JOURNAL OF BACTERIOLOGY, Nov. 1986, p. 563–567
0021-9193/86/110563-05$02.00/0

Vol. 168, No. 2

Characterization of a Manganese-Containing Catalase from the Obligate Thermophile *Thermoleophilum album*†

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Received 12 May 1986/Accepted 4 July 1986

A manganese-containing catalase has been characterized from *Thermoleophilum album* NM, a gram-negative aerobic bacterium obligate for thermophily and *n*-alkane substrates. The level of catalase in cells was increased about ninefold by growth in the presence of paraquat (2.5 μM), a superoxide-generating toxicant. Superoxide dismutase levels were unaffected by this compound. The enzyme was purified from cultures grown in the presence of paraquat to >95% homogeneity and had an M_r of 141,000. The enzyme was composed of four subunits, and each had an M_r of 34,000. There were 1.4 ± 0.4 atoms of manganese present per subunit. The catalase had a K_m for hydrogen peroxide of 15 mM and a V_{max} of 11 mM/mg. Peroxidase activity, as measured with *p*-phenylenediamine, copurified with the catalase. Inhibitors of heme-catalase were weak inhibitors of the *T. album* enzyme. The optimum pH for catalase activity was 8 to 9. The enzyme was stable from pH 6.5 to 11 and retained activity at assay temperatures from 25 to 80°C. The catalase was stable for 24 h of incubation at 60°C.

Bacterial catalases have been characterized from a number of organisms, including "*Micrococcus lysodeikticus*" (12), *Rhodopseudomonas sphaeroides* (7), and *Escherichia coli* (5, 6). Generally these enzymes are heme containing with the iron complexed with protoporphyrin. Catalases not inhibited by cyanide or azide have been observed in "*Pediococcus cerevisiae*" (9) and a number of other lactic acid bacteria (14). Johnston and Delwiche suggested that "the system was independent of the heme-iron structure" (14), and the enzyme was considered by others to be a pseudocatalase (26). Kono and Fridovich (17) characterized the catalase activity from *Lactobacillus plantarum* and reported that it is a homohexameric manganese-containing enzyme.

Previous studies (31) suggested that enzymes from members of the genus *Thermoleophilum*, a group of thermophilic obligately aerobic organisms, differed in electrophoretic mobility when compared with equivalent enzymes from other thermophilic strains. It was also reported that oxygen stress in these organisms resulted in an induction of catalase or peroxidase but did not affect superoxide dismutase levels (1, 2). In this report we characterize the inducible catalase from *Thermoleophilum album* NM.

MATERIALS AND METHODS

Organism and growth conditions. *T. album* NM (ATCC 35226), a gram-negative, obligately thermophilic bacterium that requires *n*-alkane substrates, was grown on a mineral salts medium (18) with 0.05% *n*-heptadecane as a substrate (30). Cultures were grown statically at 60°C in 2.8-liter Fernbach flasks containing 1 liter of medium. Methyl viologen (paraquat) was added (2.5 μM) to appropriate flasks (11). Cells were harvested in the late exponential phase by centrifugation, washed twice with 100 mM potassium phosphate buffer (pH 7.8), and stored at 5°C until analyzed.

Enzyme activities. Catalase activity was measured by the spectrophotometric method of Beers and Sizer (4) as described previously (28), except that 50 mM potassium phosphate (pH 8.0) was used as a buffer. One unit of catalase activity is equivalent to 1 μmol of hydrogen peroxide (H_2O_2) decomposed per min. Inhibition of catalase activity was determined by measuring oxygen production with a Clark-type polarographic electrode (YSI model 53; Yellow Springs Instrument Co.). Peroxidase activity was assayed by following the oxidation of *p*-phenylenediamine spectrophotometrically at 485 nm (20), and units were determined as previously described (1). Superoxide dismutase activity was determined by the ferricytochrome *c*-xanthine oxidase method of McCord and Fridovich (21).

