Bacterial Catalases

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All catalases, whether eukaryotic, prokaryotic, or archae, catalyze the same basic reaction, the dismutation of hydrogen peroxide to water and oxygen: $2H_2O_2 \rightarrow 2H_2O + O_2$. The enzyme presumably evolved to protect the cell by removing peroxide before cell damage was caused by peroxide radicals generated in chemical reactions. The degradation of H_2O_2 by various tissues was first studied in the early 1800s by Thénard, and the first report of a similar bacterial activity determined both in whole cells and in cell extracts appeared in 1893 (Gottstein 1893). The suggestion of a specific enzyme with the catalytic role of peroxide degradation (hence the name catalase) appeared in 1900 (Loew 1900), and this was confirmed with the purification of catalases from various sources, including beef liver (Sumner and Dounce 1937). The first report of a purified bacterial catalase from *Micrococcus luteus* appeared a decade later (Herbert and Pinsent 1948), but the simplicity of spotting a drop of H_2O_2 on the edge of a colony and watching for oxygen generation had much earlier made the presence or absence of catalase a diagnostic tool in bacterial taxonomy. Interest in bacterial catalases also derived from the potential role of the enzyme in pathogenesis wherein the infecting bacterium frequently has to survive a burst of active oxygen species, including H_2O_2 generated by the infected tissue.

There are a number of sources of H_2O_2 during the aerobic growth of any organism (Farr and Kogoma 1991). Reduction of oxygen as the terminal electron acceptor can give rise to partial reduction products, including the reactive oxygen species superoxide ion ($O_2^{\bullet -}$) and H_2O_2. The former is removed in a reaction ($2O_2^{\bullet -} + 2H^+ \rightarrow H_2O_2 + O_2$) catalyzed by one of three possible superoxide dismutases that generates additional H_2O_2. Other intracellular sources of H_2O_2 include reactions catalyzed by oxidases and dehydrogenases, as well as the oxidation of thiols, flavins, and ascorbate. Alternatively, extracellular H_2O_2 can penetrate the cell membrane, providing easy access to cellular components. The major damage from H_2O_2 arises from the highly reactive hydroxyl radicals, generated in the Fenton reaction between H_2O_2 and metal ions

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such as Fe^{++}, which can react with DNA, proteins, and lipids, making it desirable for most aerobic organisms to have catalases or peroxidases to circumvent the damage.

Having primarily a protective role, catalases are not required for cell growth in either *Escherichia coli* (Loewen 1984) or *Bacillus subtilis* (Loewen and Switala 1987), and many other bacteria have been characterized as lacking any detectable catalase activity (Buchanan and Gibbons 1974). However, a selective advantage of having the enzyme has been demonstrated in a number of ways in a number of organisms. An obvious expectation has been realized with cell survival in the presence of H_2O_2 being enhanced in catalase-proficient cells, as compared to catalase-deficient cells (Sammartano et al. 1986; Yonei et al. 1987; Abril and Pueyo 1990; McCann et al. 1991; Volkert et al. 1994). Long-term survival in stationary phase is also enhanced in catalase-proficient strains (Mulvey et al. 1990), and a selective growth advantage of the non-heme catalase from *Lactobacillus plantarum* has also been demonstrated (Kono and Fridovich 1983b). In addition, the presence of intracellular catalase reduced the rates of both spontaneous and H_2O_2-induced mutations (Abril and Pueyo 1990). Despite the apparent importance in protection against peroxide, catalase was found not to be a significant factor in either virulence or survival of either *Salmonella typhimurium* (Papp-Szabo et al. 1994; Buchmeier et al. 1995) or *Haemophilus influenzae* (Bishai et al. 1994). The reduced virulence exhibited in *katF* (*rpoS*)-containing mutants of *S. typhimurium* is thus a result of reduced expression of other virulence genes, not just catalase (Fang et al. 1992). Similarly, the sensitivity to near-UV radiation exhibited by *rpoS*-containing mutants can be ascribed to reduced expression of genes other than *katE* that are under the control of *rpoS* (Sammartano et al. 1986), although exogenous catalase was important in the protection against near-UV radiation (Sammartano and Tuveson 1984). Catalase HPI has been shown to be important in the recovery of proton-motive-force-dependent and -independent transport processes in *E. coli* after peroxide treatment (Farr et al. 1988), may be a reflection of its periplasmic location (Heimberger and Eisenstark 1988). In addition, the lack of catalase in *Bacillus larvae* has been implicated in the enhanced oxygen toxicity of the organism as compared to *B. subtilis* (Dingman and Stahly 1984).

Some catalases also have a peroxidatic mode of reaction in which an organic electron donor (or sometimes a halide ion) is employed in the reduction of H_2O_2 : $RH_2 + H_2O_2 \rightarrow 2H_2O + R$. In many catalases, this is a minor reaction that does not seem to be important, but in some bacterial catalases, the peroxidatic activity is so prominent that there is some ques-

tion as to whether the enzyme should be called a catalase or a peroxidase. As a result, considerable diversity is evident among bacterial catalases, with three or four distinct groupings possible: the monofunctional catalases, possibly subdivided on the basis of heme content and subunit size; the catalase-peroxidases; and the non-heme, Mn-containing catalases. Given the diversity in bacterial habitats, such a multiplicity of catalase types is not surprising, and this may also explain the diversity in mechanisms for controlling expression of the catalases. In this review, I summarize the broad range of catalases that have been purified and sequenced, providing a focus on the diversity in structure, function, and regulation of expression.

CATALASE DIVERSITY

Catalase Characterization and Groupings

The first report of a purified bacterial catalase from *M. luteus* appeared in 1948 (Herbert and Pinsent 1948). This was followed in 1959 by purification of the catalase from *Rhodobacter spheroides* (Clayton 1959). Another 20 years passed before the purifications of catalases (or more correctly, hydroperoxidases) HPI and HPII from *E. coli* were reported in 1979 (Claiborne and Fridovich 1979; Claiborne et al. 1979). Since then, there has been a regular appearance of reports of catalases being purified and characterized, or being cloned and sequenced from a broad range of bacterial species, including gram-negative, gram-positive, and phototrophic bacteria, mycobacteria, halophilic archaebacteria, and streptomycetes. A list of the catalases that have been characterized following purification or by sequence analysis of a cloned gene is shown in Table 1. The catalases in Table 1 have been subdivided into four groups amid the considerable heterogeneity, based on a survey of the properties and sequences of the enzymes. The first grouping of monofunctional catalases is further subdivided into the "typical" catalases (typical because of their similarity to the eukaryotic catalases) and "atypical" catalases (atypical because of certain key differences from the typical enzyme); the second grouping contains the catalase/peroxidases; and the third grouping includes the non-heme catalases. Another generalization is that it is common for bacterial species to have a multiplicity of catalase isozymes. In fact, the listing of only one catalase from a given organism in Table 1 should not be interpreted to mean that this is the only type produced by the organism. Rather, the identification of just one catalase may simply be a reflection of its predominance in the organism or the result of cloning of a particular gene. Other organisms in which catalases have been identified but not

Table 1 Summary of characterized bacterial catalases

Bacterial species	MW	Features	Designation (accession number)	References
A-1. Typical monofunctional catalases				
Gram-positive				
Bacillus firmus	60000	heme b	BfikatII	Hicks (1995)
Bacillus subtilis	54838	heme b hexamer	BsukatA (M80796)	Bol and Yasbin (1991)
Deinococcus radiodurans	60630		DrakatA (D63898)	I. Narumi et al. (unpubl.)
Lactobacillus sake	54006	hexamer	LsakatA (M84015)	Knauf et al. (1992)
Listeria seeligeri	55876		Lsekat (M75944)	Haas et al. (1991)
Micrococcus luteus	55586	heme b tetramer	Mlukat (P29422)	Herbert and Pinsent (1948)
Staphylococcus simulans	53000	tetramer	Ssikat	Fondren et al. (1994)
Gram-negative				
Bacteroides fragilis	55912	heme b dimer	BfrkatB (U18696)	Rocha and Smith (1995)
Bordetella pertussis	54514		BpekatA (U07800)	DeShazer et al. (1994)
Brucella abortus	56452	heme b tetramer	Babkat (U11439)	Sha et al. (1994)
Campylobacter jejuni	58422		CjekatA (X85130)	Grant and Park (1995)
Haemophilus influenzae	57623		HinhktE (U02682)	Bishai et al. (1994)
Klebsiella pneumoniae	62700	heme b tetramer	KpnkatT	Hochman and Goldberg (1991)
Methylobacterium extorquens	53527		MtbkatA (L48340)	L.V. Chistoserdova and M.E. Lidstrom (unpubl.)
Neisseria gonorrhoeae	56677		Ngokat (U35457)	W. Johnson and B.M. Steiner (unpubl.)
Proteus mirabilis	55621	heme b tetramer	PmikatA (P42321)	Jouve et al. (1983, 1984); Buzy et al. (1995)
Pseudomonas aeruginosa	57138	tetramer	PaekatB (U34896)	Brown et al. (1995)
Pseudomonas syringae	57332	dimer	PsycatF (U03465)	Klotz et al. (1995)
Streptomycetes				
Streptomyces coelicolor	55000	heme b tetramer	Scocat4	Kim et al. (1994); Walker et al. (1995)
Streptomyces venezuelae	54094	heme b tetramer	Svekat (X74791)	Knoch et al. (1989)

A-2. Atypical monofunctional catalases				
Gram-positive				
Bacillus firmus	80000	heme d	BfikatA (M74194)	Hicks (1995)
Bacillus subtilis	77500	heme d hexamer	BsukatB (X85182)	S. Englemann et al. (unpubl.)
Gram-negative				
Escherichia coli	84172	heme d tetramer	EcokatE (M55161)	Claiborne et al. (1979); Loewen and Switala (1986); von Ossowski et al. (1991)
Klebsiella pneumoniae	80000	heme d dimer	KpnkatA	Goldberg and Hochman (1989)
Pseudomonas putida	96000	hexamer	PpukatA	M.G. Klotz and A. Anderson (pers. comm.)
Xanthomonas oryzae	80000			Chamnongpol et al. (1995b)
Mycobacteria				
Mycobacterium avium	78184		MavkatE (L41246)	A. Milano et al. (unpubl.)
Phototrophic				
Rhodobacter spheroides	56000	heme b tetramer	Rspkat	Clayton (1959)
B. Catalase-peroxidases				
Gram-positive				
Bacillus YN-2000	73000	heme b tetramer		Yumoto et al. (1990)
Bacillus firmus	84000	heme b	BfikatI	Hicks (1995)
Bacillus stearothermophilus	82794	heme b dimer	BseperA (M29876)	Loprasert et al. (1989)
Gram-negative				
Comamonas compransoris	75000	heme b dimer	Ccokat	Nies and Schlegel (1982)
Escherichia coli	80033	heme b tetramer	EcokatG (M21516)	Claiborne and Fridovich (1979); Triggs-Raine et al. (1988)
Klebsiella pneumoniae	62500	heme b tetramer	KpnkatG	Hochman and Goldberg (1991)
Salmonella typhimurium	80042		StykatG (P177500)	Loewen and Stauffer (1990)

(Continued on following page.)

