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# Molecular Characterization of *Staphylococcus warneri* Catalase

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The catalase gene was cloned by screening a genomic DNA library of *S. warneri* ISK–1 strain with a strong catalase activity for complementation of the activity in catalase–deficient *E. coli* strain. Nucleotide sequence analysis of a 2.2–kb DNA fragment revealed an open reading frame, called *katA*, encoding a peptide of 504 amino acids with a calculated molecular mass of 58 kDa. The predicted amino acid sequence showed high similarities with the monofunctional catalases. No similarities were found between *katA* product and catalase–peroxidase type enzymes. Electrophoretic mobility of *katA* product was close to that of the previously purified ISK–1 catalase. Catalase activity was lost when the 135 amino acids were deleted from the C–terminal region.

## INTRODUCTION

Reactive oxygen species (ROS) such as superoxide anion radical ($O_2^{\cdot -}$) and hydrogen peroxide ($H_2O_2$) are produced as by–products of aerobic metabolism. The effects of ROS result in serious damages of various cellular components such as DNA, RNA, proteins and lipids (Imlay and Linn, 1988). Therefore, microorganism has evolved several enzymatic defense systems to eliminate the lethal effects of ROS (Storz *et al.*, 1990). For example, superoxide anion radical is eliminated by dismutation to hydrogen peroxide by superoxide dismutase, and hydrogen peroxide is decomposed by catalase and peroxidase (Hassen and Fridovich, 1978). Catalase catalyzes the conversion of hydrogen peroxide to water and oxygen.

*Escherichia coli* is an only microorganism the catalase of which has been studied in depth (Triggs–Raine *et al.*, 1988; von Ossowski *et al.*, 1991). This organism is known to produce two species of hydroperoxidases (HPI and HPII) which are synthesized in quite different manners. HPI encoded by *katG* exhibits a peroxidase activity in addition to the catalase activity which expresses in the exponential phase, and is governed by the global oxidative stress regulon OxyR (Loewen *et al.*, 1983; Christman *et al.*, 1989). HPII encoded by *katE* possesses only catalase activity and is synthesized in the stationary growth phase. The expression of HPII requires a functional sigma factor RpoS (Loewen and Hengge–Aronis, 1994).

The genus *Staphylococcus* comprises a group of gram–positive and catalase–positive

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facultative anaerobe. Mandell (1975) has described that the staphylococcal catalase acts as important role to tolerate against the lethal concentration of environmental hydrogen peroxide. Catalase has been used as a biochemical tool for differential identification of staphylococcal species (Raymond, 1976). But little is known about the character and structure of staphylococcal catalase, although some reports were focused on the physiological importance of this enzyme (Flowers *et al.*, 1977). Moreover, staphylococcal catalase also plays an important role in food fermentations in which hydrogen peroxide accumulated causes color defects in food and kills available bacteria. For example, *S. carnosus* catalase decomposes hydrogen peroxide formed as a metabolite by lactic acid bacteria (LAB) and may be important to reduce adverse effects on color, aroma and shelf-life of the fermented sausages (Hammes and Knauf, 1994). For these reasons, much attention is being denoted to the characteristic and structural properties of staphylococcal catalase.

*S. warneri* ISK-1 was isolated from well-aged *Nukadoko*, the rice bran packed fermentation bed for Japanese traditional pickled vegetables (Kimura *et al.*, 1997). This strain possesses a strong catalase activity compared to other aerobic or anaerobic eubacteria (Mizuno *et al.*, 2000). In *Nukadoko*, there exists many LAB, including bacteriocin-producing strains of *Lactococcus lactis* isolated from different well-aged *Nukadoko* (Ennahar *et al.*, 1999). It is also assumed that *S. warneri* ISK-1 catalase contributes to survival of LAB against the environmental hydrogen peroxide and maintains the stable quality and microflora in *Nukadoko* for a long period. In this paper, we described about the cloning and characterization of the gene encoding the catalase of ISK-1 strain. Structural properties and phylogenetic relationships of this enzyme were also discussed. In our knowledge, this is the first investigation about the genetic characterization of staphylococcal catalase.

