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Characterization of a Facultatively Psychrophilic Bacterium, *Vibrio rumoiensis* sp. nov., That Exhibits High Catalase Activity

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A novel facultatively psychrophilic bacterium, strain S-1, which exhibits extraordinarily high catalase activity was isolated from the drain pool of a fish product processing plant that uses H_2O_2 as a bleaching and microbicidal agent. The catalase activity of the isolate was 1 or 2 orders of magnitude higher than those of *Corynebacterium glutamicum*, *Staphylococcus aureus*, *Pseudomonas fluorescens*, and five other species tested in this study. The strain seemed to possess only one kind of catalase, according to the results of polyacrylamide gel electrophoresis of the cell extract. The optimum temperature for catalase activity was about 30°C, which was about 20°C lower than that for bovine catalase activity. Electron microscopic observation revealed that the surface of the microorganism was covered by blebs. Although the isolate was nonflagellated, its taxonomic position on the basis of physiological and biochemical characteristics and analysis of 16S rRNA sequence and DNA-DNA relatedness data indicated that strain S-1 is a new species belonging to the genus *Vibrio*. Accordingly, we propose the name *Vibrio rumoiensis*. The type strain is S-1 (FERM P-14531).

The response of bacteria to oxidative stress has been extensively studied for enteric bacteria (7). Most studies dealt with the response to oxidative stress or to an inducer of oxidative stress (3, 5, 7, 13, 14, 21). The gene regulation systems involved in the response to oxidative stress have also been studied (7). However, those studies investigated relatively short-term responses to oxidative stress in limited microorganisms, such as enteric bacteria. Few studies are available on the mechanisms involved in adaptation to relatively long-term oxidative stress. For example, little is known about microorganisms living in oxidative environments. Thus, we initiated studies to understand how a bacterium adapts to oxidative stress in a highly oxidative environment and why such an adaptable bacterium exists in such an environment. In addition, studies on such microorganisms will give us more opportunities to detect the proteins or genes concerned with oxidative stress. A bacterium exhibiting high catalase activity was isolated from the drain pool of a fishery product processing plant that uses hydrogen peroxide as a bleaching agent (33), and we think that the isolate might be a good candidate for studying the mechanisms of bacterial adaptation to oxidative stress.

Although there are few examples of industrial applications of cold-adapted microorganisms, there may be great potential for such applications. Hydrogen peroxide is used as a bleaching or microbicidal agent in the paper, food, textile, and semiconductor industries. In wastewater treatment in such industries, H_2O_2 should be removed before activated-sludge treatment, because H_2O_2 damages microorganisms present in the treatment system. If a cold-adapted bacterium that decomposes H_2O_2 effectively could be applied, industrial wastewater could be treated even at low temperatures. The procedure will be useful for low-energy wastewater treatment, especially in countries with cold climates. Thus, another reason for the study of such microorganisms is their possible industrial applications at low temperatures.

In the present study, we determined the taxonomic position of the isolate that exhibits high catalase activity, compared the catalase activities of several kinds of bacteria, and analyzed the catalase activity in a cell extract of the isolate.