Peroxidation of *o*-dianisidine dihydrochloride was followed at 460 nm (28), peroxidation of guiacol was followed at 436 nm (22), and peroxidation of glutathione was followed at 340 nm by coupling with the glutathione reductase reaction (24). The peroxidation of ferricytochrome (after reduction with dithionite followed by dialysis) was monitored at 550 nm (5).

Protein levels were determined with the Bio-Rad protein assay (Bio-Rad Laboratories) with bovine serum albumin as a standard. Dry weight was determined by drying thoroughly washed cells at 110°C to constant weight. All assays were performed at 25°C unless stated otherwise.

Purification of catalase. All purification procedures were performed at 5°C. Twice-washed cells (1.1 g wet weight) were suspended at 50% (wt/vol) in 50 mM potassium phosphate buffer (pH 7.8) and disrupted by sonication for 6 min with a sonic Dismembrator (Quigley-Rochester Inc.). Sonication was applied intermittently to permit cooling of the suspension. Cell-free extracts were obtained by centrifugation at 40,000 × *g* for 1 h. The pellet contained approximately 2% of the catalase activity of the supernatant. The supernatant was placed on a Sephadex G-150 column (2.5 by 76 cm) equilibrated with 50 mM potassium phosphate buffer (pH 7.8). The column was eluted with the phosphate buffer at a flow rate of 0.6 ml/min. Fractions of 4.0 ml were collected, and absorbance at 280 nm was determined. Those fractions that absorbed were assayed for catalase and peroxidase activity. The active fractions were combined and concentrated with an Amicon YM30 ultrafiltration mem-

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† Paper no. 10488 of the journal series of the North Carolina Agricultural Research Service, Raleigh, N.C.

TABLE 1. Effect of paraquat on cell yield and level of oxygen defense enzymes in *T. album*[a]

Paraquat concn (μM)	Dry wt (mg/liter)	Enzyme concn (U/mg of protein)		
		Catalase	Peroxidase	Superoxide dismutase
0	18	137	9	33
1.0	18	337	17	34
2.5	15	1,290	79	32

[a] Cells were grown (163 h) statically at 60°C with 0.05% (vol/vol) *n*-heptadecane as a substrate.

brane. The concentrated protein was placed on a DEAE Sephadex A-50 column (2.5 by 27 cm) equilibrated with 10 mM Tris hydrochloride buffer (pH 8.3). The concentrate was placed on the column with 65 ml of 10 mM Tris hydrochloride (pH 8.3) and eluted with a linear gradient of KCL (0 to 1.0 M, 750 ml) in 10 mM Tris hydrochloride (pH 8.3). The active fractions were combined and desalted on a Sephadex G-25 column equilibrated with 50 mM potassium phosphate buffer (pH 7.8). The column eluent was concentrated with an Amicon YM30 ultrafiltration membrane.

Electrophoresis. Nondenaturing discontinuous gel electrophoresis was performed with 8.0% polyacrylamide gels without sodium dodecyl sulfate (SDS) (8). Protein bands were stained with Coomassie brilliant blue R-250. Catalase bands were visualized by staining by the procedure of Woodbury et al. (27) with 2 to 3 U of catalase per gel. The bands were also determined by the procedure of Gregory and Fridovich (10) for both catalase and peroxidase activity.

SDS-gel electrophoresis was performed as described by Weber and Osborn (25). Polyacrylamide gels containing 10.0% acrylamide and 0.1% SDS were loaded with 5 to 10 μg of protein which had prior treatment with 5% 2-mercaptoethanol and 2% SDS for 10 min at 100°C. Protein staining was performed with Coomassie brilliant blue R-250. The following proteins (obtained from Bio-Rad) served as M_r standards: lysozyme (14,400), soybean trypsin inhibitor (21,500), carbonic anhydrase (31,000), ovalbumin (45,000), and bovine serum albumin (66,200).