## MATERIALS AND METHODS

### Bacterial strains, plasmid, and culture conditions

*Staphylococcus warneri* ISK-1 was isolated in our laboratory (Kimura *et al.*, 1997), and was grown at 37 °C in MRS medium (Oxoid, Hampshire, England) *Escherichia coli*

**Table 1.** Bacterial strains used in this study

| Strain | Descriptions | References |
|---|---|---|
| *Staphylococcus warneri* | | |
| ISK-1 | Isolated in our laboratory from *Nukadoko* | Kimura *et al.*, 1997 |
| *Escherichia coli* | | |
| JM109 | *recA1 supE44 endA1 hsdR17 gyrA96 relA1 thi* Δ*(lac-proAB)* | Yanisch-Perron *et al.*, 1985 |
| MC1000 | *araD139* Δ*(ara-leu)7696 galU galK* Δ*(174) rpsL thi* | Nakagawa *et al.*, 1996 |
| SN0029 | MC1000 *katG*::Cm *katE*::Km | Nakagawa *et al.*, 1996 |

JM109 (Toyobo, Osaka) and catalase-defective mutant *E. coli* SN0029 (*katG*::Cm, *katE*::Km) (Nakagawa *et al.*, 1996) were grown at 37°C with shaking in Luria-Bertani (LB) medium or on LB 1.5% agar medium containing 40 $\mu$g/ml ampicillin. When the growing was appropriate for clonal selection, X-gal (5-bromo-4-isopropyl-$\beta$-D-4-chloro-3-indol-$\beta$-D-galactopyranoside), IPTG (isopropylthio-$\beta$-D-galactosidase) and ampicillin were added at concentrations of 50, 40, 20 $\mu$g/ml, respectively. Genotypic properties of these strains are indicated in Table 1. Cloning vector pUC18 (Toyobo) was used for cloning of *S. warneri* ISK-1 catalase gene.

**DNA preparation and manipulation**

Total chromosomal DNA from *S. warneri* ISK-1 strain was isolated by applying a combination of the two methods (Marmur, 1961; Berns and Thomas, 1965). Plasmid DNA was purified from *E. coli* with Mag extractor plasmid extraction kit (Toyobo). Restriction endonuclease digestions, analyses and ligations were performed according to the methods of Sambrook *et al.* (1989). *E. coli* cells for electroporation were prepared according to the protocol recommended for the Gene Pulser apparatus (Bio-Rad, Hercules, CA, USA). Southern hybridization with nucleotide probes was performed by using the AlkPhos labeling system (Amersham Pharmacia Biotech, Uppsala, Sweden) according to the manufacture's protocol.

**Cloning of catalase gene**

The chromosomal DNA from ISK-1 strain was partially digested with restriction enzyme *Sau*3AI. A genomic library was constructed by ligating the *Sau*3AI fragments into the *Bam*HI site of pUC18 vector and transformed into *E. coli* SN0029 by electroporation. The transformants were selected on LB agar containing 40 $\mu$g/ml ampicillin. The positive clone was screened for competence of catalase activity by applying a 3% hydrogen peroxide to agar plate and looking for the generation of oxygen bubbles.

**Nested deletion and DNA sequencing**

A catalase clone of 2.2-kb *Sau*3AI fragment in both orientations was digested with *Sma*I and *Sal*I. Then nested deletion was performed with double-stranded Nested Deletion Kit outlined by the manufacture (Amesham Pharmacia Biotech). The transformants containing suitable size deletions were identified and sequenced. Sequencing reaction based on the method of Sanger *et al.* (1977) was performed with Auto Sequencer Core Kit (Toyobo) according to the instructions of the manufacture and using Cy-5 labeled M13 Universal and Reversal primers (Amersham Pharmacia Biotech). DNA sequence analyses were performed with ALF express (Amersham Pharmacia Biotech).

**Catalase activity staining**

Native PAGE was performed with 7-20% gradient polyacrylamide gels (Atto Co., Tokyo). The following staining for catalase activity was done as reported previously (Clare *et al.*, 1984): the resulting native PAGE gel was soaked for 45 min in 50 mM potassium-phosphate buffer (pH 7.0) containing 50 $\mu$g/ml horseradish peroxidase (Wako Pure

Chemical Industries, Osaka), followed by addition of 5 mM $H_2O_2$ and the incubation at 25 °C for 10 min. The gel was washed twice with distilled water and then soaked in the buffer containing 0.5 mg/ml diaminobenzidine (Nacalai Tesque) to develop the background brown color. No color would appeared in the area catalase depleted $H_2O_2$.