Table 1 (continued)

Bacterial species	MW	Features	Designation (accession number)	References
Mycobacteria				
Mycobacterium intracellulare	81428	tetramer	MinkatG (M86741)	Wayne and Diaz (1988); Morris et al. (1992)
Mycobacterium smegmatis	80000	tetramer	MsmkatG	Marcinkeviciene et al. (1995)
Mycobacterium tuberculosis	80039	heme b dimer	MtbkatG (X68081)	Zhang et al. (1992); Heym et al. (1993); Nagy et al. (1995)
Phototrophic				
Rhodobacter capsulata	61524	heme b tetramer	RcacpeA (X71420)	Hochman and Shemesh (1987); Forkl et al. (1993)
Archaebacteria				
Haloarcula marismortui	81292	heme b	Hmacpe	Cendrin et al. (1994)
Halobacterium halobium	60300	heme b tetramer	Hhacpe	Brown-Peterson and Salin (1993)
Halobacterium halobium	110000	heme b	Hhaper	Fukumori et al. (1985)
Streptomycetes				
Streptomyces sp.	78000	heme b dimer		Youn et al. (1995)
C. Non-heme catalases				
Gram-positive				
Lactobacillus plantarum	28300	Mn hexamer		Kono and Fridovich (1983a)
Gram-negative				
Thermoleophilum album	34000	Mn tetramer		Allgood and Perry (1986)

sufficiently characterized to allow inclusion in this comparison include cyanobacteria (Tel-Or et al. 1986), diverse lactic acid bacteria (Engesser and Hammes 1994), *Porphyromonas* (Love and Redwin 1994), *Rhodospirillum rubrum* (Nadler et al. 1986), and various pseudomonads (Itoh et al. 1992). From a quick perusal of Bergey's Manual (Buchanan and Gibbons 1974), it is obvious that there are far more organisms with catalases than are listed in Table 1, but the presence of these other enzymes has been deduced by the simple H_2O_2 spot diagnostic assay, not by sequencing or purification.

Monofunctional Catalases

Primarily on the basis of subunit size and heme content, the group of monofunctional catalases has been subdivided into two subgroups. The largest group contains the typical catalases that closely resemble eukaryotic catalases (Nicholls and Schonbaum 1963; Deisseroth and Dounce 1970), both in size and heme content. Normally, these enzymes are homotetramers of 55-kD to 65-kD subunits with one heme b (protoheme IX) per subunit, but a number of variants are evident, including dimeric and hexameric structures, and with varied pH and thermal sensitivities (data not tabulated). A unique heterodimeric variant has been partially characterized in *Pseudomonas syringae* where the enzyme CatF appears to contain two *catF*-encoded 67-kD subunits and two as-yet-uncharacterized 20-kD subunits. The diversity within this group is further illustrated by the catalase from *Streptomyces venezuelae*, which has an associated bromoperoxidase activity but which is closely related to typical catalases through sequence similarity.

Catalases in the second smaller subgroup of atypical enzymes generally have larger subunit sizes of 80 kD to 84 kD and contain heme d. Although its heme component has not been characterized, the *Mycobacterium avium* catalase has been included in this group on the basis of its larger size and sequence comparisons, to be discussed below. One of the catalases of *Bacillus firmus* has also been included in this group on the basis of its physical characterization and sequence comparisons, although only a portion of its sequence has been determined. The catalase from *Xanthomonas oryzae* with a subunit size of 80 kD will in all likelihood be a member of this group when its sequence is determined (Chamnongpol 1995a,b). These larger enzymes exhibit significantly enhanced stability and retain activity at 70°C, in 7 M urea or 1% SDS, and over a broad pH range from 3.0 to 11.0.

With seemingly unique properties among the atypical subgroup of catalases, why are they not treated as a separate group distinct from the typical catalases? The answer lies in the fact that both typical and atypical catalases share a very similar core sequence of approximately 350 residues, consistent with a distant ancestry. Thus, the division into two related subgroupings has been made on the basis that the two subgroups have similar sequences but slightly different physical properties. The sequence similarities of the enzymes are discussed below. It should be noted that apparently similar atypical enzymes have been characterized from *Penicillium vitale* (Vainshtein et al. 1986) and *Neurospora crassa* (Jacob and Orme-Johnson 1979).

Catalase-peroxidases

The group of six bacterial catalases that also exhibit an organic peroxidase activity, capable of utilizing *o*-dianisidine as substrate, are listed in Table 1B. They have been found in a broad range of bacteria including gram-positive, gram-negative, and phototrophic bacteria; mycobacteria; halophilic archaebacteria; and streptomycetes. Some, such as those from *Bacillus stearothermophilus*, *E. coli*, and *Haloarcula marismortui*, were isolated initially as peroxidases with an associated catalatic activity. More recently, in recognition of the ubiquity of the class, newly characterized members have been reported as catalase-peroxidases. Most commonly, the enzyme is a homotetramer of 80-kD subunits, but variants with both smaller and larger subunits and with homodimeric structures have been found. Unlike the more typical catalases, these enzymes exhibit relatively sharp pH dependency. As discussed below, there is a significant sequence resemblance to plant peroxidases, confirming the relationship of the enzymes to the family of peroxidases.

Perhaps the most notorious of this family is KatG or HPI of *Mycobacterium tuberculosis* (Zhang et al. 1992) and *M. bovis* (Wilson et al. 1995), which has been implicated as one of the determinants, along with *inhA*, in isoniazid sensitivity of the organisms. The importance of isoniazid lies in its being a front-line drug used in tuberculosis treatment, and resistance to it is an increasing problem. A certain fraction of isoniazid-resistant strains of *M. tuberculosis* have been characterized as having a defect in the *katG* gene that prevents potentiation of the antibacterial activity of isoniazid by the peroxidatic reaction of HPI (Rosner and Storz 1994; Hillar and Loewen 1995). On the basis of sequence similarities with plant peroxidases for which crystal structures are avail-

able, and the sequence characterization of a number of *katG*-containing mutants from *E. coli* (Loewen et al. 1990) and *M. tuberculosis* (Altamirano et al. 1994; Cockerill et al. 1995; Heym et al. 1995), a site-directed mutagenesis study of structure-function relationships in the enzyme is being undertaken.

Non-Heme Catalases

Originally referred to as pseudo-catalases because they were insensitive to the common catalatic inhibitors, azide and cyanide, this class is now accepted as a distinct group of catalases. This designation is quite legitimate in view of the obvious catalatic activity, and it simply increases the obvious diversity among catalases by establishing a class of non-heme enzymes. Only two enzymes in this class have been purified (Table 1C), one each from a gram-positive and a gram-negative organism. Subunit sizes are smaller than in heme-containing catalases, and both enzymes were stable at up to 80°C. The common cofactor in both cases is the Mn ion. Despite the small number in this group, other examples will undoubtedly appear when multiple catalase isozymes found in some bacteria are further characterized. Unfortunately, this class remains largely undeveloped.

Sequence Comparison of the Catalases

In 1993, the 4 then-sequenced bacterial catalases were included in a phylogenetic analysis of catalase sequences that placed them apart from the plant, fungal, and mammalian catalases (von Ossowski et al. 1993). Additional comparisons have been carried out in conjunction with the publication of new catalase sequences, but several new sequences became available in late 1995, increasing the number of bacterial catalase sequences to 20. In addition, there are 6 bacterial catalase-peroxidase sequences available for comparison. Because of the unrelatedness of the catalase and the catalase-peroxidase sequences, alignments and parsimony analyses have been carried out separately on the two groups. No sequences for non-heme catalases are yet available, making comparison impossible.

Monofunctional Catalases

An alignment of the 20 bacterial catalase sequences along with yeast catalase is shown in Figure 1 and reveals significant similarity among the sequences in a core region of about 370 residues. Using such alignments, a phylogenetic comparison of the 20 sequences was carried out using the

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3  MSQHNEKNPHQHQSPLHDSSEAKPGMDSLAPEDGSHRPAAEPTPPGA

    50                                              100                                       150
                                    LTT  GAPV    QNS TAG  RGP L QD    EKLAHFDRERIPER VHAKGSGA G F
1  --- ---MSNEREMQNKKDQQLESFRVEDEGKK  NQ LK SED-EF LK E   T ME FHFR MT  H      I   R FA H E QVYDS-----M K
2  --- MSDDQNKRVNEHSKDEQLEQYRTDNSGKKM NQ LR SED-EH LK V   T ME FHFR MT  H      V   R F VH F QVYEP-----M T
3  QPT APGSLKAPDTRNEKLNSLEDVRKGSENYA  NQ VRIADD-  LR S   T LE FILR IT  H      I   R  A H Y QPYKS-----L S
4  --- -MATDHTSDAPDPKQRDLESARFRRDTGY  QQ VR DHT-DDAL V E   T LE FHAR IT  H      V   R A  Y Y EPYDDR----L A
5  --- ----------------------MTDRRN  NQ V IGDN-  M   L K T LE YVLI  LA      V   V   R A  H K -----VTKKSM K
6  MSD ENNKGVGTAVQGVGGPRDGRTAPGEQGTT RQ H  HDN-  R V S  MTLENYQFI  S       V   R V  H V RATGKVGDEPV S
7  MNP SLNAFRPGRLLVAASLTASLLSLSVQAAT RDN   GDN-  Q   P N SV L  VQLLQ  QR      V   R T  H E -----VASADI S
8  --- MPLLNWSRHMVCLTAAGLITVPTVYATDT RDN  V GDN-  Q   A Q  V L  VQLLQ  QR      V   R T VK E -----TASADI S
9  --- ---------------------VPHATGS REN  AVSD-RE L V S E  IVLH VHLL THQ   MN  RP      F V EVTE-----DV S
10 --- ------------------------MSSNK  SW  GDN-  M   S   T I  VHLL      N   V   V      A  H Y EVTN-----DV T
11 --- ------------------------MTNQ  NE Q  ADN- H Q  AN AA S I  YQLL      N      V      A RK Y KVTK-----DM S
12 --- ----------------------MENKK  AAN R IAND-  Q   P   IML  PWLI         V      RM      Y T TVTH-----DI T
13 --- ---------------MSSQCPFSHLAATN  MGN  VND-  L   P   L A  LWLN   D V  V      RM      F T TVTH-----DI T
14 --- ------------------MTTSKCPVTH  MNN  ADN-  L   T   L T  LWLN   D V  V      RM      F T TVTR-----DI T
15 --- ----------------------MEKKK  AA   VDN-N VI   P   M L  VWFL         V      RM      F T TVTH-----DI T
16 --- --------------------MNAMTNKT  AA   AND-N TM   P   A L  VWFL         V             Y T TVTH-----DI S
17 --- ------------------------MKK  NDF NIIAND-  LS  T K  L M  YLLL     QN        T           Y EIKITA-----DL S
18 --- ---------------------MTDRPIM  SA  IPDN-  L   E   I M  YQLI    S QN       A           Y TLTITG-----DI S
19 --- -------------------------------------------  L  S   V M  YHLI   M   N      V        Y  Y T RLTR-----SL S
20 --- -----------------------MTQGP  EA   ADN-  E   V G  V V  QLLL      N      V     R A  Y T TLTR-----DV S
21 --- ---------------MNVFGKKEEKQEKVYSLQN F YSHHPYA QYSRP D  I LQDFHLL NI S     V    V     G CRLE ELTD-----SL S

