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Comparative Study of Staphylococcal Catalases

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We determined the divergence of some staphylococcal catalases on polyacrylamide electrophoresis gel. The results indicate that all *Staphylococcus* species used in this study had a single catalase in both exponential and stationary growth phases. Less variation in electrophoretic mobility of the *Staphylococcus* species suggests that this method is not useful for identification of species. Catalase activities of the seven strains were measured in both exponential and stationary growth phases. It is noteworthy that the catalase activity in exponential growth phase was higher than that in stationary growth phase for the five strains. The others maintained the catalase activity at almost the same level during the entire growth phases.

INTRODUCTION

Catalase is one of enzymes protectig against oxygen stress, which converts hydrogen peroxide to molecular oxygen and water. It is present in most aerobic organisms and probably evolved about two billion years ago when the earth developed an oxygenated atmosphere (von Ossowski *et al.*, 1993). With the universal exsistence of this enzyme, its amino acid sequence was expected to be a phylogenetic standard (Klotz *et al.*, 1997). In bacteria, catalase has been used for classification. The mobility and multiplicity on polyacrylamide electrophoresis gel of several mycobacterial catalases showed a wide variety and provided support for the identification of individual strains (Wayne and Diaz, 1986).

We have purified and characterized a catalase from *Staphylococcus warneri* ISK–1 (Mizuno *et al.*, 2000), which was isolated from well aged *Nukadoko* that is the rice bran packed fermentation bed for Japanese traditional pickled vegetables (Herawati and Ishizaki, 1997). We determined the divergence of some staphylococcal catalases on polyacrylamide electrophoresis gel and investigated whether the properties of catalase from *S. warneri* ISK–1 were conserved in other species.

MATERIALS AND METHODS

Organism and culture conditions

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The *Staphylococcus* strains used in this study were *S. epidermidis* JCM2414^T, *S. pasteuri* ATCC51129^T, *S. haemolyticus* JCM2416^T, *S. hominis* JCM2419^T, *S. aureus* NCTC8325, *S. capitis* JCM2420^T, *S. warneri* ISK-1 which was isolated from well aged *Nukadoko* (Herawati and Ishizaki, 1997) and *S. warneri* JCM2415^T. Ten ml starter cultures were grown at 37 °C for 18 h in TGC (Difco Laboratories Co., Detroit, MI, USA) medium and then used to inoculate 100 ml MRS (Oxoid Co., Hampshire, England) medium in 300-ml conical-flasks. These cultures were then grown at 37 °C on an orbital shaker at 100 rpm for 4 h. 500-ml conical-flasks containing 200 ml MRS medium were inoculated with 10 ml of the preculture. The cultures were grown at 37 °C and 100 rpm until their exponential or stationary growth phase. The cells then harvested by centrifugation were suspended in 50 mM potassium-phosphate buffer (pH 7.0) and disrupted by glass beads with Multi-Beads Shocker MB-200 (Yasuikikai Co., Osaka). The cell suspensions were centrifuged and the cell-free extracts were used for catalase activity determination and native PAGE.

For comparison of catalase activities of staphylococcal and other bacteria, *S. warneri* ISK-1, *Escherichia coli* JM109 and *Bacillus subtilis* JCM1465^T were cultured overnight with shaking in 10 ml of Luria-Bertani (LB) medium. These cultures were used to inoculate 100 ml cultures, which were grown until the absorbance at 562 nm was about 1.0. The cell-free extracts were prepared as described above.

Protein estimation

Protein was determined by the method of Bradford with bovine serum albumin (Bio-Rad, Hercules, CA, USA) as a standard (Bradford, 1976).

Catalase activity staining

The following staining for catalase activity was done as reported previously (Clare *et al.*, 1984): the resulting native PAGE gel using 7-20% gradient polyacrylamide gel (Atto Co., Tokyo) was soaked for 45 min in 50 mM potassium-phosphate buffer (pH 7.0) containing 50 μg/ml horseradish peroxidase (Wako Pure Chemical Industries Co., Osaka), followed by the 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 Co., Kyoto) to develop the background brown color. No color would appear in the area where catalase depleted H_2O_2.

Catalase assay

Catalase was assayed by following the disappearance of H_2O_2 (Santoku Chemical Industries Co., Tokyo) spectrophotometrically at 240 nm as reported previously (Beer and Sizer, 1952). The assay cocktail contained 50 mM potassium-phosphate buffer (pH 7.0) and 20 mM H_2O_2 in a final volume of 3 ml. One unit of activity was defined as the amount of enzyme catalyzing the degradation of 1 μmol H_2O_2 per min at 25 °C.