### Phylogenetic analyses

*S. warneri* ISK-1 KatA and represetative 13 bacterial monofunctional catalases were used in this analysis. The Clustal W program was used for multiple amino acid sequence comparison, and phylogenetic tree, based on neighbor-joining (NJ) method, was constructed with the program TreeView 1.5.

### Computer analyses of sequence data and nucleotide accession numbers

Computer analyses of nucleotide sequence were performed with GENETYX-MAC v. 9.0 software (Software Development, Tokyo). The search for DNA sequence homology was carried out with the BLAST program of the GenBank Nucleotide Sequence DataLibrary.

The nucleotide sequence of *S. warneri* ISK-1 *katA* has been submitted to the GenBank databases (accession number AB045340). Accession numbers of other sequences used for the analysis are as follows; *E. coli katE*, M5162; *Lactobacillus sakei katA*, M84015; *Haemophilus influenzae hktE*, U02682; *Neisseria gonorrhoeae kat* gene, U35457; *Bacteroides fragilis katB*, U187676; *Bordetella pertussis katA*, U07800; *Rhizobium meliloti katA*, U59271; *Helicobacter pylori katA*, U67458; *Bacillus subtilis katE*, X85182; *Bacillus subtilis katA*, M80796; *Streptomyces coelicolor catB*, AF000419; *Mycobacterium avium katE*, L41246; *Pseudomonas aeruginosa katA*, U34896.

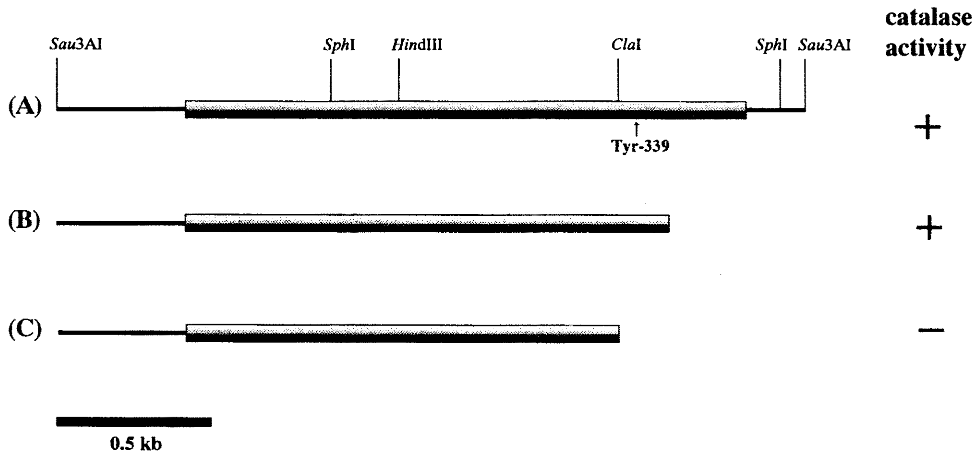


**Fig. 1.** Restriction map and deletion analysis of cloned *S. warneri* ISK-1 *katA* locus.
Ability to complement catalase activity in *E. coli* SN0029 was also noted. (A), 2.2-kb *Sau3*AI fragment; (B) and (C), deleted 2.2-kb fragments corresponding to deletion of 119 and 135 amino acids from the C-terminal region of ISK-1 KatA, respectively.