                                                  200                                          250
   YT A         GK TP F RFSTVAGE G AD  RD RGFA KFYTEEG N D VGNNTPVFFIRD  KFPD  H  KRDP               WDFW  L PESL
1  EF K KFLQDPSVK V V       SK S ETV  A     T         Y L       I       Q AI    LV AL PE HNEIPQAQSAHDTF   IA NNQ  A
2  E  R KFLQDPSVK V V       SK S  TV  A     T         Y L       I       Q AI    LV AF PE HNEMPQAATAHDTF   VA NN   A
3  DI K DFLSDPN I  V V      Q GA S  TV  I     T         IF L             Q AH    FV AV PE HWAIPQGQSAHDTF   YVS  Q  T
4  Q  A KFLTSP TR  V V      SR S  TV  V     T      Q    Y L       F      Q GI    LV AV PE HNEIPQAQSAHDTL   VS  Q  T
5  K  K QFLQEE TE EV A      IHGQHSPETL  P    SV          Y F       L        AI    VI SL P  RTNIQD----GNRY   FS  T  A
6  K  R KLFQED  E  V V      GHGTHSPETL  P    V        DA  W F V   LKI       AL    LI SQ PS TTNIQS----QERIF  FA GS  AT
7  DLSM KVFRK- EK  V V    A VHGNHSPETL  P    T       AD  W L       F T      AI    MV AF P  RSNLDD----DSRRF  FS HV  AT
8  NLSK TVFKS- EK  V V    S VHGNHSPETL  PH   T       AD  W L       F T      AI    MV AF P  RTNLDN----DSRRF  VL PRTG H
9  S  K LVFEP- VE EVLL        N SP TW  T     LR    S    Y L          I   L PM  THFIRSQ  L NSGLRDA----TME    T NN  A
10 K  K AFLSEV  R  L I        L S  TV  P      V          Y I                 AI    FI TQ    KTHLKNP----TAV    S  S
11 A  K AVFSGV  K  LIT     Q   A YP TY  V      V          Y I              VN PL    FI SQ    RTHARSQ----DMQ    S  S   V
12 K  R AIFSQV  Q EC V        R A  AE  I      M           W L              L PL    LN AV    RNNMRSA----NNN    T  L  A
13 K  R KIFSEV  K EM A        R A  AE  I      L           W L                L PR    LNKAV    RTNMRSA----TNN    T  L  A
14 K  R KIFSEV  K EM G LA     R-G  AY RV      L           W V          YP- LR    LNKAV  S-AHQYSSA----TNN    A  L  A
15 K  R KIFSEV  K EM A        R A  AE  I      L           W M          YL PL    LN IV    RTNMRNM----AYK    FS HL
16 R  R RIFAEV  Q  L L        R A  AE  V      I    D      W L                PL    FI TQ    KTNLRNA----TAA    S  N
17 A  K KIFQK- EI  L L        A A  AE  V      I    K      W L           T     AY    FI TQ    RTHLRSN----NAA    S  C
18 R  K KVLQP- AQ  MLA        L A  AE  V      L    Q      W L              V PL    FI TQ  H RTHLRSA----TAM    S  S
19 EF R KVLTEV  DV MVA      G  S  S  TA  P      L          W L         I  V  AI  S FIRTQ    RTHLKP----HWRR    S  S  AI
20 RW R AFLSEV  R ET L        SL A  AV  P  W L             Y L              K AI    FI TQ    YTGSQEA----DNV    G  S   T
21 DI Y APYQNV YKC GLV      G  S TPDTA  P  VSF    W      H W F          L  AI    VFI SQ    QSHLNQFQD-TTIY  YLT  N  I

                                          300                                                350
   HQVTI MSDRGIPASYR M GFGSHT     N  GE    VKFH K    QGI NL     EA    G D      RDL EAIE GDFP W L VQ     E  D       F PFD
1   M MWA     S  R F M  E    V  FRFV EE KAHF     W  PVL IHS VWD  QKIA K PDFHR   W S  N  Y EYE G  LIS E  EFNFD DVL
2   M MWT        R   M  E    V  FRFV EQ KARF     W  PVL VHS VWD  QKIA K PDFHR   W T  N GKVEYE G  MID E  EFKFD DIL
3   N MWA        R   T  E    I  FRLI AE KATF R   W  PLA KAS VWD  QKLT R PDFHR E W     A    EYE GF LIP E  EFKFD DLL
4   AIMWL    AL  R   M  Q    V  FRLV AR RGTF     W  PRL VHS IWD CQKIA K PDYNR   W     S QY E E G  LVA D  EFSFD DLL
5  TMI YLF  E T      EIR SSV AFKWI EE KTVY  LRWV  PKA IV  STDQ AQIQAKEFNHAS   Y    N  Y E D Y  VLDPK  LDNYD N L
6   MI LLY PW        F Q S VN YKWV DQ  GVL  Y WE  PV  VR  TQMQ DEVQATNFNHATQ  RD     Q D F KIMEDG EHPELD D L
7  RTL LLY NE T       E D NSV AYKLV AR  VHY     W  SL  QK  DPKQVAEVQ R YSHMTN  VS  RK    K D Y  VLKPE  LAKFD D L
8  PYADPVVFE  H S    F D N V AYKLV AK  VHY     W  SL  IK  DPK VAQVQSK YSHLTN  VG  KK    K D Y  VLKPE ELAKFD D L
9     YL GP   L RTW E D Y    YLWV AE AKHW  Y FI  SQE VH  SND  TQIA ENADFHRQ  F V AK V  K N YI AIPYS QGKTYR N
10       L          TL H H      FKWT AEP GVWI Y F  TE  VK  DVNT AKIA ENPDYHTE  FN    N  Y A K Y  IMPLE  ANTYR D
11       L             M H      FKWV AQ  QFW I  F  TN  IH  SNEL DELA K TDYLQN  FD    T  Y S TVA  LVLMK MAELSPKDI
12       T  P          H H      YSFL AENKRIW    L  TM  IK  TDQ  EAIIAK RESHQ   Y S   R    K KFQI LMT E EADNYRIN
13     VV              H H      YSFW EA  RFW    FR TQ  IK  TDA  AEIIAN RESHQ   Y      R    K T F  IMP A  AEKVPYH
14      V              H H      YSLW EA  RFW    FR SQ  IK  TNE  AKIIAD RESHQ   Y      R E  K TMYI VMP A  AAKVPYH
15   L  D      L L  FVH        YSFI KDN RFW    FR CQ  IK  MDD  EALV K RESSQ   F      R Y R K QI IMP K  EASTVPYN
16       L     L QN  QQH       YSFV DA  RFY    F  SQ  IACYTDG  AELV R RESAQ   FQN  Q Q  R T K  VMP A  EAATYHIN
17       L R           H H      YSFI DKN RFW    F  TQ  IK  TNQ  AELIAK RESHQ   YN   NK    K KVQ  ILA K  IEKLG N
18       L     L TDV HIN Y      YSFW DA  RYW    F  TM  HKHWTNA  EQVI RTRESTQE  FS   N E  K KVQ  IMP L  ADKTPYN
19    MFLY    T K A F N Y      FSLW DR  RHW    FH TK   Q FSAD  DEVA K PDYSSA  ST     Q    K KVS  LMP L  ADTYRIN
20    WLFG        H N Y        YQWN EA  VFW  Y F  TD  IK  TQD  NRLA E PDSHQ   R      R    T TVQ  IMPAA  AAGYR N
21   I YMFGDRGT  WAS NAYSG SFIMV KE KDTY Q  VL  SDT FET TGDK AELS SHPDYNQAK FTQLQN EK KFNCY  TMTPE QATKFRYSVN
```

Figure 1 (Continued on facing page.)

```
                                             400                                                450
    TK WP    P    G   LNRNP N FAE EQ AF P  N  VPGI  SPD MLQGRLFSY D QRYRLG  N  Q PVNRP          RDG
1   P I EEEV VKII KMT VD V T V H G H-V DFTN PL T T LI GP FHEL I VCPFHNN Q YGRQTINKGQ-V
2   P L EELV VKII KMT QD V T V H G -V DFTN PL P T T LI GP FHEI I VCPFHNN QY YHRMTINKGP-V
3   P LI EELV VQRV KMV D F N A H G H-I DFTN PL T T IS GP FHEI I TCPYHNF Q MHRMGIDTNP-A
4   A II EEQV VLPV KMV D F T V HTA -V DFTN PL F N L T LI GP FA L VAQVRTN QH YAQHAIPQGR-S
5   A D FEDVF YEHV TMT D I T SVG N G V-L MLP E RV S T H V -P YL L I S KTPVDNN Q QMPFKQQTSS--
6   D I REQF WRHV QMT E V T A GTG V-L D LDF D K T S T V -P YL L I A KKHVATN Q QMAYRVDTFEGQ
7   A I G--I ERKI QMV VD F Q T V MA S -L EP E RL A A T M V -A GLGL RSEVNTV NQ ALNAGHSTSG--
8   A I D--V EKKI QMV K VD F Q T V MA A -L EP E RL V A T M -AIGLSL Q KVAVNNG NQ ALNTGHTTSG--
9   L TISQANY RIKV VNT K F I SA S S -T GL R L A A H A L V -AHVN L S DDATHNY AFE EMWEDHTGNR--
10  V V SQKDY LIEV RMV D E Y V AT S G T-L DV K A H AH V -A HQAL I ARNKVNNY Q QMRFDDNGGGSV
11  V VISQKDY LIEI QMV DE T N EDIQEL S A -L EA KL G K AE -A YE L KVPVHNY E AMAQNQATG---
12  L V HKDF LQDV ILE E Y V S N M -I E GF K G A -V SE I K RCPFHAF H AMRVDGNYGSAK
13  L V SKKDY LIEV EFE E F DV S A S -L GA R A N A A -VCYR I RCPVHSN Q QGRVDGTYGSLP
14  L V KKDY LIEV EFE E F DV S A S -L GA K A N A A -V FR I RCPVHSN Q QGRATELRQP--
15  L V HADY LMDV YFE D Y SDV A S A -I SF K G AH -V HH I A KCPFHNY H AMRVDGNSGNG-
16  L V HADY LIEV VLE K E Y V A T A -V GF K G TH -I HH I A RCPFHSF H MGRVDGNGGAT-
17  L I HSLV LMDI EMI K Q Y N V A S S -I GF K A I P A -T YHLL AKSEVNTY NVA AMNFDSYKNDA-
18  L V HADY PIDI VME E Y T V NA S S -I GF K A I A AH H -THYESI Q KCPVHHY H QMNVYGGIKTGN
19  L V HGDY LIEI EME E Y I S E S N-V GF HK N VL A AH -V YQ I QANADVQTY H AMRTDGNHGAQV
20  L V HEDY PVEI TLE E I V SI S A H-F GP K A G AH V -I ADHL HATEART HSRDGFLYDG---RHK
21  L I HKEF LRKF TIT TE VD Y Q I V S T TCI KP N SV A P T H -A YQ L RNLGCPY SKGDSQYTAEQCPFKA