M_r determination. The M_r of purified catalase was estimated by gel filtration on a Sephadex G-150 column (2.5 by 76 cm) by the method of Andrews (3). The elution buffer was 50 mM potassium phosphate (pH 7.8) with blue dextran 2000 used to determine the void volume. Proteins (obtained from Pharmacia, Inc.) employed as M_r standards were: cytochrome *c* (13,000), ovalbumin (43,000), bovine serum albumin (67,000), aldolase (158,000), bovine catalase (232,000), and ferritin (440,000). After calibration of the column, aldolase was chromatographed as the internal standard with the purified catalase from *T. album*.

Metal content. Purified catalase (230 μg/ml) was dialyzed against 10 mM potassium phosphate–0.1 mM EDTA (pH 7.8) for 20 h at 5°C. This dialyzed enzyme (active) was analyzed by atomic absorption spectrophotometry and nuclear activation analysis (Nuclear Energy Services, North Carolina State University) by using the dialysis buffer as the control. Elemental analysis for manganese, zinc, iron, and cobalt was performed on an Instrumental Laboratories Atomic absorption Spectrophotometer with deuterium arc background correction and a pyrolytic graphite flameless furnace. The purging gas was argon. Nuclear activation analysis for manganese and iron was accomplished on an Ortec 35% GeLi detector coupled to a ND6620 computerized gamma detection system.

Enzyme characterization and stability experiments. The effect of pH on enzyme stability was determined by incubating 10 U of enzyme per ml in 50 mM buffer for 3 h followed by assay of activity. Sodium acetate buffer was employed for pH 4.0 to 5.0, potassium phosphate was used for pH 5.5 to 8.5, and glycine sodium buffer was used for pH 8.5 to 13.0. Assay for activity was as above except for the buffer added. The effect of temperature on enzyme activity was determined under standard assay conditions with selected assay temperature (15 to 90°C) maintained in a water-jacketed cuvette holder connected to a circulating bath. Temperature stability studies were with purified catalase from *T. album* NM and *E. coli* K-12 cell-free extract. Purified catalase from "*Aspergillus flavum*" and bovine liver were obtained from Sigma Chemical Co. The enzyme preparations were diluted to about 10 U/ml in 50 mM potassium phosphate buffer at pH 8.0 for the enzyme from *T. album* and at pH 7.0 for the others. The preparations were incubated at the appropriate temperatures in screw-cap vials and assayed under the conditions described above.

RESULTS

Paraquat-dependent induction. The effect of paraquat on the total growth and level of oxygen defense enzymes in *T. album* are presented in Table 1. Paraquat at a concentration of 1.0 and 2.5 μM did not significantly affect the cell yield (10 μM totally inhibited growth). Superoxide dismutase levels were unaffected by the presence of 2.5 μM paraquat, whereas catalase and peroxidase levels increased approximately ninefold.

Purification of catalase. Purification procedures for catalase are summarized in Table 2. The enzyme was purified from cells grown in the presence of 2.5 μM paraquat. Peroxidase activity for *p*-phenylenediamine copurified with catalase and increased in approximately the same ratio as catalase with each purification step. Catalase was purified 14-fold with a 17% yield of activity. The combination of paraquat induction and purification steps took the specific activity of 137 U/mg of protein in noninduced cell-free extract to 17,745 U/mg of protein in the final purified preparation. Polyacrylamide gel electrophoresis indicated the presence of one major protein band (R_f 0.46; the R_f of bromothymol blue was 1). Densitometric scans at 579 nm of gels stained for protein indicated that this band accounted for over 95% of the protein in the final preparation. Staining for catalase activity in parallel gels indicated the presence of

TABLE 2. Purification of the catalase from *T. album*

Step	Total vol (ml)	Total protein (mg)	Total activity (U × 10^3)		Sp act (U/mg)		% Yield		Purification (fold)	
			Catalase	Peroxidase	Catalase	Peroxidase	Catalase	Peroxidase	Catalase	Peroxidase
Crude extract	4.7	56.4	72.8	4.5	1,290	79	100	100	1.0	1.0
Sephadex G-150	3.5	6.7	42.7	2.7	6,379	398	59	60	4.9	5.0
DEAE-Sephadex	3.5	0.7	12.4	0.9	17,745	1,232	17	19	13.8	15.6