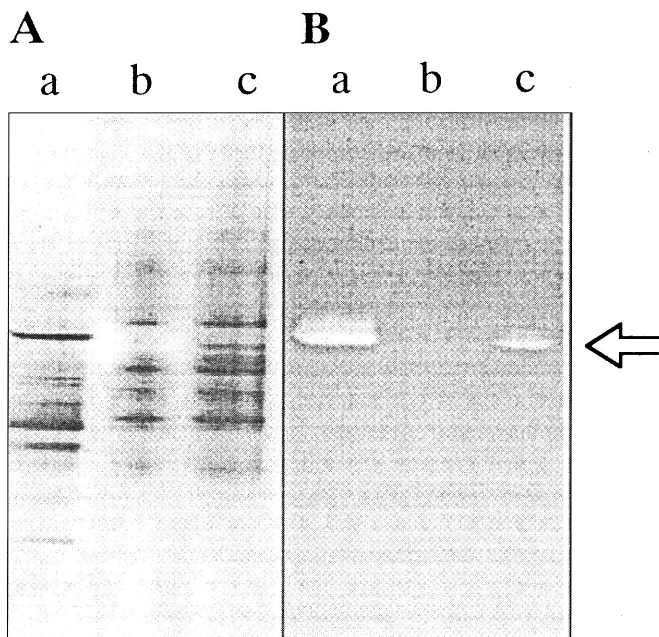


**Fig. 2.** Expression of ISK–1 *katA* in *E. coli* SN0029.
Native PAGE with 7–20% gradient; A, stained with Coomassie Brilliant Blue; B, stained with diaminobenzidine for catalase band. Lane a, partially purified ISK–1 catalase from *S. warneri* ISK–1 (Mizuno *et al.*, 2000); lane b, cell–free extract from *E. coli* SN0029; lane c, cell–free extract from *E. coli* SN0029 containing *katA* gene on plasmid pUC18. The white arrow shows the recombinant *katA* gene product expressed in *E. coli* SN0029.

