# Purification and Characterization of a Catalase from Photosynthetic Bacterium *Rhodospirillum rubrum* S1 Grown under Anaerobic Condition

By



**Yoon-Suk Kang**

**Department of Biology**

**Graduate School, Cheju National University**

# Purification and Characterization of a Catalase from Photosynthetic Bacterium *Rhodospirillum rubrum* S1 Grown under Anaerobic Condition

**By**

**Yoon-Suk Kang**



**Department of Biology**

**Graduate School, Cheju National University**

# Purification and Characterization of a Catalase from Photosynthetic Bacterium *Rhodospirillum rubrum* S1 Grown under Anaerobic Condition

**By**

**Yoon-Suk Kang**

A thesis submitted to the
Graduate School, Cheju National University
in partial fulfillment of the requirements for the degree of
Master of Science
Graduate Program in Biology



written under the direction of Professor Duck-Chul Oh and
approved by

Se-Jae Kim
Chairperson of the Committee

Duck-Chul Oh

Seok Chan Koh

December, 2002

# Abstract

*Rhodospirillum rubrum* S1 grown under anaerobic condition was found to produce two catalases. Among two catalases, higher molecular weight catalase was more stable than lower molecular weight one, regardless of the addition of reactive oxygen species inducers on the culture. In this work, we purified and characterized the higher molecular weight catalase. The specific activity of catalase of crude extracts was 88 μmol · $min^{-1}$ · $mg^{-1}$. After the final purification step, 14.3 purification fold was obtained. the specific activity of catalase was 1256 μmol · $min^{-1}$ · $mg^{-1}$. It had an estimated molecular mass of 318 kDa, consisting of four subunits of 79 kDa and showed no peroxidase activity. The purified enzyme exhibited an apparent Km value of 30.4 mM and a *Vmax* of 2,564 U(mg protein)$^{-1}$ against hydrogen peroxide. The enzyme had a broad pH(5. 0～9.0) optimum. In thermostability on catalase activity, the purified enzyme was stable in the broad range of temperatures(20 ℃～60 ℃). It sustained 90 % activity against organic solvent(ethanol/chloroform) known as an inhibitior of hydroperoxidase. When the enzyme was treated with 10 mM 3-amino-1,2,4-triazole, it showed 81% inhibition on catalase activity. It was inhibited by heme protein inhibitors, such as sodium cyanide, sodium azide and hydroxylamine. Sodium cyanide, sodium azide, and hydroxylamine inhibited the catalase activity by 50 % at the concentrations of 14.6 × $10^{-6}$ M, 6.2 × $10^{-7}$ M and 5.5 × $10^{-7}$ M, respectively.

Considering these results, the enzyme seems to be a class of monofunctional catalases.

# TABLE OF CONTENTS

# LIST OF TABLES

# LIST OF FIGURES

# INTRODUCTION

Oxygen is essential to all the aerobic microorganisms and even to facultative anaerobes for metabolism under aerobic conditions. However, it inevitably produced reactive oxygen species(ROS), such as superoxide($O^{-1}$), hydroxyl(HO・), and perhydroxyl(HOO・) radicals which could cause chemical modification of most cellular components, such as nucleic acids, proteins, lipids, etc. Such chemical modifications of the cellular components would result in various metabolic defects, aging process, mutagenesis and cell death (Halliwell and Gutteridge, 1999). Most ROS are highly toxic to biological systems and must be removed before reaching harmful levels(Levine *et al*., 1994).

The removal of reactive oxidants can be achieved by the action of several enzymes and small molecules, such as superoxide dismutase(Farr and Kogoma, 1991), glutathione(Almiron *et al*., 1992), methionine sulfoxide reductase(Moskovitz *et al*., 1995) involved in various repair processes, and SoxRS(Hengge-Aronis, 1993, Demple, 1991, Stroz and Altuvia, 1994). Catalase is also one of the representative defense systems. There are two major classes of bacterial catalases: (1) the monofunctional catalases(HPⅡ), and (2) the bifunctional catalase-peroxidases(HPⅠ), which to date have been found only in bacteria. The monofunctional catalases, which have only catalytic activity, share a number of characteristic features such as resistance to chloroform/ethanol treatment and a broad pH optimum. They exist as homotetramers or homohexamers with subunits of 60～90 kDa. The catalase-peroxidases, which are isolated from bacteria and fungi, have several properties that distinguish them from the typical catalases: they are reduced by dithionite, and they are not glycoproteins, their activity is pH

dependent, and they are more sensitive to heat, organic solvents, and $H_2O_2$ than the typical catalases, but they are insensitive to 3-amino-1,2,4-triazole(Hochman *et al*., 1991). Both classes of catalase contain protohemeⅨ with the exception of the HPⅡ catalase from *Escherichia coli*, which contains a chlorine instead(Loewen and Switala, 1986). Many prokaryotes have multiple monofunctional catalase isozymes under differential regulation(Miller *et al*., 1997).

Less is known about the catalases in anaerobic organisms(Loewen, 1992). In anaerobic conditions, generation of reactive oxygen species is thought not to be a problem because of the absence of oxygen. and the lack of protective mechanisms against oxygen toxicity in anaerobic bacteria was seen as an explanation for their sensitivity upon oxygen exposure(Morris, 1980). Many studies have shown that anaerobic bacteria are not uniformly sensitive to oxygen and there is a broad range of oxygen tolerance from species to species(Finegold and George, 1989, Morris, 1980). It is believed that in some anaerobic bacteria, as in aerobic organisms, the presence of superoxide dismutase and catalase plays a role in the detoxification of oxygen by-products(Morris, 1980).



*Rhodospirillum rubrum*, a nonsulfur photosynthetic bacterium, needs oxygen for its aerobic growth by respiration, but, in contrast, it needs strict anaerobic conditions for growth by photosynthesis or fermentation(Uffen and Wolfe, 1970). therefore, *R. rubrum* S1 capable of aerobic and anaerobic growth is considered a very useful microorganism for studying expression patterns of catalase in response to oxidative stresses.

In our previous study, we demonstrated that *Rhodospillum rubrum* S1 produces 5 distinct catalases under aerobic condition and 2 catalases among 5 catalases under anaerobic condition. We discovered a few interesting things. First, the expression of catalase, designated Cat2, was always uniformed through the growth on various

carbon sources. Secondly, the expression degree of Cat2 under anaerobic culture condition was much higher than that under aerobic one. From these results, we assumed that Cat2 might play an important role for survival and proliferation of cells as a house-keeping enzyme against various environments, especially anaerobic conditions. thus, the studies described in this study were initiated to pursue a better understanding of a catalase from anaerobically grown cells.

In this study, we purified a monofunctional catalase from anaerobically grwon *Rhodospirillum rubrum* S1 and characterized in some aspects.

# MATERIALS AND METHODS

## 1. Bacterial strain and culture condition

*Rhodospirillum rubrum* S1(ATCC 11170) was used for this study. Cells, pre-grown up to logarithmic phase on the basal medium supplemented with 0.3 % malate as a sole carbon source (Bose *et al*., 1962) under the light of 2,000 Lux at 30 ℃, were transferred to 2.5 L transparent culture bottle and cultrued under the same conditions as preculture.

## 2. Preparation of Crude extract



At logarithmic phase, cells were harvested by centrifugation and washed three times and resuspended with potassium phosphate buffer(pH 7.2). The following procedures were carried out at 4 ℃. Cells were broken with the sonicator(Bandelin Sonoplus HD 2070, Germany). and centrifuged at 16,000×g for 1h. The final supernatant was used as crude extracts.

## 3. Enzyme assays

Catalase activity was measured spectrophotometrically by monitoring the decrease at $A_{240}$ resulting from the elimination of $H_2O_2$, using a UV visible spectrophotometer(UV-1601, SHIMADZU). The ε for $H_2O_2$ at 240 nm was 43.6 $M^{-1}cm^{-1}$. The standard reaction mixture for the assay contained 50 mM potassium phosphat e buffer(pH 7.2), 20 mM $H_2O_2$, and 20 μL of catalase containing

solution for a total volume of 3.0 mL. The reaction was run at 25 ℃. The amount of enzyme activity that decomposed 1 μM of $H_2O_2$ per min was defined as 1 unit(U) of activity(Beers and Sizer, 1952).