RESULTS

We detected catalase band patterns on electrophoresis gel from eight strains grown until exponential and stationary growth phases (Fig. 1). All strains exhibited a single

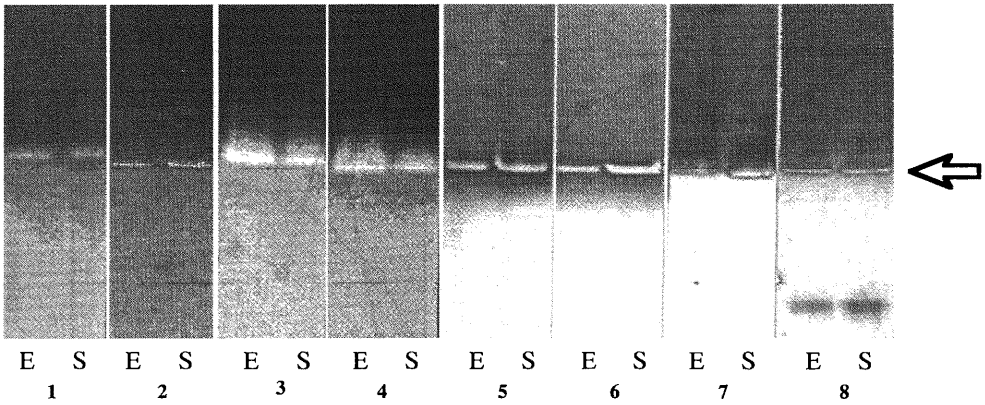

Fig. 1. Catalase zymograms of eight *Staphylococcus* strains in exponential and stationary growth phases
The gels were stained for catalase activity with diaminobenzidine as described in Materials and Methods. The white arrow indicates catalase band molecular weight of which was 125,000 (Mizuno *et al.*, 2000).
1, *S. epidermidis* JCM2414^T; 2, *S. pasteuri* JCM51129^T; 3, *S. haemolyticus* JCM2415^T; 4, *S. hominis* JCM2419^T; 5, *S. aureus* NCTC8325; 6, *S. capitis* JCM2420^T; 7, *S. warneri* ISK-1; 8, *S. warneri* JCM2415^T. E and S represent exponential and stationary, respectively.

Table 1. Catalase activity of *Staphylococcus* strains in exponential and stationary growth phases

Strains	Catalase activity (U/mg)	
	Exponential	Stationary
S. pasteuri ATCC 51129^T	57.2	10.0
S. haemolyticus JCM 2416^T	206.4	126.7
S. hominis JCM 2419^T	222.1	230.0
S. aureus NCTC 8325	191.0	114.8
S. capitis JCM 2420^T	420.3	286.6
S. warneri ISK-1	169.6	80.1
S. warneri JCM 2415^T	174.3	184.7

band both in the exponential and stationary growth phases, indicating that these strains had a single catalase. The relative mobilities of the catalase bands were identical except for those of *S. epidermidis* JCM2414^T and *S. haemolyticus* JCM2416^T which were a little less than those of the other strains. We also determined the catalase activities of the seven strains both in exponential and stationary growth phases (Table 1). The activities showed a wide variety. It is noteworthy that the catalase activity in exponential growth phase was higher than that in stationary growth phase for the five strains. The other two strains, *S. hominis* JCM2419^T and *S. warneri* JCM2415^T, maintained the catalase activities at almost the same level during the entire growth phases. On the other hand, many studies have reported an apparent increase in catalase activity upon entry into stationary growth phase.

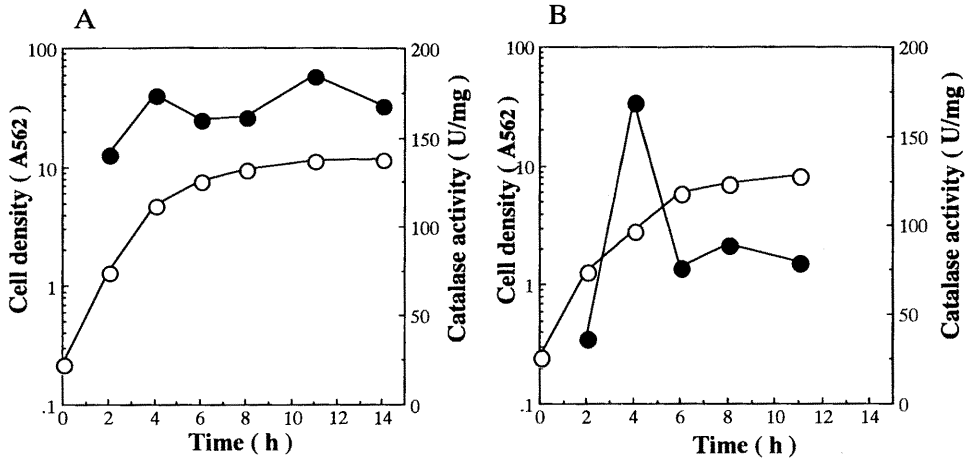

Fig. 2. Catalase activity and cell growth of *S. warneri* JCM 2415^T (A) and ISK–1 (B)
Closed circle and open circle represent catalase acitivity (U/mg) and cell density (A_{562}), respectively.