## RESULTS

### Cloning of chromosomal *S. warneri* ISK–1 catalase

The genomic library of *S. warneri* ISK–1 was screened in *E. coli* cells by the addition of 3% $H_2O_2$, and three positive clones which generated oxygen bubbles were observed. Plasmid DNA pSW103, extracted from one positive clone, contained a 2.2–kb insert DNA fragment (Fig. 1). When *E. coli* SN0029 was transformed with this plasmid, catalase activity was restored. Thus, we confirmed that expression of catalase activity was due to the presence of pSW103. The cloned catalase gene product expressed in *E. coli* SN0029 showed electrophoretic mobility identical to that of the previously purified catalase from *S. warneri* ISK–1 strain (Fig. 2). This indicates that the cloned catalase gene from *S. warneri* ISK–1 corresponds to the same enzyme protein as detected and purified from

```
   1  GATCAACAAATCGAGTAGAGTAAGAACTAAATGAACCAGATACTGGATAGAAAGTAGCTAATTCACCAATAGACGCCATTAAGAAATAAA  90
  91  GCATGATACCTATTAAGAGGTAAGCAAGGATAGCGCCACCAGGACCAGCTTCAGAAATGACACTTCCTGTAGCCACAAATAAACCAGTTC  180
 181  CAATTGCGCCACCTATAGCAATCATGGTTATGTGTCTGGAGGTTAAACCTCTTTTCATATTATTATCTTCCATATACTGTCTCCCATCTA  270
 271  TTTTAAATATATCCATCATTTTAATTCTTTTAGACCTACTATTCAATAGTAATTTAGCACTTTAAAGTAAAATTTAAAAATTTTCTGATA  360
 361  ATTCGGTTGAGATAAGGGGTTTAAGTTTCATTAAAAGAGGGGATACTTTATAATGGATACTATATTATAATAATTCTAATTTGTGTGGAG  450
 451  GGAAATTTGAATGTCTAAACAAGACGGTAAGTTAACAGGTTTATTCGGCGCACCCGTGTCAGATAGAGAAAATAGTATGACTGCTGGTCC  540
      S.D.      M  S  K  Q  D  G  K  L  T  G  L  F  G  A  P  V  S  D  R  E  N  S  M  T  A  G  P
 541  AAGAGGTCCACTTTTAATGCAGGATATTTACTTCTTAGAGCAAATGTCACATTTTGATCGTGAAGTGATTCCTGAAAGACGTATGCATGC  630
        R  G  P  L  L  M  Q  D  I  Y  F  L  E  Q  M  S  H  F  D  R  E  V  I  P  E  R  R  M  H  A
 631  TAAAGGTTCCGGTGCATTTGGAACATTTACCGTAACTAATGATATTACTCAGTATACAAGTGCTAAAATGTTCTCGGAAGTCGGTAAACA  720
        K  G  S  G  A  F  G  T  F  T  V  T  N  D  I  T  Q  Y  T  S  A  K  M  F  S  E  V  G  K  Q
 721  AACTGAAATGTTCGCGAGATTTTCTACTGTATCTGGTGAAAGAGGTGCAGCAGATGCTGAGAGAGATATTCGTGGATTTGCATTAAAATT  810
        T  E  M  F  A  R  F  S  T  V  S  G  E  R  G  A  A  D  A  E  R  D  I  R  G  F  A  L  K  F
 811  TTATACTGAAGATGGAAACTGGGATTTAGTTGGTAATAATACGCCCGTATTCTTCTTTAGAGACCCTAAATTATTCGTAAGCTTAAATAG  900
        Y  T  E  D  G  N  W  D  L  V  G  N  N  T  P  V  F  F  F  R  D  P  K  L  F  V  S  L  N  R
 901  AGCTGTTAAAAGAGATCCAAGAACGAATATGAGAAGTGCACAAAATAACTGGGACTTCTGGACAGGATTACCAGAAGCATTACACCAAGT  990
        A  V  K  R  D  P  R  T  N  M  R  S  A  Q  N  N  W  D  F  W  T  G  L  P  E  A  L  H  Q  V
 991  GACAATTTTAATGTCAGATAGAGGTATTCCAAAAGATTTACGACATATGCATGGATTCGGTTCACACACATATTCTATGTACAATGATAA  1080
        T  I  L  M  S  D  R  G  I  P  K  D  L  R  H  M  H  G  F  G  S  H  T  Y  S  M  Y  N  D  K
1081  AGGTGAACGCGTATGGGTTAAATATCATTTCAGAACACAACAAGGCATTGAAAATTTAACAGATGAAGAAGCGGCTAACGTGATTGCTAC  1170
        G  E  R  V  W  V  K  Y  H  F  R  T  Q  Q  G  I  E  N  L  T  D  E  E  A  A  N  V  I  A  T
1171  GGATCGTGATTCTTCACAAAGAGATTTATTTAATGCGATTGAAAATGGTGATTATCCTAAATGGAAAATGTATATTCAAGTAATGACAGA  1260
        D  R  D  S  S  Q  R  D  L  F  N  A  I  E  N  G  D  Y  P  K  W  K  M  Y  I  Q  V  M  T  E
1261  AGAACAAGCTAGAAATCATAAAGATAACCCATTTGATTTAACAAAAGTTTGGTATCATGGTGATTATCCATTAATTGAAGTTGGAGAATT  1350