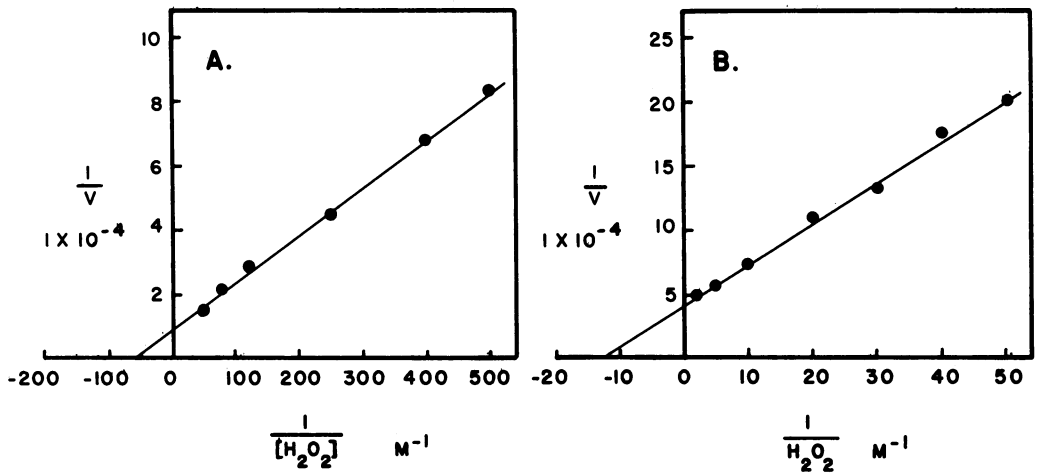

FIG. 1. Effect of H_2O_2 concentration on (A) catalase activity and (B) peroxidase activity for *p*-phenylenediamine. Enzyme activity was determined as described in Materials and Methods.

one band of activity corresponding to the major protein band. There was no difference in the relative mobility of the catalase in crude extracts grown in the presence or absence of paraquat. Peroxidase bands were not detectable in the crude extract or purified preparation in the diaminobenzidine assay.

Kinetic properties. A K_m for H_2O_2 of 15 mM and a V_{max} of 11 mM/min per mg were determined for purified catalase by using the double-reciprocal plots (19) presented in Fig. 1A. Concentrations of H_2O_2 above 20 mM inhibited the catalase reaction. The *p*-phenylenediamine peroxidase activity had a K_m for H_2O_2 of 74 μM and a V_{max} of 2.3 mM/min per mg (Fig. 1B). Concentrations of H_2O_2 above 1 mM resulted in an increased catalase activity that interfered with the peroxidase assay. Other substrates tested for peroxidase activity included *o*-dianisidine dihydrochloride, guiacol, glutathione, and reduced ferricytochrome *c*; no peroxidase activity was detected with these substrates with various H_2O_2 and substrate concentrations.

M_r and subunit analysis. The M_r of catalase was estimated to be 141,000 by using a calibrated Sephadex G-150 column. The subunit M_r was estimated by SDS-polyacrylamide gel electrophoresis. After treatment with SDS in the presence of 2-mercaptoethanol a single band with an estimated M_r of 34,000 was obtained. Complete dissociation did not occur in the absence of 2-mercaptoethanol.

Metal content and optical spectra. Purified catalase (0.7 mg/ml) from *T. album* had a single absorption band centered at 280 nm when monitored from 600 to 240 nm. The enzyme was colorless at this concentration. It did not exhibit the Soret band at 407 nm typical of heme-containing catalases (5, 12). Atomic absorption spectrophotometry and nuclear activation analysis revealed that Fe, Zn, and Co were present at less than 0.05 atoms per subunit, whereas Mn was present at 1.4 ± 0.4 atoms per subunit.