.

## 4. Protein quantification

Protein concentrations were determined with the micro-BCA protein assay (Smith *et al*., 1985) using bovine serum albumin as a standard.

## 5. Polyacrylamide gel electrophoresis(PAGE)

Non-denaturing PAGE and denaturing sodium dodecyl sulfate (SDS)-PAGE were performed as described by Laemmli(1970). After electrophoresis, proteins were detected by staining the gel with Coomassie brilliant blue. Visualization of catalase activity on non-denaturing polyacrylamide gel was performed by the ferric chloride-potassium ferric cyanide method of Wayne and Diaz(1986). In this staining method, gels were incubated initially with 5 mM hydrogen peroxide and followed by a freshly prepared mixture of 2 % ferric chloride and 2 % potassium ferric cyanide. Peroxidase activity was visualized by the method of Claiborne and Fridovich(1979) using 0.5 mM *o*-dianisidine.



## 6. Effect of ROS inducers on the catalase activitiy

After *R. rubrum* S1 was grown anaerobically to the late exponential phase at 30 ℃ under 2,000 Lux, the cells were exposed to various inducers, such as $H_2O_2$(1 mM), menadione(1 mM), benzyl viologen(500 μM), and methyl

viologen(500 μM). then, the cells exposed to inducers were incubated for a short period of time(1h, 3h) under aerobic and anaerobic condition. The cells were harvested and subjected to ultrasonic disruption to prepare the crude extracts. Effect of various inducers on the catalase activity was determined by measuring catalase activity of crude extracts obtained in stressed cultures and control cultures.

## 7. Purification of catalase

Ammonium sulfate precipitation of crude extracts of *Rhodospirillum rubrum* S1 was done in the range of 30～70 % saturation. The precipitate was collected by centrifugation(16.000 ×g for 1h) and resuspended in 50 mM potassium phosphate buffer(pH 7.2). The resuspended solution was dialyzed against the same buffer overnight. The following procedures were carried out at 4 ℃. The dialyzate was applied to a DEAE-cellulose anion exchange column which had been equilibrated with the same buffer. The adsorbed protein was eluted using a 400 mL linear 0～1 M NaCl gradient. Fractions containing catalase activity were pooled and concentrated with 70 % saturation of ammonium sulfate. The concentrated sample was dialyzed with 50 mM potassium phosphate buffer for 12hr. After dialysis, The dialyzate was loaded onto a sephadex G-200 gel filtration column equilibrated with 50 mM potassium phosphate buffer. The concentrated sample was subjected to 7.5 % non-denaturing polyacrylamide gel electrophoresis(PAGE) and electroeluted at 40 mA of constant current with a Prep-Cell 491(Bio-Rad Laboratories. Inc., Melville, N.Y.). Fractions containing catalase activity and a single protein following PAGE were pooled and concentrated.

## 8. Molecular weight determination

The molecular weight of the native protein was determined by gel filtration using superose-12HR 10/30(Amersham pharmacia Co.)column equilibrated with 50 mM potassium phosphate buffer(pH 7.4). The high-perfomance liquid chromatography(HPLC) system consisted of a solvent delivery pump(model SPD 10A vp ; SHIMADZU) and a spectrophotometric detector(model SCL 10A ; SHIMADZU) set at 280 nm. For molecular weight standards, the following proteins were used: Ferritin(386 kDa), Catalase(209 kDa), Aldolase(179 kDa), Bovin serum albumin(66 kDa), and Carbonic anhydrase(29.4 kDa).

The molecular weight of the subunit was determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis(SDS-PAGE) on a 12 % acrylamide gel according to the method of Laemmli(1970). The molecular weight standard for SDS-PAGE was Bio-Rad low range marker(Hercules, Calif.).

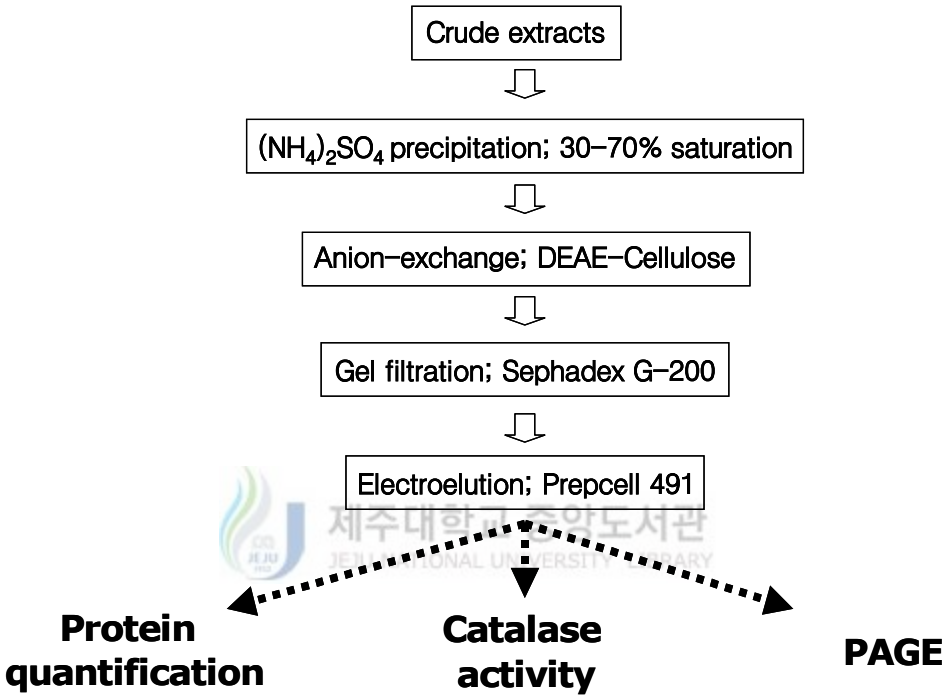


Fig 1. A scheme for the purification of a catalase from anaerobically grown *Rhodospirillum rubrum* S1.

## 9. Effect of pH and temperature on catalase activity of purified enzyme

Purified catalase were incubated for 30 min at various temperatures(20 ℃～80 ℃) and for 60 min at pHs ranging from 3.0～11.0, and the respective activities were measured as described above. The buffer systems, such as 0.5 M citrate buffer, 0.1 M phosphate buffer, 0.1 M Tris-HCl, 0.1 M carbonate buffer, were used.

## 10. Effect of inhibitors on catalase activity of purified enzyme

Enzyme solution was mixed with metal inhibitors, such as sodium cyanide, sodium azide and hydroxylamine. After 2min incubation of reaction mixtures at 25 ℃, the enzyme activity was assayed. To evaluate the effect of chemical inhibitors on catalase activity, The mixture of enzyme solution and organic solvents(enzyme solution : ethanol : chloroform = 10 : 5 : 3) was vortexed for 10min at room temperature, after then the activity was measured as described above. Also same was done with enzyme solution incubated with 10 mM 3-amino-1,2,4-triazole for 10min.

# RESULTS AND DISCUSSION

## 1. Catalase activities of crude extracts from *Rhodospirillum rubrum* S1 exposed to ROS inducers

Under anaerobic condition, the catalase activities of crude extracts from intact cells exposed to $H_2O_2$, menadione, benzyl viologen and methyl viologen for 1 hour were 135, 185, 250, and 320 U/mg, respectively. In case of 3hours cultures, those were 70, 110, 80, and 125U/mg, respectively. Under dark aerobic condition, the catalase activities of cells exposed for 1 hour to $H_2O_2$, menadione, benzyl viologen and methyl viologen were 145, 200, 45, and 260 U/mg, respectively. 3 hours after the additon of $H_2O_2$, menadione, benzyl viologen and methyl viologen, the catalase activity was 35, 20, 55, and 185 U/mg, respectively(Fig. 2,3,4,5).