        E  Q  A  R  N  H  K  D  N  P  F  D  L  T  K  V  W  Y  H  G  D  Y  P  L  I  E  V  G  E  F
1351  TGAATTAAACCGCAATCCTAATAACTATTTCCAAGATGTTGAACAAGCTGCTTTTGCACCTACAAACATCGTTCCTGGTTTAGACTATTC  1440
        E  L  N  R  N  P  N  N  Y  F  Q  D  V  E  Q  A  A  F  A  P  T  N  I  V  P  G  L  D  Y  S
1441  ACCAGATAAAATGTTACAAGGTCGTTTATTCCCTTATGGTGATGCGCAACGTTATAGATTAGGAGTTAACCATTGGCAAATACCAGTTAA  1530
        P  D  K  M  L  Q  G  R  L  F  P  Y  G  D  A  Q  R  Y  R  L  G  V  N  H  W  Q  I  P  V  N
1531  CCAACCTAAAGGTGTAGGTATTGAAAATTTATGTCCATTTAGTCGTGATGGCCAAATGCGTATTTTAGATGATAACCAAGGTGGCGGACC  1620
        Q  P  K  G  V  G  I  E  N  L  C  P  F  S  R  D  G  Q  M  R  I  L  D  D  N  Q  G  G  G  P
1621  TCATTATTATCCAAATAATCAAGGTGTTTATGATTCCCAACCTGAATTTAAAAAGCCGCCATTCCCAGCTGATGGTGATGGTTATGAATA  1710
        H  Y  Y  P  N  N  Q  G  V  Y  D  S  Q  P  E  F  K  K  P  P  F  P  A  D  G  D  G  Y  E  Y
1711  TAATCAACGTCAAGATGATGATAATTACTTTGAACAACCAGGTAAGTTATTTAGATTACAATCTGATGAAGCGAAAGAGAGAATCTTTAC  1800
        N  Q  R  Q  D  D  D  N  Y  F  E  Q  P  G  K  L  F  R  L  Q  S  D  E  A  K  E  R  I  F  T
1801  TAATACAGCCAATGCGATGGATGGTGTAACAGAGGATGTTAAAAGACGACATATTCGCCATTGCTATAAAGCAGATCCAGATTATGGTAA  1890
        N  T  A  N  A  M  D  G  V  T  E  D  V  K  R  R  H  I  R  H  C  Y  K  A  D  P  D  Y  G  K
1891  AGGTGTTGCTAAAGCATTAGGTATTGATATTAATTCAATCGATTTAGAAGGTGAGCAAGACGAAACTTATGAGAATTTTAAAAATTAAAA  1980
        G  V  A  K  A  L  G  I  D  I  N  S  I  D  L  E  G  E  Q  D  E  T  Y  E  N  F  K  N  *
1981  GTTAAGATTGCGCACTGAAGATTTATATGATAGAATTTTAAATCGTAAGTATTCCTAACTAGAAAGGGTGTTGAACGTGCGCGTAAATAT  2070
2071  CACATTAGCATGCACAGAATGTGGCGATCGTAACTATATCACTACTAAAAATAAAAGAAATAATCCTGAGCGTATTGAAATGAAAAAATA  2160
2161  TTGCCCAAGATTAAACAAATATACGTTACATCGTGAAACTAAATAATCACTTATCTTTCAAATACGACAATTTGAAATGCGAAAA       2245
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**Fig. 3.** Nucleotide sequence of the 2.2-kb *Sau*3AI fragment containing ISK-1 *katA* and the deduced amino acid sequence.

ISK–1 strain. The catalase gene located on pSW103 was named as *katA*.

### Nucleotide sequence of *katA*

The nucleotide sequence of 2.2–kb *Sau*3AI fragment revealed a single open reading frame of 1,515 bp (Fig. 3). This open reading frame encodes 504 amino acids with a predicted molecular mass of 58,000 Da. The N–terminal amino acid sequence of purified ISK–1 catalase (KatA) was Met–Ser–Lys–Gln–Asp–Gly–Lys–Leu–Thr–Gly–, which corresponds to the deduced one of *katA* gene. Thus, we confirmed the cloning of the catalase gene from *S. warneri* ISK–1.

The deletion clones used for nucleotide sequence were tested for catalase activity in *E. coli* SN0029. Deletion of 119 amino acids from the C–terminal region did not affect catalase activity. However, activity was lost when the 135 amino acids were deleted (Fig. 1). This loss of activity may result from the lack of the amino acid residue Tyr–339 which is one of the essential residues for monofunctional catalase activity as mentioned below.

### Comparison of amino acid sequences

The predicted amino acid sequence obtained for ISK–1 KatA was compared to other catalase sequences in the GenBank–EMBL/Swiss–Prot databases. The KatA showed the highest amino acid identity with *Bacteroides fragilis* catalase KatB (Rocha and Smith, 1995) (identity 64% and positive 77%) and *Haemophilus influenzae* catalase HktE