Table 2. Comparison of the activity of catalase from *S. warneri* ISK–1 with those from other bacteria

Bacteria	Catalase activity (U/mg–protein)
Staphylococcus warneri ISK–1	310
Escherichia coli JM 109	65.0
Bacillus subtilis JCM 1465^T	43.0

Catalase activities of *S. warneri* ISK–1 and JCM2415^T were measured during the entire growth phases (Fig. 2). Both strains are representatives of each group that showed different patterns of catalase activity during cell growth (Table 1). Although maximum catalase activities in the exponential growth phase (4 h) were almost same (ISK–1, 169.6 U/mg and JCM2415^T, 174.3 U/mg), the activities in stationary growth phase (11 h) were quite different (ISK–1, 80.1 U/mg and JCM2415^T, 184.7 U/mg). Hence, the regulation of catalase expression in the stationary growth phase may not be conserved in the two strains.

Furthermore, we compared the catalase activity of *S. warneri* ISK–1 to those of *E. coli* JM109 and *B. subtilis* JCM1465^T by using LB medium under the conditions described in the Materials and Methods section. The activity of *S. warneri* ISK–1 was 5 and 7 times those of *E. coli* JM109 and *B. subtilis* JCM1465^T, respectively (Table 2).

DISCUSSION

Comparative study of staphylococcal catalases on electrophoresis gel has previously been reported (Zimmerman, 1976). In the study, however, cultivation time of the cells

used was 24 h, indicating that the cells might be in their stationary growth phase. Additionally, they used starch gel in which the catalase bands were so diffused that it was difficult to estimate the differences in mobilities of each band. Recently, it has been shown that many bacteria possess multiple catalases and do not always express them simultaneously. In *E. coli*, *katE* encoding HP II monofunctional catalase is expressed specifically in the stationary growth phase (Loewen *et al.*, 1985). We therefore determined the catalase band patterns on polyacrylamide gradient gel with eight *Staphylococcus* strains both in exponential and stationary growth phases. The results indicate that all *Staphylococcus* species used in this study had a single catalase in both growth phases. It might be attributed to the possible relationship between the singleness of catalase and the character of its host as described previously (Klotz *et al.*, 1997). Bacteria possessing only a single catalase have a restricted environment in parasitic life (*Helicobacter pyroli* (Odenbreit *et al.*, 1996), *Brucella abortus* (Sha *et al.*, 1994), *Compylobacter jejuni* (Grant and Park, 1995), *Bordetella pertussis* (Deshazer *et al.*, 1994), *Proteus mirabilis* (Jouve *et al.*, 1983), *Haemophilus influenzae* (Fleischmann *et al.*, 1995), *Nesseria gonorrhoeae* (Archibald and Duong, 1986)). In addition, these catalases have a mutual sequence and form a distinct group within 74 catalase protein sequences from bacteria, fungal, animal and plant sources (Klotz *et al.*,1997). We have cloned and sequenced the catalase gene of *S. warneri* ISK–1 and found that it belongs to this group (Fukuda *et al.*, 2000). The physiological significance of the singleness of catalase is, however, not known yet. Less variation in electrophoretic mobility of catalases of the *Staphylococcus* species used in this study suggests that this method is not useful for identification of species.

From the results of the determination of catalase activities of the seven staphylococcal species in two different growth phases, the activities of all the strains used in this study decreased or maintained at almost the same level on entering the stationary phase. In *E. coli*, the catalase activity in stationary phase increased from 2 to 3 fold that in the exponential phase (Loewen *et al.*, 1985). This increase in activity during stationary phase was also found in *Streptomyces coelicolor* A3(2) although it has a single catalase (Walker *et al.*, 1995). So, it can not be attributed solely to the expression of another specific catalase gene in the stationary growth phase. Similar increases in catalase activity during the stationary growth phase have been described for *Bacillus subtilis* (Loewen and Switala, 1987) and *Pseudomonas fluorescens* (Rodriguez–Bravo and Pionetti, 1981). Therefore, the decrease in staphylococcal catalase activity during the stationary growth phase is interesting and should be investigated in further detail.

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