*R. rubrum* S1 possesses two constitutive catalases under anaerobic growth. Especially, the expression pattern of the higher molecular weight catalase was always regular against various inducers such as hydrogen peroxide, menadione and methyl viologen(paraquat), as a superoxide anion generator, and the redox cycling agents benzyl viologen compared with the lower molecular weight catalase. Regardless of kinds of ROS inducers, the catalase activity of the crude extracts obtained from the cells incubated for 1 hour under anaerobic condition after the addition of inducers was much higher than control. However, the catalase activity of the crude extracts obtained from cells incubated for 3 hours was substantially decreased. Similarly, All inducers showed same results under aerobic condition except benzyl viologen. That is, the catalase activity increased in the early stage after the addition of inducers, as time goes on the catalase

activity slowly decreased. In short, Cell death might be caused by the addtion of too many inducers. Like most typical catalase(Mongkolsuk *et al*., 1997), catalases of *Rhodospirillum rubrum* S1 also was induced by ROS inducers. In case of benzyl viologen, the induction of catalase by benzyl viologen was not detected under aerobic condition. Benzyl viologen acts as a electron acceptor of hydrogenase activated under anaerobic condition(Detlef and Blaut, 1998). Based on these facts, benzyl viologen was not reduced by hydrogenase under aerobic condition. Accumulated benzyl viologen might cause cell death.

## 2. Purification of catalase

The results of the purification procedure are summarized in Table 1. The purified native catalase from the bacterial crude extracts on non-denaturing PAGE is shown in Fig. 6. The catalase was purified by one-step anion-exchange chromatography, one-step gel filtration, and one-step electroelution. By the final step, an 14.3 fold purification was obtained. The specific activity of catalase in crude extracts of *R. rubrum* was 88 μmol · $min^{-1}$ · $mg^{-1}$. After purification step, the specific activity of purified enzyme was 1256 μmol · $min^{-1}$ · $mg^{-1}$(Table 1).

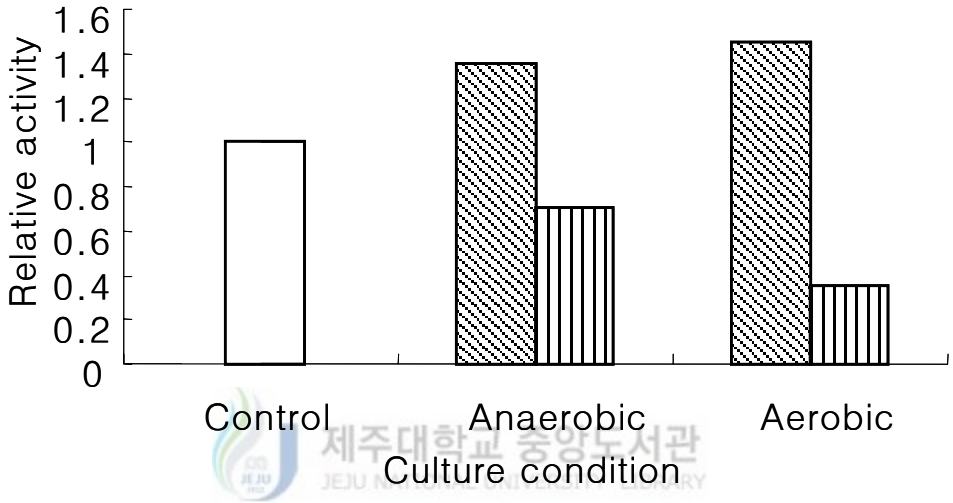




Fig. 2. Catalase activity of the crude extracts from *Rhodospirillum rubrum* S1 treated with $H_2O_2$. Cells were harvested after 1 or 3 hours incubation with $H_2O_2$. □; control, ▨; 1 hour incubation, ▥; 3 hours incubation.

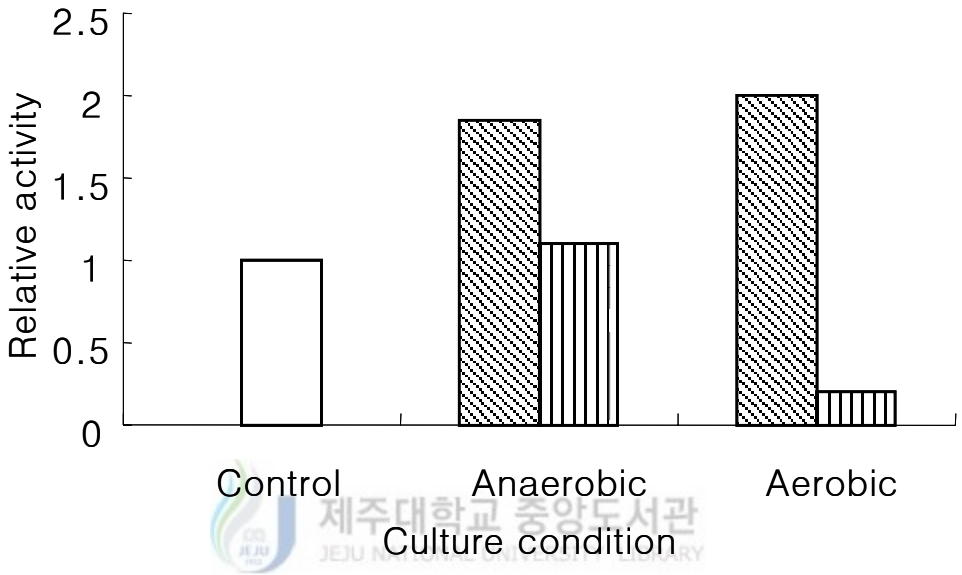




Fig. 3. Catalase activity of the crude extracts from *Rhodospirillum rubrum* S1 treated with menadione. Cells were harvested after 1 or 3 hours incubation with menadione. □; control, ▨; 1 hour incubation, ▥; 3 hours incubation.

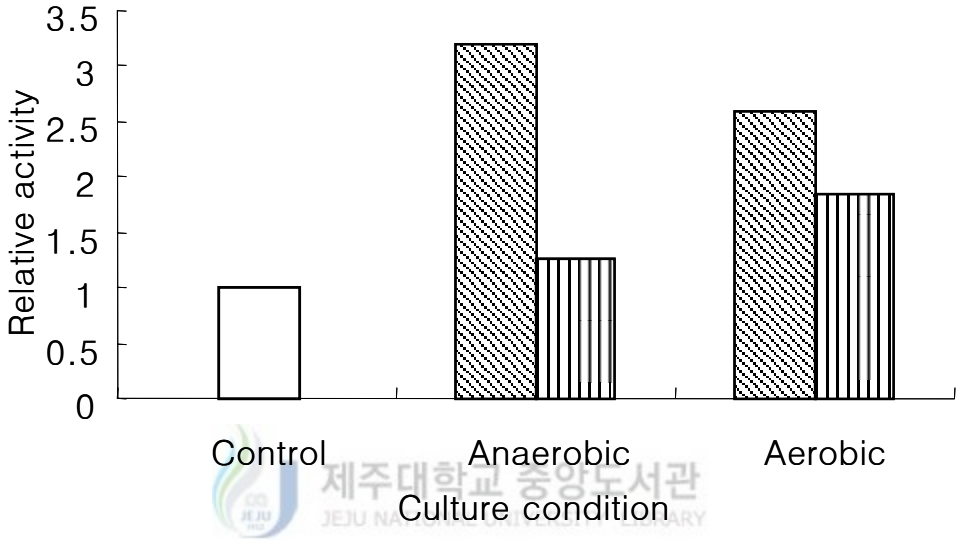




Fig. 4. Catalase activity of the crude extracts from *Rhodospirillum rubrum* S1 treated with methyl viologen. Cells were harvested after 1 or 3 hours incubation with methyl viologen. ▭; control, ▨; 1 hour incubation, ▥; 3 hours incubation.