```
                        49    dd                                                  108
S.warneri ISK-1  KatA   VIPERRMHAKGSGAFGTFTVTNDITQYTSAKMFSEVGKQTEMFARFSTVSGERGAADAER
B.fragilis       KatB   VIPERRMHAKGSGAYGTFTVTHDITKYTRAAIFSQVGKQTECFVRFSTVAGERGAADAER
H.influenzae     HktE   VIPERRMHAKGSGAFGTFTVTHDITKYTRAKIFSEVGKKTEMFARFTTVAGERGAADAER
L.sakei          KatA   RIPERVVHAKGAGAKGYFKVTKDMSAYTKAAVFSGVGKKTPLITRFSQVAGEAGYPDTYR
E.coli           KatE   RIPERIVHARGSAAHGYFQPYKSLSDITKADFLSDPNKITPVFVRFSTVQGGAGSADTVR

                        109                 d    d       d                      164
S.warneri ISK-1  KatA   DIRGFALKFYTEDGBWDLVGNNTPVFFFRDPKLFVSLNRAVKRDPRTNMR----SAQNNW
B.fragilis       KatB   DIRGFAMKFYTEEGNWDLVGNNTPVFFLRDPLKFPDLNHAVKRDPRNNMR----SANNNW
H.influenzae     HktE   DIRGFALKFYTEEGNWDLVGNNTPVFFLRDPRKFPDLNKAVKRDPRTNMR----SATNNW
L.sakei          KatA   DVRGFAVKFYTEEGNYDIVGNNTPVFFVNDPLKFPDFIHSQKRDPRTHAR----SQDMQW
E.coli           KatE   DIRGFATKFYTEEGIFDLVGNNTPIFFIQDAHKFPDFVHAVKPEPHWAIPQGQSAHDTFW