MATERIALS AND METHODS

Bacterial strains and cultivation. The strain that we examined was strain S-1 (33). The organism was cultivated aerobically until the late logarithmic growth phase at 27°C in PYS-2 medium (pH 7.5), unless otherwise stated. The PYS-2 medium contained (per liter of deionized water) 8.0 g of polypeptone (Nihon Pharmaceuticals, Tokyo, Japan), 3.0 g of yeast extract (Kyokuto, Tokyo, Japan), 5.0 g of NaCl, and 15 g of agar (when necessary). For the comparative study of catalase activity, *Alcaligenes faecalis* (laboratory strain), *Corynebacterium glutamicum* IAM 12432, *Staphylococcus aureus* IAM 12544^T, *Aeromonas hydrophila* subsp. *hydrophila* JCM 1027^T, *Escherichia coli* IAM 1264, *Pseudomonas fluorescens* JCM 5963^T, *Bacillus subtilis* IAM 1026, and *Vibrio parahaemolyticus* JCM 2147 were used. These microorganisms were also grown in PYS-2 medium until the stationary growth phase. For DNA-DNA hybridization, *Vibrio halioticoli* IAM 14596^T, *Vibrio alginolyticus* V477 (a kind gift from the Tokyo Metropolitan Research Laboratory of Public Health), *Vibrio pelagius* ATCC 25916^T, *Vibrio campbellii* ATCC 25926^T, *Vibrio fischeri* ATCC 7744^T, *Vibrio splendidus* ATCC 33125^T, *V. parahaemolyticus* JCM 2147, *Vibrio harveyi* NCMB 1280^T, *Photobacterium leiognathi* NCMB 391, and *Photobacterium phosphoreum* IAM 12085 were used. These microorganisms were grown in ZoBell 2216E broth (26) without $FeSO_4 \cdot 7H_2O$.

Phenotypic characterization of strain S-1. For phenotypic characterization, PYS-2 medium was used as the basal medium, the culture was incubated at 27°C for 2 weeks unless otherwise stated, and the experiment was performed more than twice. Morphological, physiological, and biochemical tests were performed as described previously (2). Carbohydrate metabolism was tested by the method

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of Leifson (22). The results were checked daily until 2 weeks after inoculation. Alginase activity was determined by 10 days of culture on an agar plate containing alginic acid and overlaid with ethanol. Sensitivity to the vibriostatic compound O/129 (2,4-diamino-6,7-di-*iso*-propylpteridine phosphate) was determined after agar plate culture for 1 week by using diagnostic discs (10 and 150 μg) (Oxoid Ltd., Basingstoke, Hampshire, England). Determination of the utilization of the substrate as the sole carbon and energy source was performed in US medium (pH 7.5) containing 1% substrate, 2 g of NH_4Cl, 2 g of Na_2HPO_4, 1 g of KH_2PO_4, 0.1 g of $MgSO_4 \cdot 7H_2O$, 0.05 g of $CaCl_2 \cdot 2H_2O$, and 1 ml of trace minerals. The trace minerals included (per 100 ml) 1.8 g of EDTA · 2Na, 5.0 g of $ZnSO_4 \cdot 7H_2O$, 5.0 g of $FeSO_4 \cdot 7H_2O$, 1.5 g of $MnSO_4 \cdot 4H_2O$, 0.4 g of $CuSO_4 \cdot 5H_2O$, 0.25 g of $Co(NO_3)_2 \cdot 6H_2O$, and 0.1 g of H_3BO_3.

Electron microscopy. Cells grown on PYS-2 agar slants were suspended in physiological saline. A small drop of the suspension was placed on a carbon-coated copper grid and negatively stained with 1% phosphowolframic acid for observation with a transmission electron microscope (Hitachi H-800).

16S rRNA sequencing. The 16S rRNA gene was amplified by PCR. The sequences of the primers used for amplification were 5′-AGAGTTTGATCCTG GCT-3′ and 5′-AAGGAGGTGATCCAGCCGCA-3′, corresponding to positions 8 to 24 and 1521 to 1540, respectively, in the 16S rRNA sequence of *E. coli* (4). The 1.5-kb PCR product (positions 29 to 1520 in the 16S rRNA sequence of *E. coli*) was directly sequenced by the dideoxynucleotide chain termination method with a DNA sequencer (model 377; Applied Biosystems, Inc.). Multiple alignments of the sequence were performed, nucleotide substitution rates (K_{nuc} values) were calculated, and a neighbor-joining phylogenetic tree (18, 28) was constructed by using the CLUSTAL W program (31) with the determined 1494-bp sequence. The similarity values of the sequences were calculated by using the GENETYX computer program (Software Development Co., Ltd., Tokyo, Japan).