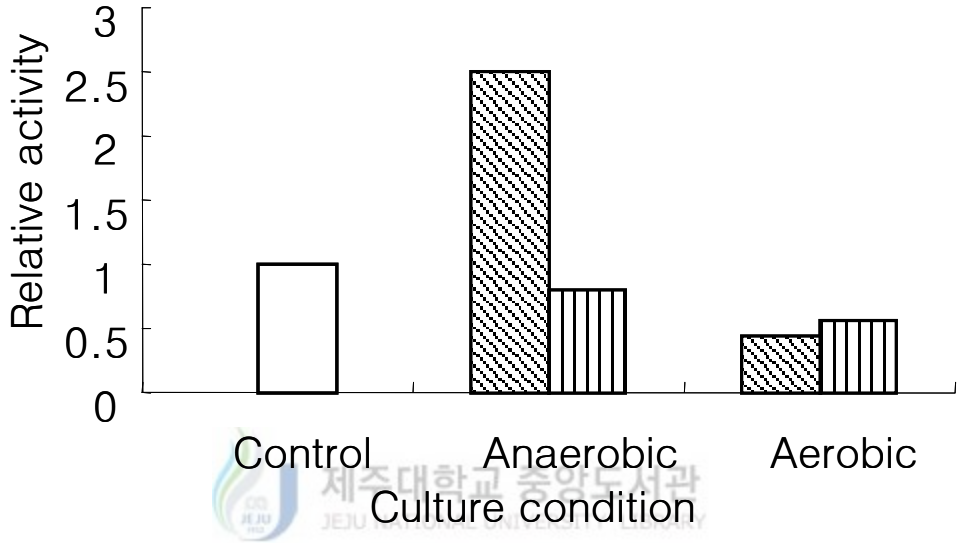




Fig. 5. Catalase activity of the crude extracts from *Rhodospirillum rubrum* S1 treated with benzyl viologen. Cells were harvested after 1 or 3 hours incubation with benzyl viologen. □; control, ▨; 1 hour incubation, ▥; 3 hours incubation.

Table 1. Purification procedure for a catalase from *Rhodospillum rubrum* S1 grown anaerobically

| Step | Protein Conc. (mg/mL) | Total protein (mg) | Specific activity (U/mg) | Total U | Purification (fold) | Enzyme yield(%) |
|---|---|---|---|---|---|---|
| Crude extracts | 4.6 | 634.8 | 88 | 55862 | 1 | 100 |
| Ammonium sulfate | 16 | 160 | 129.7 | 20752 | 1.5 | 25.2 |
| DEAE-cellulose | 5.6 | 56 | 349.8 | 19589 | 4 | 8.8 |
| Sephadex G-200 | 1.1 | 3.3 | 447.1 | 1475 | 5.1 | 0.5 |
| Prepcell 491 | 0.045 | 0.103 | 1256 | 129 | 14.3 | 0.02 |

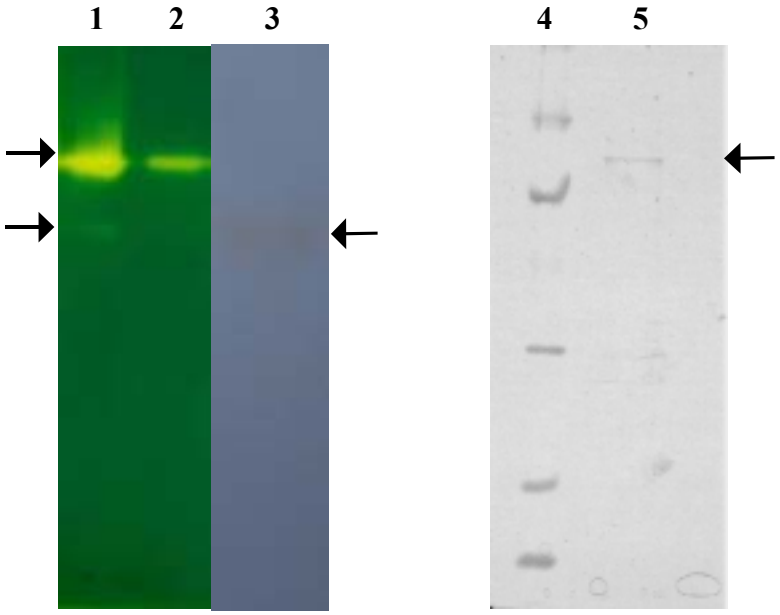




Fig. 6. Identification of catalase from *Rhodospirillum rubrum* S1 by non-denaturing PAGE(A, 7.5 % acrylamide) and SDS-PAGE(B, 12 % acrylamide). Lane 1. Crude extract, 2. Purified enzyme; staining for catalase activity, 3. Crude extract; staining for peroxidase activity, 4. The protein markers; phosphorylase b(94 kDa), bovine serum albumin (66.2 kDa), ovalbumin(45 kDa), carbonic anhydrase(31 kDa), soybean trypsin inhibitor(21.5 kDa), lysozyme(14.4 kDa), 5. Purified enzyme stained with silver.

### 3. Molecular weight of purified catalase and Km value for $H_2O_2$

The catalase activity staining on non-denaturing PAGE of crude extracts from *R. rubrum* S1 grown anaerobically revealed 2 distinct bands, whereas only one band by peroxidase activity staining(Fig. 6). Bacteria with more than one catalase regulate these enzymes individually. For example, the *E. coli* HPⅡ catalase and the spore-specific catalase-2 from B. subtilis are expressed in the stationary phase(Loewen *et al*., 1987). A strain of *S. coelicolor* is known to have six electrophoretically distinguishable catalases, all of which are regulated independently during the different phases of growth(Kim *et al*., 1994). These results indicate that *R. rubrum* S1 grown anaerobically could produce monofunctional catalase and a peroxidase expressed in different growth phase. The molecular weight of a subunit of the catalase was estimated to be 79 kDa by SDS-PAGE(Fig. 6,8). The native molecular weight of the enzyme was estimated to be 318 kDa by gel filtration using superose-12HR(Fig. 7). These results suggest that the purified catalase is composed of four identical units. The subunit molecular weight determined by SDS gel analysis was 79 kDa, and this agreed closely with the 706 amino acid residues reported to the gene database. Although catalases containing two subunits have been frequently reported in prokaryotes, such as *Klebsiella pneumoniae* kpa(Goldberg and Hochman, 1989), *Mycobacterium tuberculosis*(Wayne and Diaz,1986), and *Bacteroides fragilis*(Rocha and smith, 1995), three and six subunits catalases have been found in *Desulfovibrio gigas*(Wagner *et al*., 2000) and *Haenophilus influenzae*(Bishai *et al*., 1994), respectively.

A Lineweaver-Burk plot of purified catalase from anaerobically grown S1 cells

showed about 30.4 mM of Km value and the *Vmax* of 2,564 U/mg for hydrogen peroxide(Fig. 9). This is less small value compared to that of other typical catalases. For example, the Km value of *Brucella abortus*, *Listeria seeligeri*, and *Pseudomonas aeruginosa* was 84 mM, 49 mM, and 38 mM(Switala and Loewen, 2002), respectively. This suggests that purified enzyme is more efficient in scavenging hydrogen peroxide of high concentrations than other typical catalases.

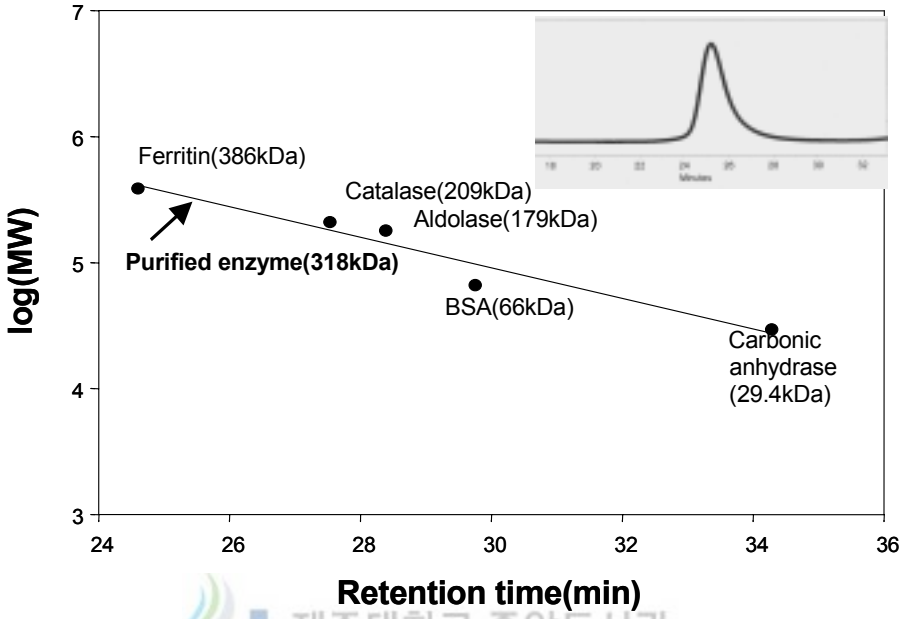




Fig. 7. Determination of native molecular weight of catalase of *Rhodospirillum rubrum* S1 by Gel filtration HPLC. The protein markers; ferritin(386 kDa), bovine liver catalase(209 kDa), aldolase(179 kDa), bovine serum albumin(66 kDa), carbonic anhydrase(29.4 kDa).