                                  500                                                          550
1   SYHKNSLAANTP--QPASEEEGGYAHYQEKVEGR KVRKRSES--FKD------------------------------------- ---------------------
2   AYHKNSLQNNDP--SPATAEEGGYVHYQEKVEGK KIRQRSDS--FNDYYSQAKLFWNSMS-PVEKQHIISAFCFEVGKVKSKDV QRQVVDVFSNVDADLAEEIFH
3   NYEPNSINDNWPRETPPGPKRGGFESYQERVEGN KVRERSPS--FGEYYSHPRLFWLSQT-PFEQRHIVDGFSFELSKVVRPYI RERVVDQLAHIDLTLAQAVAK
4   SYFKNSIGGGCP----ALADEDVFRHYTQRVDGQ TIGKRAEA--FQNHYGQARMFFKSMS-PVEAEHIVAAFAFELGKVEMPEI RSAVVAQLARVDDQLAAQVAA
5   --INYEPNSYDTE---PKENPAYIEPEQEIRGDI SGRLVAE---KPNNFGHAKEVWKRYS-DAERAALVKNIVDDWEGVR-EDI KIRNLRNFYQVEPEFAERVAA
6   DQRVNYEPSLLSG---PKEAPRRAPEHTPRVEGN LVRAAIE---RPNPFGQAGMQYRNFA-DWERDELVSNLSGALAGVD-KRI QDKMLEYFTAADADYGQRVRE
7   ---VNYQPSRLDP----REEQASARYVRTPLSGT TQQAKIQ---REQNFKQTGELFRSYG-KKDQADLIASLGGALAITD-DES KYIMLSYFYKADSDYGTGLAK
8   ---VNYEPSRLEP----RPADDKARYSELPLSGT TQQAKIT---REQNFKQAGDLFRSYS-AKEKTDLVQRFGESLADTH-TES KNIMLSVLYKEDPALRHSGSR
9   --STYVPNSDGNS----WSNEVGPTNNGWEAVGV LTREAEALRANDNNFGEAGTLTREVFSNEERNNFVQTVAGALKGVR-SNV EAEAFVYWKLVNATNGERIQN
10  ---YYEPNSFGG-----PKESPEDKQAAYPVQGI ADSVSYDHY---DHYTQAGDLYRLMS-EDERTRLVENIVNAMKPVEKEEI KLRQIEHFYKADPEYGKRVAE
11  --VNYEPNSQDG-----PTEVPAAKIHGDQLSGT TGNFSADP----DYYSAAGKLYRLLS-ADEQTRLIENIRMNLGQVTKPEI QIREVKQFYQADPEYGRRVAT
12  ---GYEPNSY-GE----WQDSPEKKEPPLKVHGD VFNYNERE-YDDDYYSQPGDLFRLMP-ADEQQLLFENTARAMGDAE-LFI KQRHVRNCYKADPAYGTGVAQ
13  ---HYEPNSF-SQ----WQQQPDFAEPPLRISGD AAHWDYRT-DDNDYFSQPRALFNLMN-AEQKQSLFNNTAAAMGDAP-DFI KYRHIRNCHWCDAAYGEGVAK
14  --AHYEPNSF-GQ----WSQQPDFAEPPLKINGD AAHWDYRQ-DDDDYFSQPRALFNLMN-DAQKQALFDNTAAAMGDAP-DFI KYRHIRNCYRCDPAYGEGGSK
15  --ITYEPNSG-GV----FQEQPDFKEPPLSIEGA ADHWNHRE--DEDYFSQPRALYELLS-DDEHQRMFARIAGELSQAS-KET QQRQIDLFTKVHPEYGAGVEK
16  --LNYEPNSF-GE----WREAKHAAEPPLALDGQ AADRWNHR-VDEDYYSQPGALFRLMN-DDQKQQLFGNIGRHMAGVP-EEI QRRQLEHFRRADPAYAAGVAK
17  --AYYEPNSYDNS----PKEDKSYLEPDLVLEGV AQRYAP---LDNDFYTQPRALFNLMN-DDQKTQLFHNIATSMEGVD-EKI ITRALEHFEKISPDYAKGIKK
18  PD-AYYEPNSFNG----PVEQPSAKEPPLCISGN ADRYNHR--IGNDDYSQPRALFNLFD-AAQKQRLFSNIAAAMKGVP-GFI VERQLGHFKLIHPEYEAGVRK
19  ---DYEPNSF-GG----PKQDPSFSEPPLRIQGD AGRYGWPG-DDEDLYGQPKLFWTKVLDEGGRKRLVENIVTSMGDSP-RHI QERMIAHWFKVHEDFGRGVAQ
20  GA-KNYEPNSFGGP---VQTDRPLWQPTPVTGVT GDHAAPSH-AEDDDFTQAGDLYRLMS-EDEKGRLIDNLSGFIAKVSRDDI AERAIGNFRRADEDFGKRLEA
21  VNFQRDGPMSYYNFGPEPN--YISSLPNQTLKFK NEVNDEVSDKFKGIVLDEVTEVSVRKQEQDQIRNEHIVDAKINQYYYVYG ISPLDFEQPRALYEKVYNDEQ

                              600                                          650
1   ---------------------------- ------------------------------------------------ ---------------------
2   AKGVGVAAPAKRKASKEN------LTSPA LSQART-VKTASTRKVAVLAGNGEKELQTVLEALKQEGITVDIISQNLG- YMTSGSGQQLEASGTFLTVDSVLYDA
3   NLGIELTDDQLNITPPPDVNGLKKDPSLS LYAIPDGDVKGRVVAILLNDEVRSADLLAILKALKAKGVHAKLLYSRMG- EVTADDGTVLPIAATFAGAPSLTVDA
4   KLGLPEPPEEQVDESA------PVSPALS QVTDGGDTIASRRIAVLAADGVDVVGTQRFTELMEQRGAVVEVLAPVAGG TLAGGSGGELRVDRSFTTMASVLYDA
5   GTGINLAEHVIDLK-------------- ------------------------------------------------ ---------------------
6   GIQAKEAEMKGQKQEA---------PVYG TEASSLY----------------------------------------- ---------------------
7   VAGADLQRVRQLAAKL---------QD-- ------------------------------------------------ ---------------------
8   SRQRRSEQGQVPGCKP---------QGLM I----------------------------------------------- ---------------------
9   TVKRKSGNGIPGVEAGGEARM------- ------------------------------------------------ ---------------------
10  GLGLPIKKDS------------------ ------------------------------------------------ ---------------------
11  SVKLRFSSV------------------- ------------------------------------------------ ---------------------
12  ALGIDLEEALKE---------------- ------------------------------------------------ ---------------------
13  ALGLTVEDALKARDTDPALGQGGLL---- ------------------------------------------------ ---------------------
14  ALGLTVEEPQAARATDPALGQGGLL---- ------------------------------------------------ ---------------------
15  AIKVLEGKDAK----------------- ------------------------------------------------ ---------------------
16  ALGLK----------------------- ------------------------------------------------ ---------------------
17  AFPIVSRIRAWRNHGHRCFLCEIVIRSQF HTTYEPEA---------------------------------------- ---------------------
18  ALKDAHGYDANTIALNEKITAAE----- ------------------------------------------------ ---------------------
19  GLGIDTAQAAAE
20  AVQALRG--------------------- ------------------------------------------------ ---------------------
21  KKLFVHNVVCHACKIKDPKVKKRVTQYFG LLNEDLGKVIAEGLGVPWEPVDLEGYAKTWSIASAN------------- ---------------------