Reference sequences. The accession numbers for the sequences used as references are as follows: *Salinivibrio costicola* ATCC 35508^T, X74699; *Photobacterium angustum* ATCC 25915^T, X74685; *P. leiognathi* ATCC 25521^T, X74686; *P. phosphoreum* ATCC 11040^T, X74687; *V. fischeri* ATCC 7744^T, X74702; *Vibrio logei* ATCC 15832, X74708; *Vibrio cholerae* ATCC 14035^T, X74695; *Vibrio vulnificus* ATCC 27562^T, X76333; *V. splendidus* ATCC 33125^T, X74724; *Vibrio anguillarum* ATCC 43313, X74719; *Vibrio fluvialis* ATCC 33809^T, X74703; *Vibrio orientalis* ATCC 33934^T, X74714; *Vibrio nereis* ATCC 25917^T, X74716; *Vibrio proteolyticus* ATCC 15338^T, X74723; *V. pelagius* ATCC 25916^T, X74722; *V. campbellii* ATCC 25920^T, X74692; *V. parahaemolyticus* CIP 73.30, X74721; *V. alginolyticus* ATCC 17749^T, 74706; *V. harveyi* ATCC 14126^T, X74706; *Vibrio cincinnatiensis* ATCC 35912^T, X74698; *Vibrio gazogenes* ATCC 29988^T, X74705; and *V. halioticoli* IAM 14596^T, AB000390.

DNA base composition and DNA-DNA hybridization. DNA was prepared from bacterial cells by the method of Marmur (25). The G+C content of the DNA was determined by the method of Tamaoka and Komagata (30). The prepared DNA was digested with nuclease P1 (Yamasa Shoyu, Choshi, Japan). The resulting nucleotides were analyzed by high-pressure liquid chromatography (HPLC) with a 4.6- by 250-mm Inertsil C_4 column (GL Science) at room temperature. The HPLC system used consisted of a solvent delivery pump (model CCPM-II; Tosoh) and a UV spectrophotometer as the detector (model UV-8020; Tosoh) at 235 nm. An equimolar mixture of four deoxyribonucleotides (Yamasa Shoyu) was used as the standard.

Levels of DNA-DNA relatedness were determined fluorometrically by the method of Ezaki et al. (6) with photobiotin-labeled DNA probes and microplates.

Growth conditions and preparation of cell extracts. Cells for catalase activity determination were incubated in a 2,000-ml flask containing 800 ml of PYS-2 broth medium, which was set on a rotary shaker (100 rpm · min^{-1}) and maintained at 27°C for 48 h. For comparison, cells of other strains were also incubated under the same conditions. A cell extract was obtained as follows. The cells were harvested by centrifugation at 10,000 × *g* for 15 min and suspended in a buffer containing 20 mM $MgSO_4$ and 50 mM Tris-HCl, pH 8.0 (buffer A). The cells were disrupted by passage through a French pressure cell (SLM-AMINCO) at 20,000 lb/in^2 at 4°C. The suspension was then centrifuged at 10,000 × *g* for 15 min to remove unbroken cells. The resulting supernatant was used as a cell extract. The protein content was determined by the method of Lowry et al. (24), with bovine serum albumin as a standard.

Enzyme assay conditions. Catalase activity was measured spectrophotometrically by monitoring the decrease in absorbance at 240 nm caused by the disappearance of H_2O_2, using a Hitachi U-3210 spectrophotometer. The ε value at 240 nm for H_2O_2 was assumed to be 43.6 M^{-1} cm^{-1} (16). The standard reaction mixture for the assay contained 50 mM potassium phosphate buffer (pH 7.0), 30 mM H_2O_2, and 3 μl of a catalase-containing solution, in a total volume of 1.0 ml. The amount of enzyme activity that decomposed 1 μmol of H_2O_2 per min was defined as 1 U of activity. Enzyme activity was estimated more than four times for each sample, using at least two independent samples.