Effect of inhibitors on activity. Catalase activity was strongly inhibited by hydroxylamine hydrochloride, by mercuric chloride, and by high concentrations of histidine (Table 3). Sodium azide, potassium cyanide, and 3-amino-1,2,4-triazole were weak inhibitors at concentrations of 10 mM. EDTA did not reduce activity at a concentration of 5 mM. H_2O_2 caused significant inhibition at a concentration twice that normally used in the catalase assay, and *p*-phenylenediamine was not an inhibitor of catalase at the concentration normally used for the peroxidase assay.

Effect of pH on catalase. Catalase activity occurred over a pH range of 5.5 to 12.0, with the optimum between 8.0 and 9.0. Purified catalase (10 U/ml) retained 100% activity after 3 h of incubation at 25°C in buffers over a pH range of 6.5 to 11.0. Less than 20% activity remained when the enzyme was incubated in buffers with a pH of 5.0 or less and buffers with a pH of 12.0 or greater.

Effect of temperature on catalase. Purified *T. album* catalase had maximum activity at assay temperatures from 25 to 35°C with 56% of this activity at 60°C; no activity was observed at 85°C and above. *T. album* catalase retained 90%

TABLE 3. Effect of inhibitors on the activity of the catalase from *T. album*

Inhibitor	Final concn (mM)	% Inhibition[a]
Hydroxylamine hydrochloride	0.01	56
	0.1	100
$HgCl_2$	1.0	100
Histidine	5.0	40
	50.0	100
Sodium azide	10.0	45
	25.0	84
KCN	10.0	19
	100.0	37
3-Amino-1,2,4-triazole	10.0	0
	150.0	13
EDTA	5.0	0
	10.0	18
H_2O_2	40.0	43
p-Phenylenediamine	3.0	0

[a] Catalase was assayed with an oxygen electrode in a reaction mixture (3 ml) containing 50 mM potassium phosphate (pH 8.0), 20 mM H_2O_2, and 5 μg of catalase.

TABLE 4. Comparison of catalases isolated from several bacterial species

Species and catalase	Reference	Crude extract activity (U/mg of protein)	Metal	M_r ($\times 10^3$)	No. of subunits
E. coli hydroperoxidase I	(5)	38	Fe	337	4
E. coli hydroperoxidase II	(6)	32	Fe	312	4
R. sphaeroides	(7)		Fe	232	4
"*M. lysodeikticus*"	(12)		Fe	232	4
L. plantarum	(17)	107	Mn	172	6
T. album	(This study)	137	Mn	141	4

of its initial activity after incubation for 1 h at 80°C and 93% activity after incubation 24 h at 60°C. The catalase was stable at room temperature for 168 h, although significant activity (43%) was lost on freezing and thawing. The catalases from bovine liver, "*A. flavum*," and *E. coli* were less stable at 60°C but more stable to freezing and thawing. Bovine liver catalase was completely unstable at 60°C for 1 h.

DISCUSSION

The induction of catalase to eliminate hydrogen peroxide was previously reported to be an important response of thermophilic bacteria to oxygen toxicity (2). Induction of catalase levels in *T. album* during growth in the presence of 2.5 μM paraquat protected the organism from the oxygen stress caused by this toxicant and facilitated the purification of the enzyme.

Catalases which are insensitive to 10 mM sodium azide and potassium cyanide have been called pseudocatalases (26). Johnston and Delwiche (15, 16) objected to this designation since there is nothing "false" about the activity of such catalases. The level of catalase activity in crude extracts of *T. album* and *L. plantarum* is relatively high when compared to the catalase activity found in *E. coli* (Table 4). *T. album* catalase has a lower M_r than previously isolated catalases and, like the heme-containing catalases, is apparently a tetramer. Similar to the manganese-containing catalase of *L. plantarum* (17), sodium azide, potassium cyanide, and 3-amino-1,2,4 triazole were weak inhibitors of the manganese catalase of *T. album*. Histidine, which stimulated *E. coli* HP-1 catalase (5), was inhibitory to *T. album* catalase. Hydroxylamine, a strong inhibitor of *L. plantarum* manganese catalase (17), was also a strong inhibitor of *T. album* catalase. The pH optimum of *T. album* catalase is higher than that of previously isolated catalases (5, 17). It could not be purified by lowering of the pH to 5.0 (13) because this resulted in loss of activity.