                          700                                              750
2   VYAAGG----LELKDNKQAMAFIR EAYNHYKAIGAANEGIDLLQSS---------VGTTEGLGIVTAKDEPDYT --AFSKAFI DAVAAHRH WDRRI----
3   VIVPCG--NIADIADNGDANYYLM EAYKHLKPIALAGDARKFKATIKIAD-------QGEEGIVEADSADGSFM DELLTLMAA HRVWSRIPKIDKIPA---
4   VVVACGPRSVSTLSDDGYAVHFVT EAYKHLKPIGAYGAGVDLLRKAGIDNRLAEDTDVLNDQAVVTTKAAADEL PERFAEEFA AALAQHRCWQRRTDAVPA
```

Figure 1 (For legend, see following page.)

highly conserved core of 340 amino acids that gave rise to the relationships shown in Figure 2A. The sequence of the typical catalase from the yeast *Saccharomyces cerevisiae* was used as an outgroup for comparison. A similar phylogenetic tree was obtained using the complete sequence of the enzymes rather than just the core (M.G. Klotz and P.C. Loewen, unpubl.). The most striking feature of Figure 2A is the existence of two main groups that are inconsistent either with the groupings based on molecular comparison of 16S rRNA sequences or with common distinguishing structural features. Instead, the groupings appear to be based on the ecological niche of the organism. One group is made up of normally nonpathogenic bacteria that are widely spread in nature, including such organisms as *B. subtilis, E. coli, Pseudomonas aeruginosa,* and *P. syringae*. The second group is composed mainly of pathogenic bacteria, including *H. influenzae, Neisseria gonorrhoeae, Bordetella pertussis,* and *Brucella abortus*. Such a division would imply evolutionary pressures on catalases that differ between these two environmental niches. One rationale might be that there are greater demands on catalases of pathogenic bacteria in the hostile intracellular environment which frequently experience defensive bursts of H_2O_2. Unfortunately, there are no obvious catalytic differences among enzymes from the two groups to support this concept, and the inclusion of more catalase sequences in the future may provide other interpretations. The distance tree was also determined and is shown in Figure 2B. Perhaps the most noteworthy features of this tree are the long branches to the individual catalases combined with the relatively short distances between the branch points in the core of the tree, suggesting that most evolution took

Figure 1 Alignment of 20 bacterial catalase sequences and the yeast T catalase generated by CLUSTAL-W (Thompson et al. 1994) and refined by eye. Where a residue is conserved in more than half of the 21 sequences, the residue is placed above the sequences and replaced with a dotted rectangle. The accession numbers and other information about the following aligned sequences are summarized in Table 1: (1) *B. firmus* KatA, (2) *B. subtilis* KatE, (3) *E. coli* KatE, (4) *M. avium* KatE, (5) *L. seeligeri* Kat, (6) *D. radiodurans* KatA, (7) *P. aeruginosa* KatB, (8) *P. syringae* CatF, (9) *M. luteus* Kat, (10) *B. subtilis* KatA, (11) *L. sake* KatA, (12) *B. fragilis* KatB, (13) *H. influenzae* HktE, (14) *N. gonorrhoea* Kat, (15) *P. mirabilis* KatA, (16) *B. pertussis* KatA, (17) *C. jejuni* KatA, (18) *B. abortus* Kat, (19) *M. extorquens* KatA, (20) *S. venezuelae* Kat, (21) *S. cerevisiae* CatT (accession number P06115). The numbering is consecutive throughout the sequence starting from the amino-terminal residue of the *E. coli* KatE sequence, the terminal 47 residues of which are shown alone at the top of the figure.

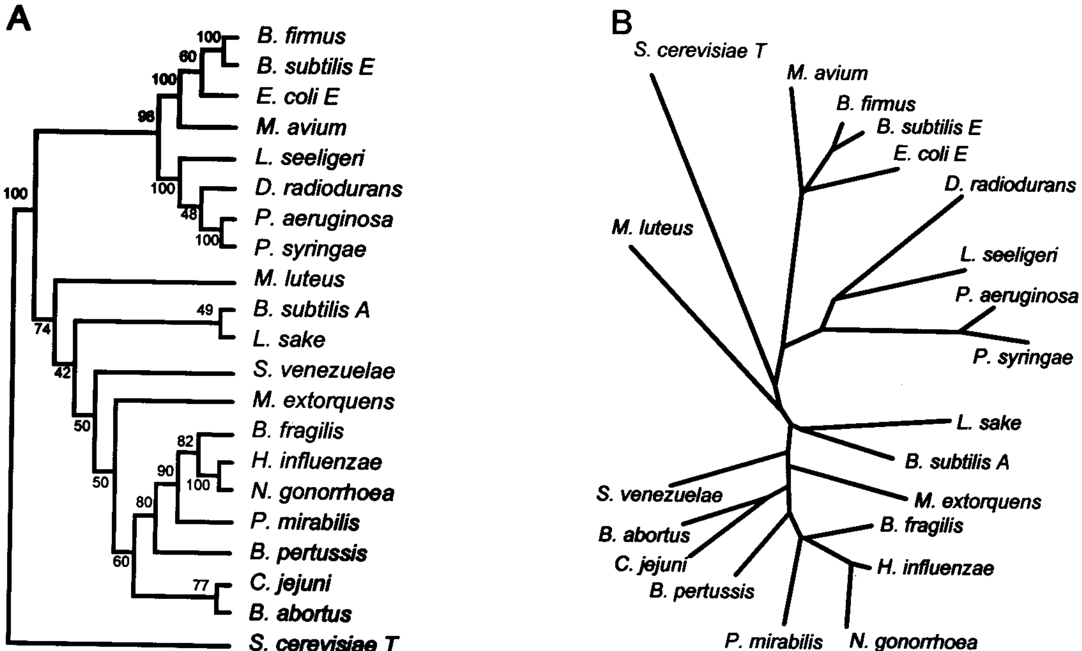


Figure 2 Unrooted phylogenetic tree based on the amino acid sequences of 21 catalases aligned in Fig. 1 constructed by (*A*) parsimony or (*B*) neighbor-joining methods. The core sequences from residue 101 to 446 in Fig. 1 were used for comparison. The numbers at the nodes in *A* are an indication of the level of confidence for the major branches as determined by bootstrap analysis using programs contained within the PHYLIP package version 3.5 (Felsenstein 1994) including SEQBOOT, PROTPARS, and CONSENSE. In *B*, the lengths of the arms are drawn to scale to indicate the branch lengths as determined by the programs PROTDIST and NEIGHBOR, also in the PHYLIP package.

```
                                                          50
                                                     DWWPNQL    LHQH    NPLG      FDY  EF
1  ---------MSTSDDIHNTTATGKCPFHQGGHDQSAGAGTTTR        RVDL N  SNRS    ED----   RK  S
2  ---------MSTTDDTHNTLSTGKCPFHQGGHDRSAGAGTASR        RVDL N  SNRS    ED----   RK  S
3  MSSDTSSSRPPQPDSGTASKSESENPAIPSPKPKAHAP-LTNR     D V DVSS  P  SPLS    DD----   AA  A
4  ----MPEQHPPITETTTGAASNG-CPVVGHMKYPVEG--GGNQ      R  NLKV   NPAVAD M AA----   AA VA
5  ------------MENQNRQNAAQCPFHESVTNQSSNR-TTNK         NLSI   DRKT  HDEE----N AE  Q
6  -------------MDGKDKATGKCPVMHGAMTAAGVS---NTS     A  NLDI   DTKG   NG-----   RAAVK
7  --MTTAVRLLPSLGRTAHKRSLYLFSAAAAAAAAATFAYSQSQKRSSSSP GGGSN GWNNWGKAAALASTTPLVHVASVE

                  100                                                     150
   KLD   ALK DL  LMT SQ   PWWPADYGHYGGLFIRMAWH AGTYR  DGRGGAG G  QRFAPLNSWP DN SLDKARR
1     YY-G  K  KA L E     -        W S A            G        SI         R Q-             V
2     YYS    G  KA L D    -        W S V            G        SI         R Q-            TV
3     VE-   A MIS   T     -D                   S  A       IH          Q M-             A
4  TSRLD-  TR IEEV  T     -            P             A       IH          G M-             A
5     YW-    E  RK   E    -D           P             S       IG         ST T-            AN
6  G  VG--  RA  HA   D    -        W         M        A  S  AA       GNT KPA               V
7   GRSYEDFQKVYNAIALKLR EDDEYDN IG  PVLV L   TS  WDKH NT  SYG T-Y  KKEFND  S AG QNGFK

                                                 200
   LLWPIK KYG K SWADL    AGNVALE  G  TFGFGAG REDVWEP      G  E                         PL
1       Q   Q I        FIL        NS FR                 DL-DVNWGD KAWLTHR------HPEALAKA
2       Q   Q I        FIL        NS FR                 DL-DVNWGD KAWLTHR------HPEALAKA
3       K   N I        ITY        SM FK      F          EE-ILW EE EWLGTDKR----YSGERELAQ Y
4      V K   K L       IVF   RCARNRWASRRSGS S GV Q  TDE-VYW KEATWLGDDG-----YS-VSDLEN
5  CYGRS RNT T SLGPICSFWRAMSL NRWVEKRLDSA     PLTSGIRKK-TFI DRKKSGSPLN------AIPVIASSKT
6        K    NAV      ILF   T  Y SM LK      F      I  A EKDVYW A KDWLAPSDGRYGDLAKPETMEN
7  F E  HKEFPWIS G-   FSLG VT VQEMQGPKIPWRC  RVDTPEDTT------------PDN---------------

         250                                                   300
   GA  MGLIYV NPEGP   PDP     AA  IRETF RMGMNDEETVALIAGGHTLGKTHG  G A  VGP PEAAPIE QGL
1    TE              DHSGE LS--  AA  A  GN                          A  PTSN   D         E
2    TE D       T    NHSGE LS--  AA  A  GN                          P AA SH  AD          A
3    TT               EGK   IA--  ID     G  A        A   V   SF     A  D DL   E          Q
4  A VQ              A NGN  MA--  VD      R  A    V  A    V     F     A  P DL   E        L QM
5  RSPRANGVNL RQ--  RRAGRQAGSKSRGISA   R                      F  A RG  P TH   E          A
6  A VQ              VNGQ   AR--T LH      A                  T     V  A  N  D KAL  D    DVTVRA
7  ---------  -----GRL  ADK--D DYV TF Q LN   R -  VALM A AL     LK -------------------

                                   350                                           400
   GW SSYG G G DAITSGLEVVWT TPT W  N F E L GYEW LTKSPAGAWQ  AVD      IPDPFD     R PTM
1    A TY S V A             Q   Q  S Y F N FK    VQ R         I FE    AP--EI       PSKK K
2    A    S V A             Q   Q  S Y F N FK    VQ R         I FE    AP--DI       PSKK K
3    K    T S K             P   K  D S L T Y      E              FT K GAGAGT       G-GAG A
4    K    T T K        I     N   K  D S L I Y      E              YT K GAGAGT       G-GPG S
5    I    K K S TI     I GA  P   Q  DTSYFDM F  D W             WM   PDEKDLA  AE PSKKVPTM
6  AGRTRIWAARRRRPSPR SRAPGPRIRRAG TWAISRCSS HD E               WKP TIAEEAKPL AT LTTRHD L
7  ---------------NS Y GP GAANNVF T E YLN LNED K E ND NNE WDSKSGY--MML TDYSLIQDPKYLS
```

Figure 3 (*Continued on facing page.*)

```
                                                       450
  LVTDL LR DP  E I RRFL  PE    AFA AW KLTHRDMGP  RY  GP VPK   IWQDP P
1      T  F  EF K S    ND QAFNE   R  F          KS  I   E   EDL      L QPIYNPT-EQDIID
2      T  F  EF K S    ND QAFNE   R  F          KA  I   E   EDL      L QPLYQPT-QEDIIN
3     IS  ES IYAD T  W DH  ELAD   K  Y  I       VS  L   W AEP-QL     V AVDHELVDDNDVAA
4   A   S  V  IY R T  W EH  ELADE RK  Y         VA  L   L   QTLL     V AVSTTSS-AKQIAS
5  MT   A  F  EY K A   HQN  EFAE   R  F         KT  L   E   EDF      I EVDYELT-EAEIEE
6  TDA MAMKV  STMRSV SSWPIRPPSTTLSRAPGSSCCIATW RRRATS A MC PR--  SAGPGAAGPTGW---DVAK
7  I KEYANDQ KFFKDFSKA EKLL NGITFPKD PSPFIFKTLEEQGL-- -------------------------


                 500                                                  550
  LK   I   SGLSVS LV TAW ASA   FR   DKRGGANG R RL PQ   W VN         L VLE IQ
1   FA AD       E  SV        ST  GG         A LA M  RD D  --AAAVRA P   K  KES GK-------A
2   AA AA      I EM SV       ST  GG         A LA A  RD D  --AVAARV P   E  KTT NK-------A
3   KKVLD     IPQ  K      SA ASY NT         G L  Q  RS E  EPSELDKV P   K  QDF NASASGGKKI
4   SQ RA      T  Q  S      A SS  GS        G I  Q  VG E  DPDGSAQGHSHP E  ESF TR--RGNIKV
5  I AK LN     T  E  K        ARS-ATRISAAT  R I  A  KD E  EPERLAKV S  RGHPART AE------KS
6  V AQ AA       AD  A     D  RT  QS Y       A I  A  KD AG EPERLAGACGARTDRGGAG AS-------
7  ------------------  ------------------------------------------------  ---------


