Clin. Microbiol. **8:**553–557.
75. **Woodbury, W., A. K. Spencer, and M. A. Stahmann.** 1971. An improved procedure using ferricyanide for detecting catalase isozymes. Anal. Biochem. **44:**301–305.
76. **Zhang, Y., B. Heyn, B. Allen, D. Young, and S. Cole.** 1992. The catalase-peroxidase gene and isoniazid resistance of *Mycobacterium tuberculosis*. Nature (London) **358:**591–593.