Peroxidase activity by catalase has been described previously. *E. coli* has two distinct hydroperoxidases; hydroperoxidase I has both catalase and peroxidase activity, whereas hydroperoxidase II is an effective catalase lacking peroxidase activity (6). Hydroperoxidase I was considered to be predominately a catalase mostly because it showed activity with diansidine but not with glutathione, ferricytochrome *c*, or nitrite (5). Catalase purified from the yeast *Candida tropicalis* had peroxidase activity for β-(3,4-dihydroxyphenyl)-L-alanine but not for guiacol, one of the general substrates for peroxidase (29). Catalase purified from human erythrocytes was devoid of peroxidase activity for guiacol until it was dissociated with 8 M urea. This resulted in a loss of catalase activity and augmentation of peroxidase activity (23). The catalase from *T. album* had peroxidase activity for *p*-phenylenediamine but not for guiacol, *o*-diansidine dihydrochloride, glutathione, or ferricytochrome *c*. Dissociation with 8 M urea resulted in loss of catalase activity and peroxidase activity for *p*-phenylenediamine.

The enzyme from an obligate thermophile is expected to be thermostable (32); the catalase from *T. album* is thermostable and thermoactive. Commercially available purified catalases were not very stable at high temperatures for periods of 24 h. The manganese catalase of *L. plantarum* had little thermostability (17). H_2O_2 incubated at the concentration that was used for the catalase assay and that permits maximum measurable catalase activity was quite stable at 60°C for 1 h but broke down rapidly at 90°C (unpublished data).

ACKNOWLEDGMENTS

This study was supported in part by Public Health Service Training Grant ESO7046 from the National Institute of Environmental Health Sciences.

LITERATURE CITED

1. **Allgood, G. S., and J. J. Perry.** 1985. Oxygen defense systems in obligately thermophilic bacteria. Can. J. Microbiol. **31**:1006–1010.
2. **Allgood, G. S., and J. J. Perry.** 1985. Paraquat toxicity and effect of hydrogen peroxide on thermophilic bacteria. J. Free Rad. Biol. Med. **1**:233–237.
3. **Andrews, P.** 1964. Estimation of the molecular weights of proteins by Sephadex gel-filtration. Biochem. J. **91**:222–233.
4. **Beers, 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.
5. **Claiborne, A., and I. Fridovich.** 1979. Purification of the o-diansidine peroxidase from *Escherichia coli* B. Physiochemical characterization and analysis of its dual catalatic and peroxidatic activities. J. Biol. Chem. **254**:4245–4252.
6. **Claiborne, A., D. P. Malinowski, and I. Fridovich.** 1979. Purification and characterization of hydroperoxidase II of *Escherichia coli* B. J. Biol. Chem. **254**:11664–11668.
7. **Clayton, R. K.** 1959. Purified catalase from *Rhodopseudomonas spheroides*. Biochim. Biophys. Acta **36**:40–47.
8. **Davis, B. J.** 1964. Disc electrophoresis. II. Methods and application to human serum proteins. Ann. N.Y. Acad. Sci. **121**:404–427.
9. **Delwiche, E. A.** 1961. Catalase of *Pediococcus cerevisiae*. J. Bacteriol. **81**:416–418.
10. **Gregory, E. M., and I. Fridovich.** 1974. Visualization of catalase on acrylamide gels. Anal. Biochem. **58**:57–62.
11. **Hassan, H. M., and I. Fridovich.** 1979. Paraquat and *Escherichia coli*. Mechanism of production of extracellular superoxide radical. J. Biol. Chem. **254**:10846–10852.
12. **Herbert, D., and J. Pinsent.** 1948. Crystalline bacterial catalase. Biochem. J. **43**:193–202.
13. **Jacob, G. S., and W. H. Orme-Johnson.** 1979. Catalase of *Neurospora crassa*. 1. Induction, purification, and physical properties. Biochemistry **18**:2967–2975.
14. **Johnston, M. A., and E. A. Delwiche.** 1962. Catalase of the *Lactobacillaceae*. J. Bacteriol. **83**:936–938.