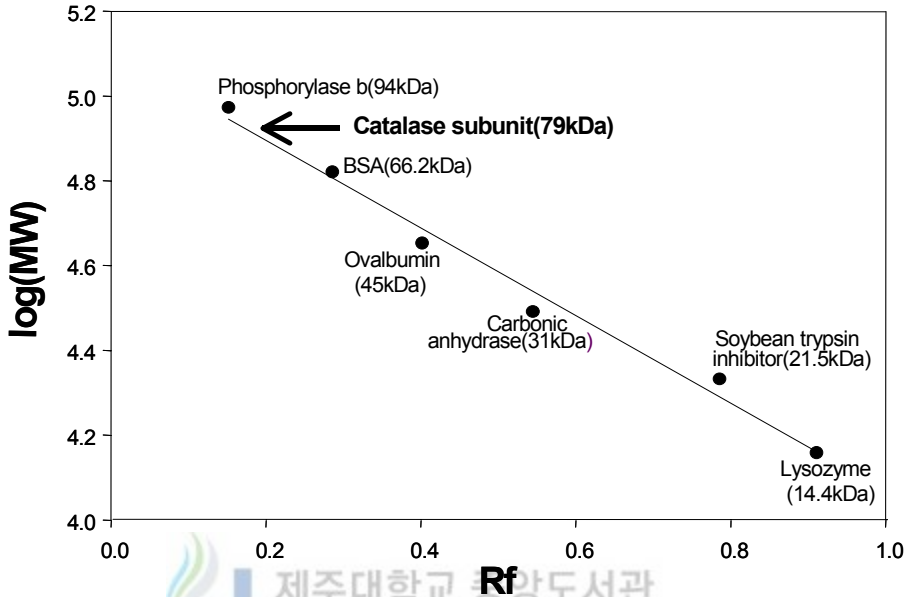



Fig. 8. Determination of molecular weight of the catalase subunit of *Rhodospirillum rubrum* S1 by SDS-PAGE. The protein markers; phosphorylase b(94 kDa), bovine serum albumin (66.2 kDa), ovalbumin(45 kDa), carbonic anhydrase(31 kDa), soybean trypsin inhibitor(21.5 kDa), lysozyme(14.4 kDa).

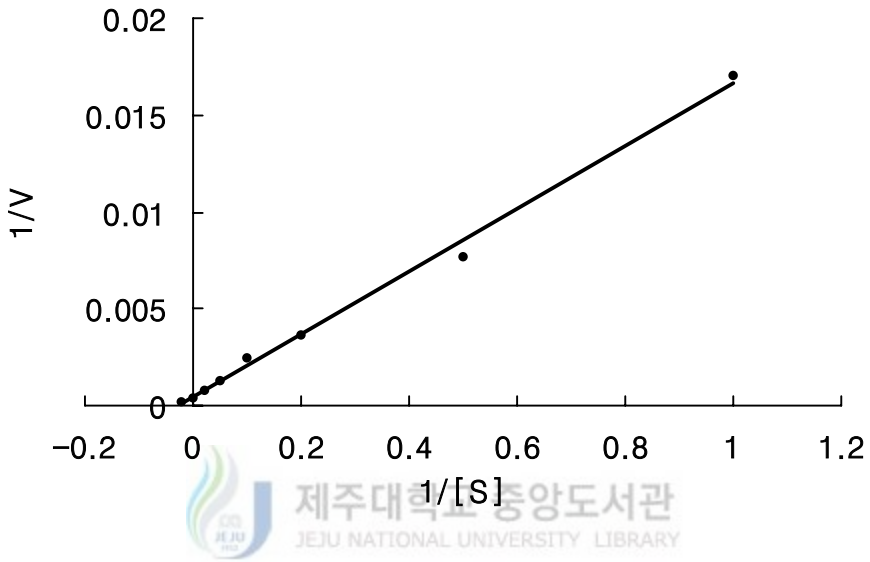



Fig. 9. Lineweaver-Burk plot of the reaction velocity of a catalase from *Rhodospirillum rubrum* S1.

## 4. Effect of pH on catalase activity and pH stability of the catalase

A broad optimum pH range of purified catalase was observed from pH 5.0 to 9.0. The catalase activity was remained approximately 40 % below pH 3.0(Fig. 10). When the enzyme was incubated in a buffer solution(pH range, 3.0 to 11.0) at 25 ℃ for 60 min, the catalase activity showed the most high value at pH 7.0. Although the purified enzyme showed a weak pH dependence. The enzyme was generally stable in the pH range from 5.0 to 9.0 and more than 50 % of the activity remained at pH 5.0. At pH 11.0, enzyme activity remained about 20 %(Fig. 11). The monofunctional catalase of *R. rubrum* S1 is known to be broad pH range(Nadler *et al*., 1986). the catalase activity of the enzyme used this study showed a weak pH dependence. Although the purified enzyme appeared the most high catalase activity at pH 7.0, Catalase activities remained by 60 % on the whole, except pH 3.0, pH 10.0, and pH 11.0, show that the purified enzyme is somewhat stable in the pH range of pH 5.0～9.0.

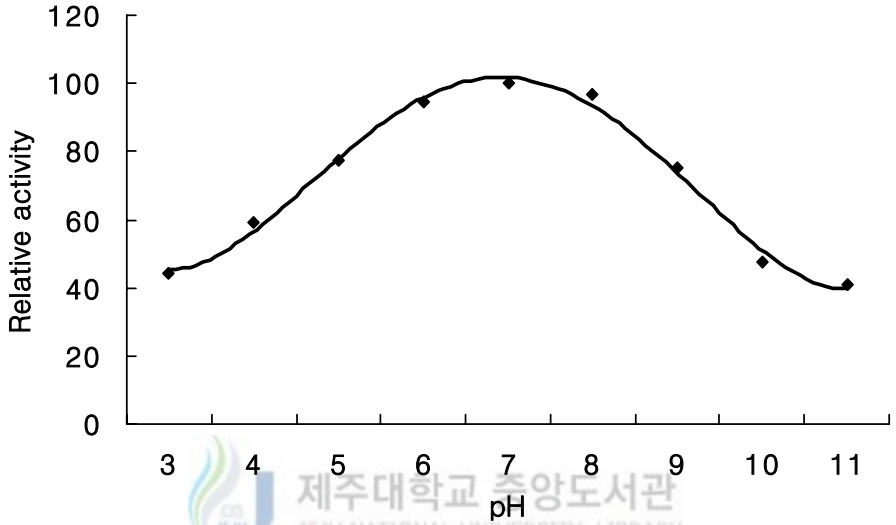



Fig. 10. Effect of pH on the activity of a catalase purified from *Rhodospirillum rubrum* S1.