                        285               p                                p   p 344
S.warneri ISK-1  KatA   YHGDYPLIEVGEFELNRNPNNYFQDVEQAAFAPTNIVPGLDYSPDKMLQGRLFPYGDAQR
B.fragilis       KatB   PHKDFPLQDVGILELNRNPENYFAEVEQSAFNPMNIVEGIGFSPDKMLQGRLFSYGDAQR
H.influenzae     HktE   SKKDYPLIEVGEFELNRNPENFFADVEQSAFAPSNLVPGIGASPDRMLQARLFNYADAQR
L.sakei          KatA   SQKDYPLIEIGQMVLDENPTNNFEDIQELAFSPANLVPGIEASPDKLLQGRLFGYKDAER
E.coli           KatE   PEELVPVQRVGKMVLNRNPDNFFAENEQAAFHPGHIVPGLDFTNDPLLQGRLFSYTDTQI
```

**Fig. 4.** Multiple alignment of the deduced amino acid sequence of ISK–1 KatA with other bacterial catalases.
The active site residues are indicated by underbar. The proximal and distal heme ligand sites are indicated by p and d.

(Bishai *et al.*, 1994) (identity 66% and positive 78%). The KatA also exhibited high homologies to catalases of *Lactobacillus sakei* (Knauf *et al.*, 1992), *Bacillus subtilis*, and *E. coli* HPII. No similarities were found to bacterial catalase–peroxidase type enzymes like *E. coli* HPI.

To identify important structural motifs in ISK–1 KatA, multiple alignment of the amino acid sequence of ISK–1 KatA with other catalases was investigated (Fig. 4). The active sites (His–74, Ser–113, and Asn–147) of bovine liver catalase, which is a monofunctional catalase well–investigated in structure (Murthy *et al.*, 1981), were conserved in the KatA at His–56, Ser–95 and Asn–129 positions. Moreover, the proximal heme sites, Pro–335, Arg–353 and Tyr–357 as ligands in bovine liver catalase, were localized as Pro–303, Arg–335 and Tyr–339 in the KatA. Distal heme site ligands in bovine liver catalase were also conserved as His–56, Asn–129, Phe–134 and Phe–142, except that Val–73 was replaced by Met–55. The same replacement was also observed in

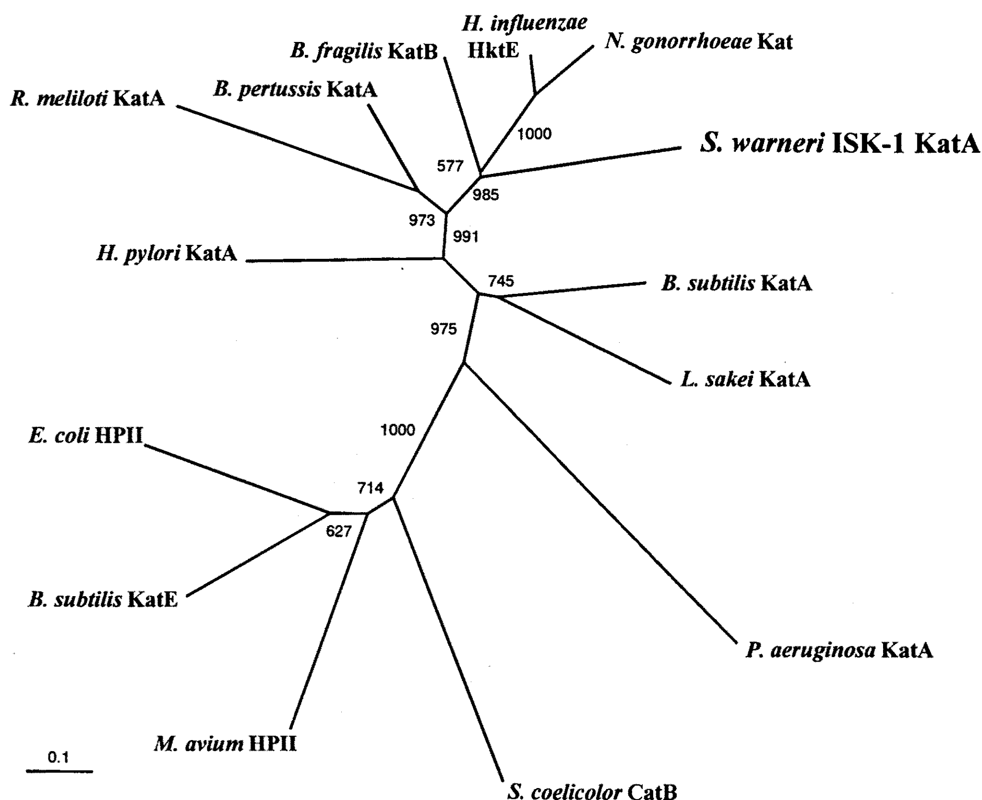


**Fig. 5.** Phylogenetic relationship of *S. warneri* ISK–1 KatA and representative microbial monofunctional catalases.
Catalase amino acid sequences used in this analysis were listed in Materials and Methods.

*B. fragilis* KatB and *H. influenzae* HktE.

Phylogenetic relationship between ISK–1 KatA and 13 bacterial monofunctional catalases was determined by the neighbor–joining (NJ) method (Fig. 5). The KatA showed a close relationship with *Neisseria gonorrhoeae* catalase, *B. fragilis* KatB and *H. influenzae* HktE.

## DISCUSSION

In this paper, we cloned and characterized *S. warneri* ISK–1 catalase gene, *katA*. Expression of *katA* in *E. coli* SN0029 and catalase activity restored have strongly indicated that cloned *katA* encodes the catalase of *S. warneri* ISK–1. The KatA structural gene was 1,515 nucleotides long and encoded a protein of 504 amino acids. The previously purified catalase subunit revealed the molecular mass of 64 kDa in the SDS–PAGE gels (Mizuno *et al.*, 2000), which was higher than that of *katA* gene product (58 kDa) calculated from its predicted amino acid sequence. This discrepancy might be due to a feature of specific protein structure.

The multiple amino acid sequence alignments and phylogenetic analysis showed a strong relationship between ISK–1 KatA and monofunctional catalases. Especially, the KatA showed a high degree of amino acid sequence identity with *B. fragilis* KatB and *H. influenzae* HktE. Based on 16S rDNA phylogenetic analyses, staphylococci are not closely related to these bacteria, although codon usage patterns of catalase genes *katA*, *katB* and *hktE* are highly similar. These results strongly indicate that there might be a horizontal transfer of the catalase gene from a common ancestor into these strains, and there is no relationship to phylogenies based on phenotype or 16S rDNA sequence.

Catalase activity is universal among *Staphylococcus* spp. Southern blot hybridization analysis revealed that single catalase gene was present in *S. warneri* ISK–1 chromosome (data not shown). On the other hand, high catalase activity of *S. simulans* strain resulted from both of catalase genes encoded on plasmid as well as chromosome (Lynn *et al.*, 1994). The catalase gene on the plasmid contributed to the high catalase activity of *S. simurans*. Generally, genes encoded on plasmid DNA are known to transfer with high frequencies, also suggesting that some staphylococcal strains gained several kinds of catalase genes from different ancestors.

In our previous report (Mizuno *et al.*, 2000), we described that the expression behavior of *S. warneri* ISK–1 KatA was similar to that of *B. fragilis* KatB, in the way up–regulated by oxygenation but not peroxides. But unlike *B. fragilis* KatB, *S. warneri* ISK–1 KatA was mainly expressed in the exponential growth phase and down–regulated in the stationary growth phase. These suggested that aerobic induction of catalase in *S. warneri* ISK–1 is independent of peroxides and may be induced via a pathway, which is related to aerobic vegetative metabolism and has not been reported previously as a regulator of catalase expression like RpoS of OxyR. The regulatory system involved in the regulation of *S. warneri* ISK–1 *katA* should be interesting and is presently under investigation. Thus, the further experiments which include the regulatory analysis of catalase by Northern blotting and construction of catalase–deficient *S. warneri* ISK–1 strain will be needed to give a clear solution about the role of catalase to survive against toxic ROS.

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