                                      600
  S AS   VLAG     E AA   A    I V F PG   DA     TD E F   LEP ADGFRNY               L  KA  L
1   L DII     VVGV K   SA GLS H P A   RV   RQDQ   I M EL   I         RARLDVSTTESL ID  QQ T
2   L DII     VVGI Q   AA RVS H P P   RV   RHDQ   I M SL   I         RARLDVSTTESL ID  QQ T
3   LADLI     SAAV K   KD GYE S H A   RT   SQES   V S AV   R         IRPGEKAPLEQL IER YL G
4   F DLV  G CAPL K   KA GHN T P T   PH   SQEQ   V S AV   K         LGKGNRCRPSTS LD  NL T
5  KHRRLDR G TLRWKRQPATPALMSKCH SLAAAMRHKSK PMSKALPCWNRSQMASATIKSKSTRFRRKSCSST P-SSS
6  -V DVI     NLGVEQ --------------------- ------------A VSRWRCPS----------------
7  ------------------------------------- ------------------------------------


        650                                                    700
    PEMT LV GG R LGAN        GVFTD   G L NDFFVNLLDM   EW          EGRDR   G VK TA R DLV
1  LTA    A     M V      FDGSKN     RV V S            RY  KATDESKELF       ET E  F  S A
2  LTA    V     M V   T FDGSQN     KP V ST    A       RY  KPTDDANELF       LT E  Y  I A
3  VTG    V     L A      HGSSKH     RP A T            GT  KASETAENVY       AS AL W  TAN
4  LSA    V     L V      YKRLPL    EASES T            GIT EPSPADDGTY Q K -GS K  W GS V
5  ADR RNDGLS WRFARV P YRHLPH     RI V T            NY  VPTDSG--IY  I   KT E RW  I V  I
6  --P------ ------------------------------------------- ----------VA M AAMT
7  --------- ------------------------------------------- -----------------