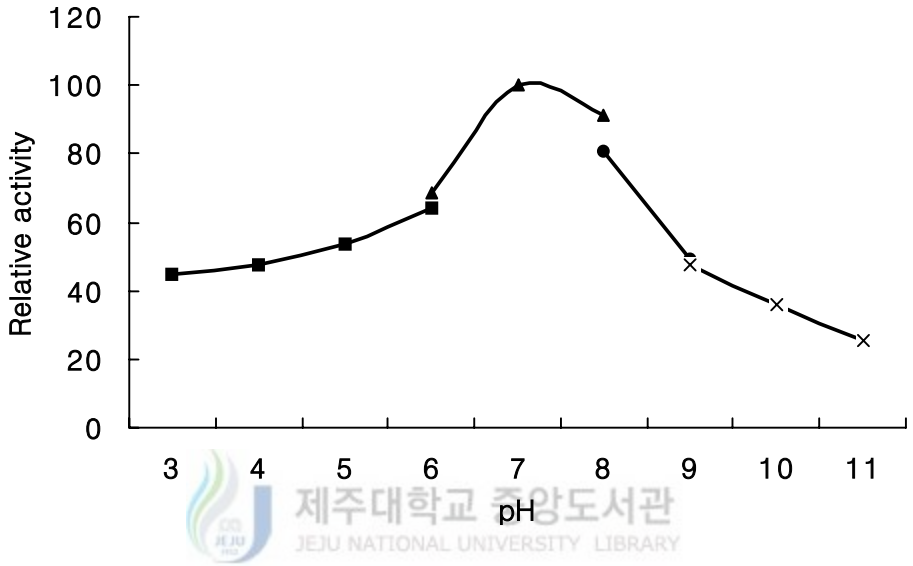



Fig. 11. Effect of pH on the stability of a catalase purified from *Rhodospirillum rubrum* S1.(Citrate buffer; -■-, Phosphate buffer; -●-, Tris-HCl; -▲-, Carbonate buffer; -×-). The enzyme solution was incubated for 1h at 25℃.

## 5. Temperature dependence and thermal stability of catalase

Catalase activity was assayed at various temperatures using the enzyme purified from *R. rubrum* S1. Like most monofunctional catalase(Loewen and Switala, 2002), the temperature dependence of catalase activity was not great. The optimum temperature for the enzymatic activity in *R. rubrum* S1 was approximately 30 ℃(Fig. 12). Even when the purified enzyme was incubated at 40 to 50 ℃, its catalase activity was slightly suppressed. The catalase activity of *R. rubrum* S1 was remained approximately 20 % by incubation at 70 ℃(Fig. 13). These results show that the purified enzyme is very stable at high temperature.

## 6. Effect of metal and chemical inhibitors on catalase activity



Sodium azide, sodium cyanide, and hydroxylamine are known as heme inhibitors of catalase. the effect of metal inhibitors on the purified enzyme was examined(Fig. 14). At the concentrations of $14.6\times10^{-6}$ M, sodium cyanide inhibited the activity of *R. rubrum* S1 catalase by 50 %. Sodium azide and hydroxylamine inhibited the catalase activity by 50 % at the concentrations of $6.2\times10^{-7}$ M, $5.5\times10^{-7}$ M, respectively. which were similar to those of a typical catalase of yeast(Yumoto *et al*., 1990). These values are much lower than those of catalase-peroxidase(Hochman and Shemesh, 1987).

*R. rubrum* S1 catalase was mixed with organic solvent(ethanol/chloroform). After incubation of the enzyme solution with organic solvent for 10 min 25 ℃, only 10 % of the catalase activity was lost, showing that *R. rubrum* S1 catalase is quite resistant to organic solvent(Table 2). On the contrary, the catalase activity of the purified enzyme was inhibited by 81 %, after incubation for 10 min with

10 mM 3-amino-1,2,4-triazole(Table 2). From these result, the purified enzyme seems to be insensitive to organic solvents(ethanol/chloroform) and sensitive to 3-amino-1,2,4-triazole like other typical catalases(Loewen and Switala, 2002).

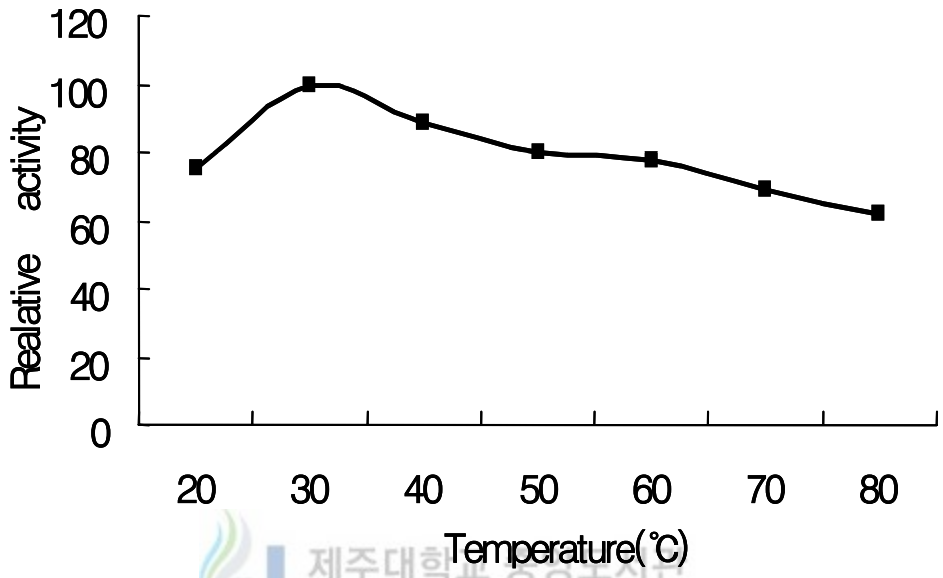


Fig. 12. Effect of temperature on the activity of a catalase purified from *Rhodospirillum rubrum* S1.

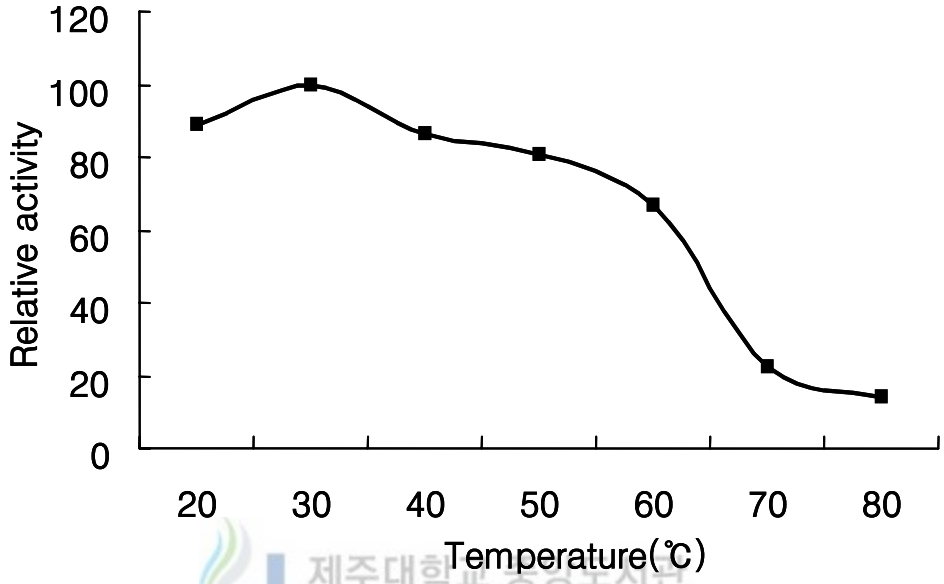




Fig. 13. Thermostability on the activity of a catalase purified from *Rhodospirillum rubrum* S1. The enzyme activity was assayed after 30 min incubation at each temperature.