15. **Johnston, M. A., and E. A. Delwiche.** 1965. Distribution and characteristics of the catalases of *Lactobacillaceae*. J. Bacteriol. **90**:347–351.
16. **Johnston, M. A., and E. A. Delwiche.** 1965. Isolation and characterization of the cyanide-resistant and azide-resistant catalase of *Lactobacillus plantarum*. J. Bacteriol. **90**:352–356.
17. **Kono, Y., and I. Fridovich.** 1983. Isolation and characterization of the pseudocatalase of *Lactobacillus plantarum*. A new manganese-containing enzyme. J. Biol. Chem. **258**:6015–6019.
18. **Leadbetter, E. R., and J. W. Foster.** 1958. Studies on some methane-utilizing bacteria. Arch. Microbiol. **30**:91–118.
19. **Lineweaver, H., and D. Burk.** 1934. The determination of enzyme dissociation constants. J. Am. Chem. Soc. **86**:658–666.
20. **Luck, H.** 1963. Peroxidase, p. 895–897. *In* H. U. Bergmeyer (ed.), Methods of enzymatic analysis. Academic Press, Inc., New York.
21. **McCord, J. M., and I. Fridovich.** 1969. Superoxide dismutase: an enzymic function for erythrocuprein (hemocuprein). J. Biol. Chem. **244**:6049–6055.
22. **Putter, J.** 1974. Peroxidases, p. 685–690. *In* H. U. Bergmeyer (ed.), Methods of enzymatic analysis, vol. 2. Academic Press, Inc., New York.
23. **Scherz, B., E. J. Kuchinskas, S. R. Wyss, and H. Aebi.** 1976. Heterogeneity of erythrocyte catalase. Dissociation, recombination, and hybridization of human erythrocyte catalases. Eur. J. Biochem. **69**:603–613.
24. **Stults, F. H., J. W. Forstrom, D. T. Y. Chiu, and A. L. Tappel.** 1977. Rat liver glutathione peroxidase: purification and study of multiple forms. Arch. Biochem. Biophys. **183**:490–497.
25. **Weber, K., and M. Osborn.** 1969. The reliability of molecular weight determinations by dodecyl sulfate-polyacrylamide gel electrophoresis. J. Biol. Chem. **244**:4406–4412.
26. **Whittenbury, R.** 1964. Hydrogen peroxide formation and catalase activity in the lactic acid bacteria. J. Gen. Microbiol. **35**:13–26.
27. **Woodbury, W., A. K. Spencer, and M. A. Stahmann.** 1971. An improved procedure using ferricyanide for detecting catalase isozymes. Anal. Biochem. **44**:301–305.
28. **Worthington Biochemical Corporation.** 1972. Worthington Enzyme Manual, p. 41–42. Worthington Biochemical Corporation, Freehold, N.J.
29. **Yamada, T. A., Tanaka, and S. Fukui.** 1982. Properties of catalase purified from whole cells and peroxisomes of *n*-alkane-grown *Candida tropicalis*. Eur. J. Biochem. **125**:517–521.
30. **Zarilla, K. A., and J. J. Perry. 1984.** *Thermoleophilum album* gen. nov. and sp. nov., a bacterium obligate for thermophily and *n*-alkane substrates. Arch. Microbiol. **137**:286–290.
31. **Zarilla, K. A., and J. J. Perry.** 1986. Deoxyribonucleic acid homology and other comparisons among obligately thermophilic hydrocarbonoclastic bacteria, with a proposal for *Thermoleophilum minutum* sp. nov. Int. J. Syst. Bacteriol. **36**:13–16.
32. **Zeikus, J. G.** 1979. Thermophilic bacteria: ecology, physiology, and technology. Enzyme Microb. Technol. **1**:243–252.