                         750
  FGSNS LR    EVYA       KFV DFVAA WVKVMN DRFDL
1       V  AVA     SSDAHE   K           L     L------- --
2       V  ALA     CSDAHE   K           L     Q------- --
3       V  GLV     QDDAHG   E           S     K------- --
4       E  ALV     PMTRQA   TG          L     VR------- --
5       I  SYA F   QDDNQE   R  IN       A     VKKARESV TA
6  DGPS--------------------LTC  SRC TASATG--------- --
7  ------------------------  ------------------ --
```

Figure 3 (*For legend, see following page.*)

place after the sequences diverged. The branching of *Deinocococcus radiodurans* and *Listeria seeligeri* is slightly different in the trees in Figure 2A and B, a minor variation that reflects the lower bootstrap value at that particular branch.

Both typical and atypical catalases are included in the alignment in Figure 1 and the trees in Figure 2. The close sequence similarity among all members of both subgroups is the main reason for keeping them in the same group in Table 1. However, the fact that the two atypical heme-d-containing enzymes for which sequence is available (*E. coli* and *B. subtilis*) are grouped phylogenetically with the third enzyme that was identified as a potential member of the group (*M. avium*), provides support for the subgrouping. The inclusion of the *B. firmus* enzyme in the atypical subgrouping, despite the shorter sequence, is also expected, based on its physical characterization as containing heme d and having an 80-kD subunit (Hicks 1995). The reason for the shorter sequence is that only a portion of the gene was cloned and sequenced.

Catalase-peroxidases

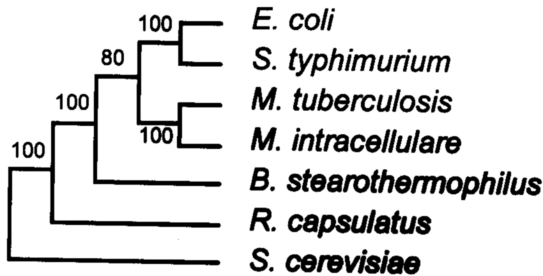
The alignment of the six catalase-peroxidase sequences is shown in Figure 3, and parsimony analysis of this alignment gives rise to the relationships shown in Figure 4 using yeast cytochrome *c* peroxidase as an outgroup. The yeast enzyme was aligned only with the amino-terminal 420 amino acids from the catalase-peroxidases, but the complete sequences of the latter enzymes were used in this comparison. This restriction was necessitated by the second region of weak similarity to the yeast enzyme in the carboxyl end of the apparently sequence-duplicated catalase-peroxidases (Welinder 1991). These results contain no surprises, with the enteric bacteria and mycobacteria grouping separately.

Figure 3 Alignment of 6 bacterial catalase-peroxidase sequences and yeast cytochrome *c* peroxidase generated by CLUSTAL-W (Thompson et al. 1994) and refined by eye. Where a residue is conserved in 4 or more of the 7 sequences, the residue is placed above the sequences and the residue is replaced with a dotted rectangle. The accession numbers and other information about the following aligned sequences are summarized in Table 1: (1) *E. coli* KatG, (2) *S. typhimurium* KatG, (3) *M. intracellulare* KatG, (4) *M. tuberculosis* KatG, (5) *B. stearothermophilus* PerA, (6) *R. spheroides* Kat, (7) *S. cerevisiae* cytochrome *c* peroxidase (accession number P00431). The numbering is consecutive throughout the sequence starting from the amino-terminal residue of the *M. intracellulare* KatG sequence.

REGULATION OF CATALASE SYNTHESIS

The general phenomenon of two regulatory pathways controlling bacterial catalase synthesis was inferred from the mechanisms operating in *E. coli* and *S. typhimurium,* where HPI synthesis is induced by H_2O_2 and

A



B

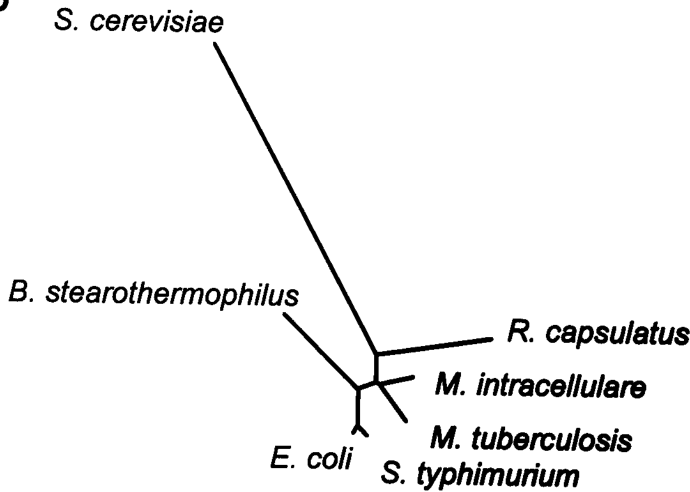


Figure 4 Unrooted phylogenetic trees based on the amino acid sequences of 6 catalase-peroxidases as aligned in Fig. 3 constructed by (*A*) parsimony or (*B*) neighbor-joining methods using yeast cytochrome *c* peroxidase as an outgroup. The numbers at the nodes in *A* are an indication of the level of confidence for the major branches as determined by bootstrap analysis as described in Fig. 2. In *B*, the lengths of the arms are drawn to scale to indicate the branch lengths as determined by the neighbor-joining, also as described in Fig. 2.

to a lesser extent in stationary phase, and HPII synthesis is induced in stationary phase (Loewen et al. 1985; Loewen 1992). In the past 5 years the regulation of catalase levels in a number of other organisms has been reported, and the two main factors influencing catalase levels in a majority of bacteria remain H_2O_2 in the medium and growth into stationary phase (Table 2). The rationale for why catalase levels should respond directly to H_2O_2 is that it allows the cell to remove H_2O_2, the substrate for catalase, before it has a chance to damage cellular components (Yonei et al. 1987). In the case of *E. coli* and *P. aeruginosa,* it makes further sense that the peroxide-inducible catalases, HPI and KatB, respectively, should be periplasmic enzymes, where they can protect respiratory-chain components and degrade incoming peroxide before it fully enters the cell (Heimberger and Eisenstark 1988; Brown et al. 1995). The parallel rationale for why catalase would be an asset in stationary phase cells is that the enzyme will serve as a protectant against peroxide that may be generated or encountered during periods of low metabolic activity. The importance of this response was demonstrated by the more rapid drop in viability of *katE*-containing mutants as compared to wild type when subjected to prolonged incubation under starvation conditions (Mulvey et al. 1990).

Despite the well-characterized nature of these responses in *E. coli,* there is clearly no uniformity in response patterns in other bacteria, with almost as many different apparent responses as there are bacteria. For example, HtkE from *H. influenzae* is a typical catalase but is induced by H_2O_2; CatB from *D. radiodurans* and KatA from *B. subtilis* respond to both H_2O_2 and stationary phase; and Cat-I, the catalase-peroxidase from *B. firmus,* is not induced by either peroxide or stationary phase. Therefore, it is possible to generalize that bacteria will respond to hydrogen peroxide and starvation with the synthesis of catalases, but each organism appears to have adapted the responses of its unique arsenal of catalases to specific needs arising from its environment.

It has become evident that oxygen levels may be a third general moderator of catalase synthesis in some organisms (Table 2). In fact, just such a role for oxygen in *E. coli* has been suggested (Hassan and Fridovich 1978; Meir and Yagil 1990), but because reports to the contrary (Yoshpe-Purer et al. 1977; Mulvey et al. 1990) cast doubt on the reproducibility of the phenomenon, *E. coli* has not been included in the part of Table 2 listing oxygen responses. The rationale for why catalase should be induced under aerobic conditions is that a cell is more likely to encounter or generate H_2O_2 during growth on O_2 than in its absence. The molecular mechanisms controlling the response of catalase to oxygen

Table 2 Regulatory responses of catalases

Bacterial species	Catalase or response	References
A. H_2O_2 response		
Bacillus firmus	Cat II	Hicks (1995)
Bacillus subtilis	KatA	Ishida and Sasaki (1981); Bol and Yasbin (1994)
Deinococcus radiodurans	CatB	Wang and Schellhorn (1995)
Escherichia coli	KatG	Loewen et al. (1985)
Haemophilus influenzae	HpkE	Bishai et al. (1994)
Neisseria gonorrhoeae	Cat	Zheng et al. (1994)
Pseudomonas aeruginosa	KatB	Brown et al. (1995)
Rhizobium leguminosarum		Crockford et al. (1995)
Salmonella typhimurium	KatG	Christman et al. (1985); Morgan et al. (1986)
Xanthomonas oryzae	Kat	Chamnongpol et al. (1995b)
B. Stationary phase response		
Bacillus firmus	Cat III	Hicks (1995)
Bacillus subtilis	KatA	Bol and Yasbin (1994)
Deinococcus radiodurans	CatB	Wang and Schellhorn (1995)
Escherichia coli	KatE	Loewen et al. (1985); Mulvey et al. (1990)
Pseudomonas syringae	CatF	Klotz and Hutcheson (1992)
Salmonella typhimurium	KatE	Fang et al. (1992)
Staphylococcus aureus		Martin and Chaven (1987)
Streptomyces coelicolor	Cat1, 4, 5	Kim et al. (1994); Walker et al. (1995)
C. Oxygen response (induction by oxygen)		
Bacillus coagulans		Vassilyadi and Archibald (1985)
Bacteroides fragilis	Cat	Rocha and Smith (1995)
Rhodobacter capsulatus	Cat-per	Hochman et al. (1992)
Rhodobacter spheroides		Clayton (1960a,b)
Staphylococcus simulans		Fondren et al. (1994)
Xanthomonas oryzae	Kat	Chamnongpol et al. (1995a)
D. Other responses		
Bacillus subtilis	sporulation	Loewen and Switala (1987)
Bacteroides fragilis	glucose repression	Gregory et al. (1977)
Haemophilus influenzae	stationary phase repression	Bishai et al. (1994)
Rhizobium leguminosarum	cell density repression	Crockford et al. (1995)
Xanthomonas oryzae	superoxide induction	Chamnongpol et al. (1995b)

remain unknown, but systems to sense and respond to aerobic/anaerobic changes have been identified in *E. coli* (Shaw and Guest 1982; Iuchi et al. 1986; Kalman and Gunsalus 1988), suggesting that similar systems exist in other organisms and may have been adapted to control catalase levels.

At a molecular level, the most detailed picture of catalase synthesis remains the one developed in *E. coli* (for reviews, see Loewen 1992; Loewen and Hengge-Aronis 1994; Schellhorn 1994). HPI, the catalase-peroxidase, is expressed constitutively in exponential phase under the control of the alternate sigma factor σ^S (Ivanova et al. 1994), and its levels increase two- to fourfold as cells enter stationary phase, presumably because of elevated σ^S levels. HPI levels also increase five- to tenfold in response to added H_2O_2 in a reaction mediated by OxyR, a member of the LysR family of regulatory proteins that responds to oxidant levels in the cell (Christman et al. 1989; Tartaglia et al. 1989; Storz et al. 1990; Kullik et al. 1995).

In *B. subtilis*, where *katA* is induced in response to H_2O_2, a somewhat different mechanism has been proposed (Chen et al. 1995). This has arisen from the identification of a potential operator sequence upstream of the *katA* promoter, the promoter of *mrgA*, encoding a protective DNA-binding protein, and the promoter of *hemAXCDBL*, the heme biosynthetic operon, all of which are coordinately induced by H_2O_2 and repressed by Mn^{++} ion. Interaction of this operator with a peroxide- and Mn^{++}-sensing repressor has been proposed as the controlling element of *katA* expression, although the repressor has yet to be identified. A sequence very similar to the hypothetical operator of *B. subtilis* has also been identified upstream of the *kat* promoter in *L. seeligeri*, suggesting that the operator-repressor sensor of peroxide may be a common alternative to the OxyR sensor of enteric bacteria (Chen et al. 1995).

HPII levels are expressed at a low level in exponential phase under the control of σ^S, and there is an increase in expression of eight- to tenfold as cells enter stationary phase. The principal and possibly sole determinant in the increase of HPII expression seems to be σ^S, with only indirect evidence suggesting the involvement of additional transcription factors (Meir and Yagil 1990; Mulvey et al. 1990). Therefore, any description of HPII regulation requires a discussion of σ^S regulation, a story with many facets. Starvation or medium depletion causes the formation of ppGpp by RelA as a result of ribosome stalling, and this has been correlated with the appearance of σ^S (Gentry et al. 1993). ppGpp appears to affect σ^S expression by enhancing both transcriptional elongation and a less clearly defined posttranscriptional process (Lange et al.

1995). In addition, an indirect involvement of ppGpp has been suggested to involve homoserine lactone (Huisman and Kolter 1994), and other mechanisms have UDP-glucose (Boehringer et al. 1995) and H-NS interfering with σ^S expression (Barth et al. 1995). Notwithstanding its possible uniqueness to *E. coli* and a limited number of related bacteria, the general picture of control of catalase levels in response to starvation and oxidative stress is summarized in Figure 5. The third control pathway involving oxygen levels is included despite a lack of detailed information and the possibility that it may simply be a manifestation of the oxidative stress response.

In addition to these three general mechanisms controlling catalases, there are a number of mechanisms that appear to be unique to individual organisms. These include superoxide induction, repression in stationary phase, cell density repression involving an as-yet-unidentified heat-stable repressor, and glucose repression (Table 2). Because of the limited number of examples of each, these latter instances may reflect adaptations to unique environments experienced by specific bacteria. The response to superoxide generation can be rationalized in terms of a need to respond to potential peroxide generation from superoxide dismutase. The reason

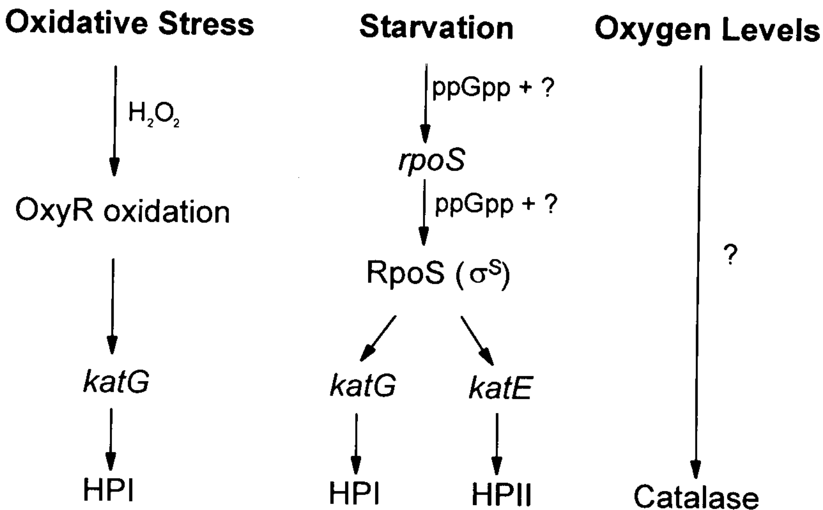


Figure 5 Schematic summary of the common responses of bacterial catalases to oxidative stress, starvation, and oxygen levels. The detailed mechanisms for oxidative stress and starvation regulation are from *E. coli,* and no mechanism has been determined for the response to oxygen levels, although it may be related to the oxidative stress response.

catalase synthesis should be repressed in stationary phase or as cell density reaches a certain level, the opposite of what seems to be more common in most other bacteria so far studied, may be related to the reduction in effective H_2O_2 concentration experienced by an individual cell in a very dense culture (Ma and Eaton 1992). Elucidation of the molecular mechanism controlling the cell density response may ultimately supply the rationale. It seems likely that bacteria not exhibiting the apparently more normal response of increased catalase in response to H_2O_2 or starvation may have evolved in environments where peroxide is not a factor in stationary-phase metabolism.