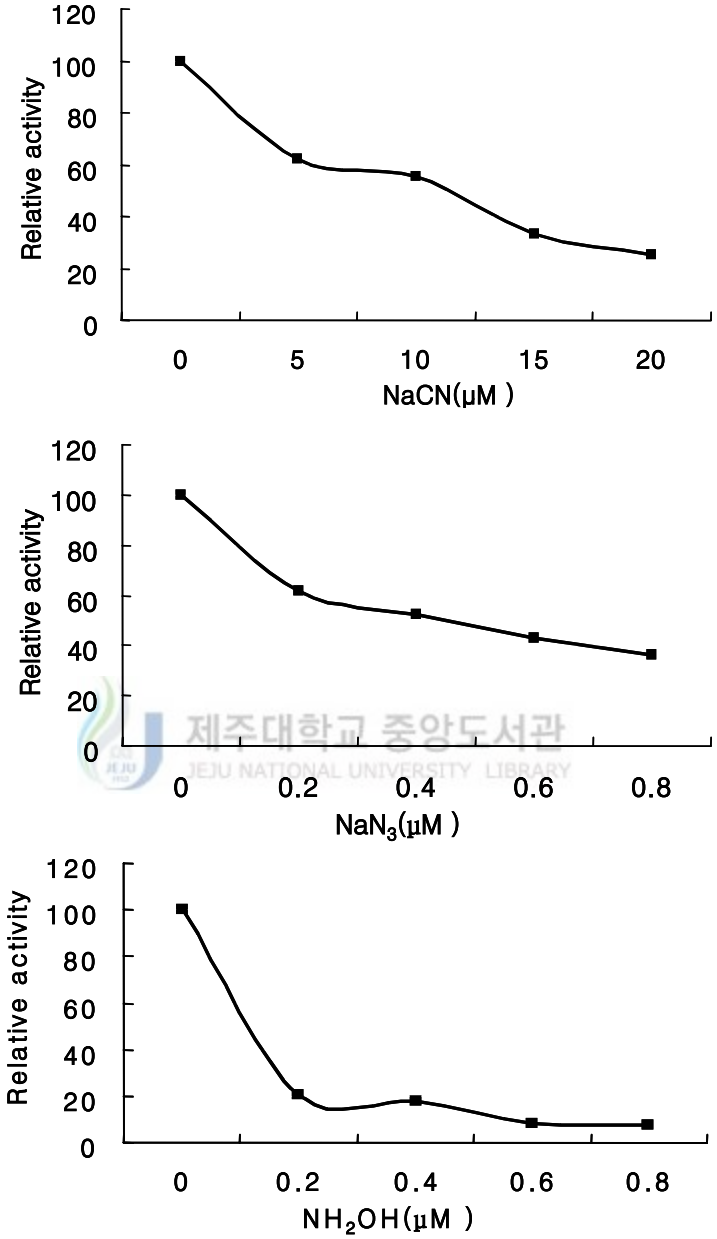




Fig. 14. Effect of heme protein inhibitors on the activity of a catalase purified from *Rhodospirillum rubrum* S1.

Table 2. Effect of chemical inhibitors on the activity of catalase purified from *Rhodospirillum rubrum* S1

| Chemical inhibitors | Catalase activity(%) |
|---|---|
| Control | 100 |
| Organic solvent[a] | 89.4 |
| 3-amino-1,2,4-triazole[b] | 18.6 |

a : The enzyme solution was mixed with organic solvents to give a final composition, enzyme solution: ethanol: chloroform = 10: 5: 3, and then vortexed for 10 min at room temperature.



b : The enzyme solution was incubated in 10 mM 3-amino-1,2,4-triazole for 10min.

# REFERENCES

Beers, Jr. R. F., and I. W. Sizer. 1952 A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. *J. Biol. Chem.* 195: 133-140.

Bose, S. K., H. Gest., and J. G. Ormerod. 1962 Light-activated hydrogenase activity in photosynthetic bacterium : A permeability phenomenon. *J. Biol. Chem.* 236: 13-14.

Brown-Peterson, N. J., and M. L. Salin. 1995 Purification and characterization of mesohalic catalase from the halophilic bacterium *Halobacterium halobium*. *J. Bacteriol*. 177: 378-384.



Brown, S. M., M. L. Howell, M. L. Vasil, A. J. Anderson, and D. J. Hassett. 1995 Cloning and characterization of the KatB gene of *Pseudomonas aeruginosa* encoding a hydrogen peroxide-inducible catalase: Purification of KatB, cellular localization, and demonstration that it is essential for optimal resistance to hydrogen peroxide. *J. Bacteriol*. 177: 6536-6545.

Claiborne, D. A., and Fridovich. 1979 Purification of o-dianisidine peroxidase from *Escherichia coli* B. *J. Biol. Chem*. 254: 4245-4252.

Finegold, S. M., and W. L. George. 1989 Anaerobic infections in humans Academic Press. San Diego. Calif.

Hertel, C., G. Schmidt, M. Fischer, K. Oellers, and W. P. Hammes. 1998 Oxygen-dependent regulation of the expression of the catalase gene KatA of *Lactobacillus sakei* LTH677. *Appl. Environ. Microbiol*. 64: 1359-1365.

Hedrick, S. L., and A. J. Smith. 1968 Size and charge isomer separation and estimation of molecular weights of proteins by disc gel electrophoresis. *Arch. Biochem. Biophys*. 126: 239-243.

Hicks, D.B. 1995 Purification of three catalase isozymes from facultatively alkaliphilic *Bacillus firmus* OF4. *Biochim Biophys Acta.* 1229: 347-355.

Hochman, A., and I. Goldberg. 1991 Purification and characterization of a catalase-peroxidase and a typical catalase from the bacterium *Klebsiella pneumoniae*. *Biochim Biophys Acta.* 1077: 299-307.



Hochman, A., A. Figueredo, and J. D. Wall. 1992 Physiological functions of hydroperoxidase in *Rhodobacter capsulatus*. *J. Bacteriol*. 174: 3386-3391.

Hochman, A., and A. Shemesh. 1987 Purification and characterization of a catalase-peroxidase from the photosynthetic bacterium *Rhodopseudomonas capsulata*. *J. Biol. Chem.* 262: 6871-6876.

Imlay, J. A., and S. Linn. 1988 DNA damage and oxygen radical toxicity. *Science*. 240: 1302-1309.

James, A. Imlay. 2002 What biological purpose is served by superoxide reductase? *J. Biol. Inorg. Chem.* 7: 659-663.

Jeong, J. H., E. S. Kwon, and J. H. Roe. 2001 Characterization of the Manganese-containing superoxide dismutase and its gene regulation in stress response of *Schizosaccharomyces pombe*. *Biochem. Biophys. Res. Commun.* 283: 908-914.

Kim, H., J. S. Lee, Y. C. Hah, and J. H. Roe. 1994 Characterization of the major catalase from *Streptomyces coelicolor* ATCC 10147. *Microbiology* 140: 3391-3397.

Laemmli, U.K. 1970 Cleavage of structural proteins during the asembly of the head of bacterophage T4. *Nature* 227: 680-685



Lee, I. J., and Y. N. Lee. 1995 Purification and characterization of catalase-3 of *Deinococcus radiophilus*. *J. Microbiol.* 33: 239-243.

Lee, J. S., Y. C. Hah, and J. H. Roe. 1993 The induction of oxidative enzymes in *Streptomyces coelicolor* upon hydrogen peroxide treatment. *J. Gen. Microbiol.* 139: 1013-1018.

Lee, S. I., J. Lee, and J. H. Roe. 2000 Subcellular localization of catalase encoded by the *ctt*1$^{+}$ gene in *Schizosaccharomyces pombe*. *J. Microbiol.* 38: 156-159.

Lim, H. K., Y. M. Kim, D. H. Lee, H. Y. Kahng, and D. C. Oh. 2001 Analysis of Catalases from photosynthetic bacterium *Rhodospirillum rubrum* S1. *J. Microbiol.* 39: 168-176.

Loewen, P. C., and J. Switala. 1986 Purification and characterization of catalase HPⅡ from *Escherichia coli* K12. *Biochem Cell Biol.* 64: 638-646

Loewen, P. C. 1992 Regulation of bacterial catalase synthesis. p.97-115 *In* J.G. Scandalios(ed.). Molecular biology of free radical scavenging systems. Cold Spring Harbor Laboratory Press. Cold Spring Harbor. N.Y.

Michaela, K., L. M. Harvey, and B. McNeil. 2002 Oxidative stress response of a recombinant *Aspergillus niger* to exogenous menadione and $H_2O_2$ addition. *Enzyme and Microbial Technology*. 30: 346-353.



Miller, C. D., Y. C. Kim, and A. J. Anderson. 1997 Cloning and mutational analysis of the gene for the stationary phase inducible catalase(cat c) from *Pseudomonas putida. J. Bacteriol*. 179: 5241-5245.

Mongkolsuk, S., P. Vattanaviboon, and W. Praituan. 1997. Induced adaptive and cross-protection responses against oxidative stress killing in a bacterial phytopathogen, *Xanthomonas oryzae* pv. *oryzae*. *FEMS Microbiology Letters*. 146: 217-222.

Morris, J. G. 1980 Oxygen tolerance/intolerance of anaerobic bacteria. p.7-15. *In* G. Gottschalk. N. Penning. and H. Werner(ed.). Anaerobes and anaerobic

infections. Symposia held at the XII International Congress of Microbiology in Munich. September 3-8. 1978. Gustav Fisher Verlag. Stuttgart. Germany.

Nadler, V., Goldberg, I., and A. Hochman. 1986 Comparative study of bacterial catalase. *Biochim Biophys Acta* 82: 234-241.

Nestor, C., N. Carrillo, C. Pasternak, A, Balzer, and G. Klug. 1998 Molecular cloning and expression analysis of the *Rhodobacter capsulatus* sodB gene, encoding an iron superoxide dismutase. *J. Bacteriol*. 180: 5413-5420.

Obinger, C., G. Regelsberger, G. Strasser, U. Burner, and G. A. Peschek. 1997 Purification and characterization of a homodimeric catalase-peroxidase from the cyanobacterium *Anacystis nidulans*. *Biochem. Biophys. Res. Commun*. 235: 545-552.



Odenbreit, S., B. Wieland, and R. Haas. 1996 Cloning and genetic characterization of *Helicobacter pylori* catalase and construction of catalase-deficient mutant strain. *J. Bacteriol*. 178: 6960-6967

Oh, K.A., and Y. N. Lee. 1998 Purification and characterization of catalase-2 from *Deinococcus radiophilus*. *J. Biochem & Mol. Biol*. 31: 144-148.

Rocha, E. R., and C. J. Smith. 1995 Biochemical and genetic analyses of a catalase from the anaerobic bacterium *Bacteroides fragilis*. *J. Bacteriol.* 177: 3111-3119

Rocha, E. R., and C. J. Smith. 1997 Regulation of *Bacteroides fragilis* KatB mRNA by oxidative stress and carbon limitation. *J. Bacteriol*. 179: 7033-7039.

Schnell, S., and H. M. Steinman. 1995 Function and stationary phase induction of periplasmic copper-zinc superoxide dismutase and catalase/peroxidase in *Caulobacter crescentus*. *J. Bacteriol*. 177: 5924-5926.

Shin, K. J., Y. T. Ro, and Y. M. Kim. 1994 Catalase in *Acinetobacter* sp. strain JCI DS 3803 growing on glucose. *Kor. J. Microbiol*. 32: 155-162.

Silgia, A. C., and A. Cavaco-Paulo. 2002 Studies of stabilization of native catalase using additives. *Enzyme and Microbial Technology*. 30: 387-391.



Smith, P. K., Krohn, R. I., Hermanson, G. T., Mallia, A. K., Gartner, E. K., Goeke, N. M., Olson, B. J., Klenk, D. C. 1985 Measurement of Protein Using Bicinchoninic Acid. Anal. Biochem. 150, 76-85.

Steinman, H. M., F. Fareed., and L. Weinstein. 1997 Catalase-peroxidase of *Caulobacter crescentus* : Function and role in stationary-phase survival. *J. Bacteriol*. 179: 6831-6836.

Switala, J. and P. C. Loewen. 2002 Diversity of properties among catalases. *Arch. Biochem. Biophys*. 401: 145-154.

Terzenbach, D. P. and M. Blaut. 1998 Purification and characterization of a catalase from the nonsulfur phototrophic bacterium *Rhodobacter sphaeroides* ATH

2.4.1 and its role in the oxidative stress response. *Arch. Microbiol*. 169: 503-508.
Uffen, R. L. and R. S. Wolfe. 1970 Anaerobic growth of purple nonsulfur bacteria under dark conditions. *J. Bacteriol*. 104: 462-472.

Vattanaviboon, P., S. Mongkolsuk. 2000 Expression analysis and characterization of the mutant of a growth phase- and starvation-regulated monofunctional catalase gene from *Xanthomonas campestris* pv. *phaseoli*. *Gene*. 241: 259-265.

Wagner, G. D., and J. Legall. 2000 Purification and Characterization of an Iron superoxide dismutase and a catalase from the sulfate-reducing bacterium *Desulfovibrio gigas*. *J. Bacteriol*. 182: 796-804.

Wayne, L. G., and G. A. Diaz. 1986 A double staining method for differentiating between two classes of mycobacterial catalase in polyacrylamid gels. *Anal. Biochem.* 157: 89-92.



Yun. E. J., and Y. N. Lee. 2000 Production of two different catalase-peroxidases by *Deinococcus radiophilus*. *FEMS Microbiology Letters.* 184: 155-159

Yumoto, I., D. Ichihashi, H. Iwata, A. Istokovics, N. Ichise, H. Matsuyama, H. Okuyama, and K. Kawasaki. 2000 Purification and characterization of a catalase from the facultatively psychrophilic bacterium *Vibrio rumoiensis* S-1$^{T}$ exhibiting high catalase activity. *J. Bacteriol*. 182: 1903-1909.

Yumoto, I., H. Iwata, T. Sawabe, K. Ueno, N. Ichise, H. Matsuyama, H. Okuyama, and K. Kawasaki. 1999 Characterization of a facultatively psychrophilic

bacterium, *Vibrio rumoiensis* sp. nov., that exhibits high catalase activity. *Appl. Environ. Microbiol*. 65: 67-72.

Yumoto, I., Y. Fukumori, and T. Yamanaka. 1990 Purification and Characterization of catalase from a facultative alkalophilic bacillus. *J. Biochem*. 108: 583-587.

# 초 록

비유황광합성 세균인 *Rhodospirillum rubrum* S1이 혐기적인 조건에서 생장할 때 2종류의 catalase를 생산한다. 혐기적인 조건에서 지수기 말기까지 자란 *R. rubrum* S1에 활성산소족의 유도제인 $H_2O_2$, menadione, methyl viologen, 그리고 benzyl viologen을 처리한 후, 호기적과 혐기적 조건에서 1시간과 3시간동안 배양하였다. 배양조건에는 관계없이 유도제 첨가후 1시간 배양한 세포에서 추출한 조효소액의 catalase 활성이 가장 높게 나타났다. 이러한 실험을 기초로 2종류의 catalase 중에서 높은 분자량을 가진 catalase를 분리하고 그 특성을 알아보았다. 조효소액 단계에서는 88U/mg의 catalase 특이 활성은 최종단계를 거치면서 1,256 U/mg의 활성에 이르렀으며, 처음 단계에 비해 14.3배의 효소를 얻었다. 분리된 catalase는 수용액 상태에서 318 kDa의 분자량을 보였으며, 전기영동을 통해 79 kDa의 분자량을 가진 소단위 4개로 이루어졌음을 알 수 있었고 peroxidase 활성은 보이지 않았다. 분리된 catalase의 과산화수소에 대한 Km은 30.4 mM이며, 2,564 U의 *Vmax*를 보였다. pH 7에서 가장 높은 catalase 활성을 보이는 약한 pH 의존성을 보였지만, pH 전반에 걸쳐 안정적인 catalase 활성을 보였다. 온도에 대한 영향에서는 60 ℃에서 30분을 배양한 효소에서도 최대 활성의 60 % 이상이 남아 있는 것으로 보아 열에 상당히 강한 안정성을 보였다. hydroperoxidase의 저해제로 알려진 유기용매에 대해서는 90 %이상의 활성을 유지하였다. catalase의 저해제인 3-amino-1,2,4-triazole을 10 mM의 농도로 처리했을 때에는 활성의 81 %가 저해되었다. NaCN, $NaN_3$, $NH_2OH$같은 heme 저해제를 처리했을 때, NaCN의 경우에는 $14.6\times10^{-6}$ M의 농도에서 활성의 50 %가 저해되었고, $NaN_3$와 $NH_2OH$의 경우에는 $6.2\times10^{-7}$M과 $5.5\times10^{-7}$ M의 농도에서 각각 50 %의 활성이 저해되었다. 이러한 결과들을 종합해볼 때, 분리된 효소는 전형적인 catalase로 보여진다.