The instance of catabolite repression of catalase expression is also difficult to explain because it is not clear what advantage there would be in turning on or off a nonmetabolic enzyme in response to carbon source changes. Different metabolic pathways may lead to different levels of active oxygen species as by-products leading to an organism producing more or less protective enzyme depending on the particular carbon source. However, external sources of H_2O_2 would remain a threat and require the potential for enzyme synthesis regardless of carbon source. *E. coli* is not listed as experiencing catabolite repression of catalase because of the conflicting reports in support (Yoshpe-Purer et al. 1977; Hassan and Fridovich 1978; Meir and Yagil 1990) and to the contrary (Epps and Gale 1942; Loewen et al. 1985), and because of evidence that cAMP does not affect catalase expression. It is clear that a definition of catabolite repression of catalase in *E. coli* is complicated by the influence of metabolite changes and starvation on σ^S levels. For example, growth phase, medium pH, and acid content (Schellhorn and Stones 1992; Mukhopadhyay and Schellhorn 1994) all affect σ^S levels and, indirectly, catalase levels. Therefore, any demonstration of catabolite repression of catalase expression must differentiate the response from more general metabolic or pH effects on gene expression.

BACTERIAL CATALASE STRUCTURE

The crystal structures of three bacterial catalases have been solved, including those from *M. luteus* (Murshudov et al. 1992), *E. coli* (Bravo et al. 1995), and *Proteus mirabilis* (Gouet et al. 1995). In all three cases, there is extensive similarity to the structures of the two eukaryotic catalases from bovine liver (Murthy et al. 1981) and *P. vitale* (Vainshtein et al. 1986), and the bacterial structures were determined in large part by molecular replacement using the bovine liver enzyme as a model. To date, no catalase-peroxidase has been crystallized, and the only structural

information available has been gleaned from the structure of yeast cytochrome *c* peroxidase which, as noted above, exhibits some sequence similarity with the catalase-peroxidases.

HPII

Multimeric Structures

Because the structures of catalases are discussed elsewhere in this monograph, only a very brief description of the significant features is presented here. Despite the existence of dimeric and hexameric enzymes, the three structures so far solved have been of tetramers. Because HPII had earlier been characterized as a hexamer, and its initial crystals were consistent with the hexameric structure, the tetrameric structure was a surprise, although it was consistent with the structures of other crystallized catalases. Two explanations for this discrepancy are based on differences in salt and pH used in crystal formation as compared to enzyme characterization. The enzyme was originally characterized in a relatively low-salt environment of 50 mM potassium phosphate, pH 7, whereas the crystals were isolated from 1.5 M LiCl–0.25 M Tris.HCl, pH 9. When the enzyme was characterized by gel filtration at high salt, the apparent size was consistent with a tetrameric structure, whereas at low salt it remained consistent with a hexamer (P.C. Loewen, unpubl.). Thus, the size difference between high and low salt could have arisen from a change in subunit composition, or alternatively, the change in salt could have induced a change in the Stokes radius or conformation of the protein, affecting the apparent size of the protein. Electrospray mass spectrometry of HPII has revealed a tetrameric structure even at very low salt concentrations (5 mM ammonium carbonate), confirming that the enzyme is most likely a tetramer under all conditions, and that it is the size of the tetramer that is changing with salt (Chernushevich et al. 1995).

Heme Location and Structure

In all catalases, the heme is deeply buried and relatively inaccessible to larger substrates, providing an explanation for its lack of reactivity with larger organic molecules. This lack of reactivity is enhanced further in HPII, where the access channel is even more occluded, making access for anything larger than H_2O_2 almost impossible. Heme b (protoheme IX) is found in the typical catalases of *M. luteus* and *P. mirabilis*, but heme d is present in HPII of *E. coli* (Chiu et al. 1989). What is even

more striking about the heme of HPII is the flipped orientation compared to the other enzymes (Bravo et al. 1995). This has the effect of placing the reactive histidine (His-128) over ring IV of the heme, as compared to the equivalent His-54 in the *P. mirabilis* catalase being situated over ring III (Fig. 6). The other key catalytic residues (Asn-201 and Ser-167) and the heme iron are in the same spatial relationship to His-128 as in other catalases, thereby providing an explanation for the similar catalatic activities of atypical and typical catalases.

The flipped conformation does create another problem, however, and that is that the site of oxidation on ring III and the proximal location of the oxygens of the spirolactone and hydroxyl group on ring III are distant from the catalytic residues. As shown in Figure 6A, the modification site on the heme (denoted by spirolactone and OH on ring III) is a significant distance, and on the opposite side of the heme, from His-128, which is an essential residue in the heme modification reaction (Loewen et al. 1993). The mechanism of catalysis involved in the heme modification is now being investigated.

NADPH Binding

The binding of NADPH to catalase was first demonstrated for the bovine enzyme (Kirkman and Gaetani 1984). Subsequent studies of bacterial enzymes have determined that the nucleotide is bound to the *P. mirabilis* enzyme (Jouve et al. 1989) but not to *E. coli* HPII (Hillar et al. 1994). Thus, we have an atypical catalase without NADPH and a typical catalase with NADPH, leading to the speculation that the difference in nucleotide binding may be another difference between the two groupings. However, generalizations based on a sample size of two are tenuous, and the analysis of additional members of each group will be required to verify or disprove this idea.

The crystal structures have shown that NADPH is not bound immediately adjacent to the heme pocket and active site in either the bovine structure (Fita and Rossman 1985) or the *P. mirabilis* enzyme (Gouet et al. 1995). Furthermore, it is not required for enzymatic activity, raising the question of its role in catalase. Because NADPH prevents and may even reverse the accumulation of compound II, an inactive form of bovine liver catalase (Kirkman et al. 1987), it has been proposed that the role of NADPH is to reduce a labile free-radical precursor in the pathway leading to compound II through either a two-electron transfer (Hillar and Nicholls 1992) or a more unusual one-electron transfer (Almarsson et al.

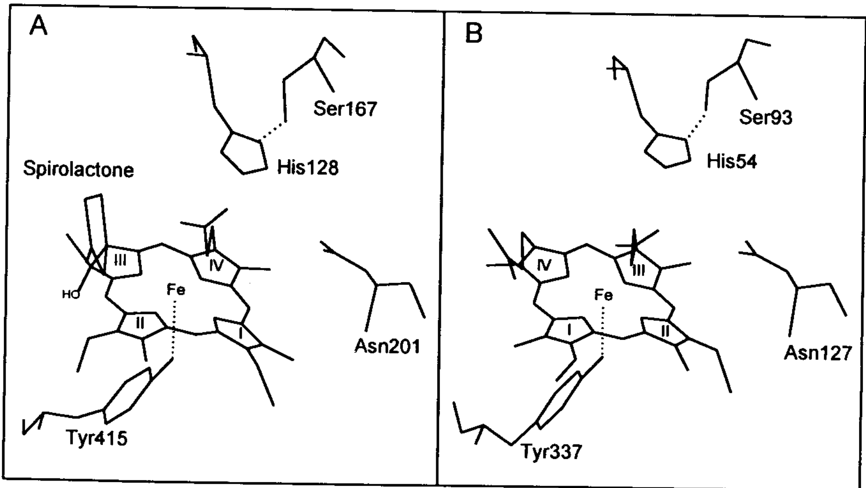


Figure 6 Comparison of the three-dimensional relationship of active-site residues and heme in catalase HPII of *E. coli* (*A*) and the catalase of *P. mirabilis* (*B*). The active-site residues are numbered according to their location in the sequence of the individual enzymes, and the spirolactone and hydroxyl modifications in the heme d of HPII are also indicated in *A*. The rings of the heme are numbered (I–IV) according to convention to illustrate the opposite orientation in the two enzymes. The dashed lines between His-128 and Ser-167 in *A* and His-54 and Ser-93 in *B* denote a potential hydrogen bond. The dashed lines between Tyr-415 (*A*) or Tyr-337 (*B*) and the iron atom of the heme indicate fifth ligand association on the proximal side of the heme. The three-dimensional relationships of the various residues were generated from the atomic coordinates using QUANTA (Molecular Simulations Inc, Boston, Massachusetts).

1993). Regardless of the mechanism, it is interesting to speculate that HPII, and possibly others in the atypical group, do not bind NADPH because compound II formation is rare, having never been demonstrated in HPII, making NADPH an unnecessary accessory.

Function of the Extended Sequence of HPII

HPII and other atypical catalases differ from the smaller typical bacterial catalases in having approximately 75 additional residues at the amino terminus and 175 residues at the carboxyl end. In this respect, they resemble the *P. vitale* catalase, and there is even extensive structural similarity between the two enzymes, with the carboxy-terminal sequence residing in a unique flavodoxin-like domain that does not seem to be in-

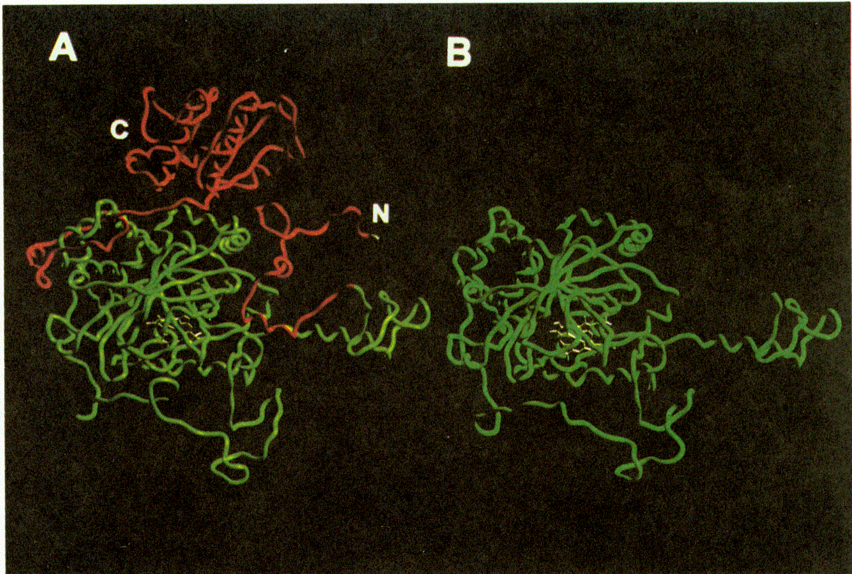


Figure 7 Diagrams of catalase HPII of *E. coli* (*A*) and the catalase of *P. mirabilis* (*B*) to illustrate the locations of the extended sequences of HPII relative to the similar core structures of the two enzymes. The amino-terminal extension of 75 residues and the carboxy-terminal domain of 160 residues in HPII that are absent in the *P. mirabilis* catalase are indicated with N and C, respectively, and are colored red. The diagrams were generated from the atomic coordinates using the program SETOR (Evans 1993).

volved in subunit interactions. Diagrams depicting the structure of *E. coli* HPII and the *P. mirabilis* catalase are shown in Figure 7, where the large carboxy-terminal domain and the amino-terminal extension are clearly visible in the HPII structure. HPII is remarkably resistant to heat and pH denaturation, and the subunits do not dissociate even under the extreme voltages of mass spectrometry. In the latter case, the multimeric state proved to be so stable that monomers are not seen, and larger complexes of two, three, and four tetramers were evident, the largest having a mass of 1.3×10^6 D (Chernushevich et al. 1995). Additional stabilizing interactions involving the amino and carboxyl termini are an obvious explanation for the enhanced stability; this is currently being investigated through the construction of truncated mutants of HPII. It is tempting to speculate that the additional sequence may interfere with compound II formation, thereby eliminating the need for NADPH, but further study is needed to confirm such conjecture.

Modified Residues

The catalase from *P. mirabilis* has been found to contain a methionine sulfone (Buzy et al. 1995) at residue 53, immediately adjacent to the key active-site residue His-54. It was concluded that the modified residue did not significantly influence catalysis on the basis of similarities in kinetic properties between the *P. mirabilis* and bovine catalases. However, one of the sulfone oxygens appears to be involved in a hydrogen bond with an adjacent asparagine, suggesting a role in protein folding. It was also suggested that the modification may explain the reduced sensitivity of the *P. mirabilis* enzyme to aminotriazole. The more normal residue at this location in 15 of 20 catalases is valine, but three apparently related catalases from *B. fragilis, H. influenzae*, and *N. gonorrhoeae* (Fig. 1) also have a methionine in the same location. Whether the sulfone modification occurs in these other enzymes remains to be determined.

One of the two surface-situated cysteines of *E. coli* HPII has been found to be modified (Sevinc et al. 1995), but the identity and role of the modification remain unknown. The modification on Cys-438 is alkali-labile and has a mass of 44 ± 2 D, but otherwise does not have properties consistent with being a disulfide, an acetyl ester, a carbamoyl derivative, or an oxidized sulfur. Both cysteines, and therefore the modification, could be replaced with either alanine or serine without a significant effect on catalysis; perhaps not surprising, given the remoteness from the deeply buried active site.

HPI

The use of the crystal structure of yeast cytochrome *c* peroxidase (CCP) as a model for the structure of the catalase-peroxidases was first proposed by Welinder (1991, 1992). A number of specific residues of yeast CCP were identified as having a catalytic role, including His-175 (the heme iron fifth ligand), Asp-235 (hydrogen-bonded to His-175 on the proximal side of the heme), His-52 and Arg-48 on the distal side (which catalyze H_2O_2 cleavage), and Asn-82 (which is hydrogen-bonded to His-52). The equivalent residues in HPI are all conserved as His-267, Asp-377, His-106, Arg-102, and Asn-136. By analogy with the strong similarity in structures between the shorter bovine catalase and larger HPII, with most of the extra sequence of HPII residing in a separate domain, it is possible that HPI will be organized with the amino-terminal, active-site-containing 400 residues residing in a domain that is structurally similar to yeast CCP, and the remaining 300 residues resid-

ing in a separate domain. However, there is currently no evidence to support such a hypothesis, and the additional sequence could just as well be folded into the CCP domain, causing significant changes. Consequently, the larger size of HPI as compared to yeast CCP requires a note of caution when attempts to draw structural analogies are made. With this in mind, site-directed mutagenesis studies of specific residues in HPI are now under way.

SUMMARY

Catalases have been purified and characterized from a large number of bacterial species. A phylogenetic comparison of the sequences and physicochemical properties of the enzymes has resulted in the identification of four groups: the monofunctional catalases broken down into typical and atypical groups, the catalase-peroxidases, and the non-heme catalases. Great diversity in the number and types of catalases present in different bacteria has been reported, with anywhere from zero to six isoenzymes being identified, although a maximum of three catalases have ever actually been purified from any one organism. Associated with this diversity of catalases is a diversity of control patterns. Induction of catalase synthesis in response to H_2O_2, starvation, and oxygen occurs commonly in different organisms. However, there is no consistent association of a particular induction mechanism with catalase type, and other responses have been identified that seem to be unique to specific organisms. It would appear that the diversity of catalases and control mechanisms have evolved in response to the diversity of environments that different bacteria experience. We can expect even more diversity to appear as more catalases are studied. This mixture of similarity and diversity extends even to structure, where there is a strong resemblance between the typical and atypical monofunctional catalases, but also significant differences that may be responsible for the higher catalytic rate at high [H_2O_2], the enhanced stability, and the lack of NADPH in atypical HPII as compared to the typical catalases. This diversity evident in the family of bacterial catalases will continue to fascinate workers and, as more is learned, should provide insights into the adaptation of bacteria to different environments.

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