Catalase activity staining. The cell extract was centrifuged at 105,000 × *g* for 1 h. The resulting supernatant was separated by native gel electrophoresis with a 12.5% polyacrylamide gel according to the method of Laemmli (20). Staining for catalase activity was performed as follows (12): the electrophoresed gel was soaked in 50 mM potassium phosphate buffer (pH 7.4) containing 1 mg of 3,3-diaminobenzidine tetrachloride per ml and 10 U of horseradish peroxidase per ml for 45 min in the dark, and then 30% H_2O_2 was added to the reaction mixture.

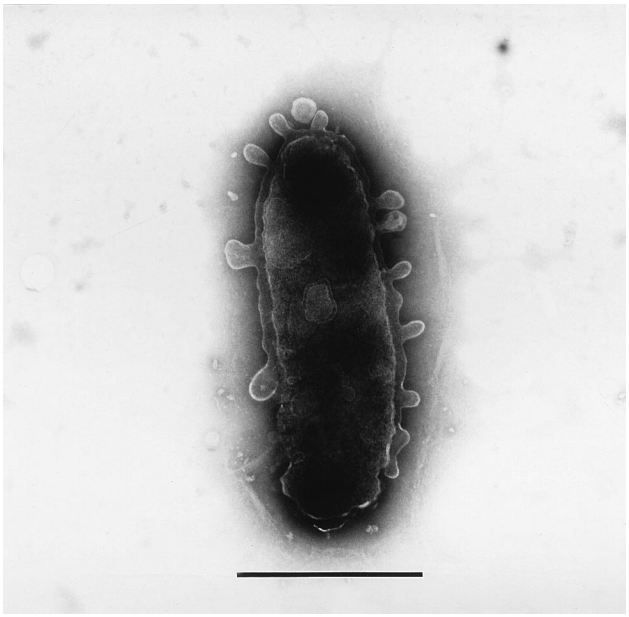

FIG. 1. Electron micrograph of a negatively stained cell of *V. rumoiensis* S-1^T, showing blebs on the cell surface. Bar, 1 μm.

Nucleotide sequence accession number. The nucleotide sequence data determined in this study have been deposited in the DDBJ, EMBL, and GenBank nucleotide sequence databases under accession no. AB013297.

RESULTS

Morphology. After incubation at 27°C on PYS-2 agar medium, colonies of strain S-1^T were circular and colorless, and cells appeared as nonpigmented, nonflagellated rods 0.5 to 0.9 by 0.7 to 2.1 μm in size. Spore formation was absent, and Gram staining was negative. In electron microscopic analysis, numerous blebs were observed on the cell surface (Fig. 1). Microscopic observations of other strains (controls) were also performed. However, there were no blebs on the cell surfaces of the other strains.

Cultural characteristics. The microorganism can grow at temperatures ranging from 2 to 34°C, with an optimal temperature of around 30°C (data not shown). The growth rate under optimal conditions gave a doubling time of about 100 min (data not shown).

Phenotypic characteristics. Strain S-1^T exhibited the following physiological and biochemical characteristics. It was positive for oxidase and catalase. It fermented D-glucose, L-arabinose, D-fructose, D-maltose, D-mannose, sucrose, D-xylose, D-mannitol, and D-galactose but not *myo*-inositol and L-rhamnose. No growth was observed in the absence of NaCl in the culture medium; in contrast, prolific growth was observed in the medium supplemented with 3, 4, or 6% NaCl. Susceptibility to vibriostatic compound O/129 (10 and 150 μg) was observed. The strain was positive for methyl red, citrate utilization, and reduction of NO_3 to NO_2 but negative for the Voges-Proskauer test, arginine dihydrolase, and indole and H_2S production. It hydrolyzed chitin, starch, DNA, and Tweens 20, 40, 60, and 80 but not casein, gelatin, or alginic acid. The strain utilized L-arabinose, D-fructose, D-glucose, glycerol, lactose, and D-gluconate as the sole carbon and energy source for growth but not melibiose, raffinose, and D-sorbitol.

DNA base composition. The G+C content of strain S-1^T was 43.2%.

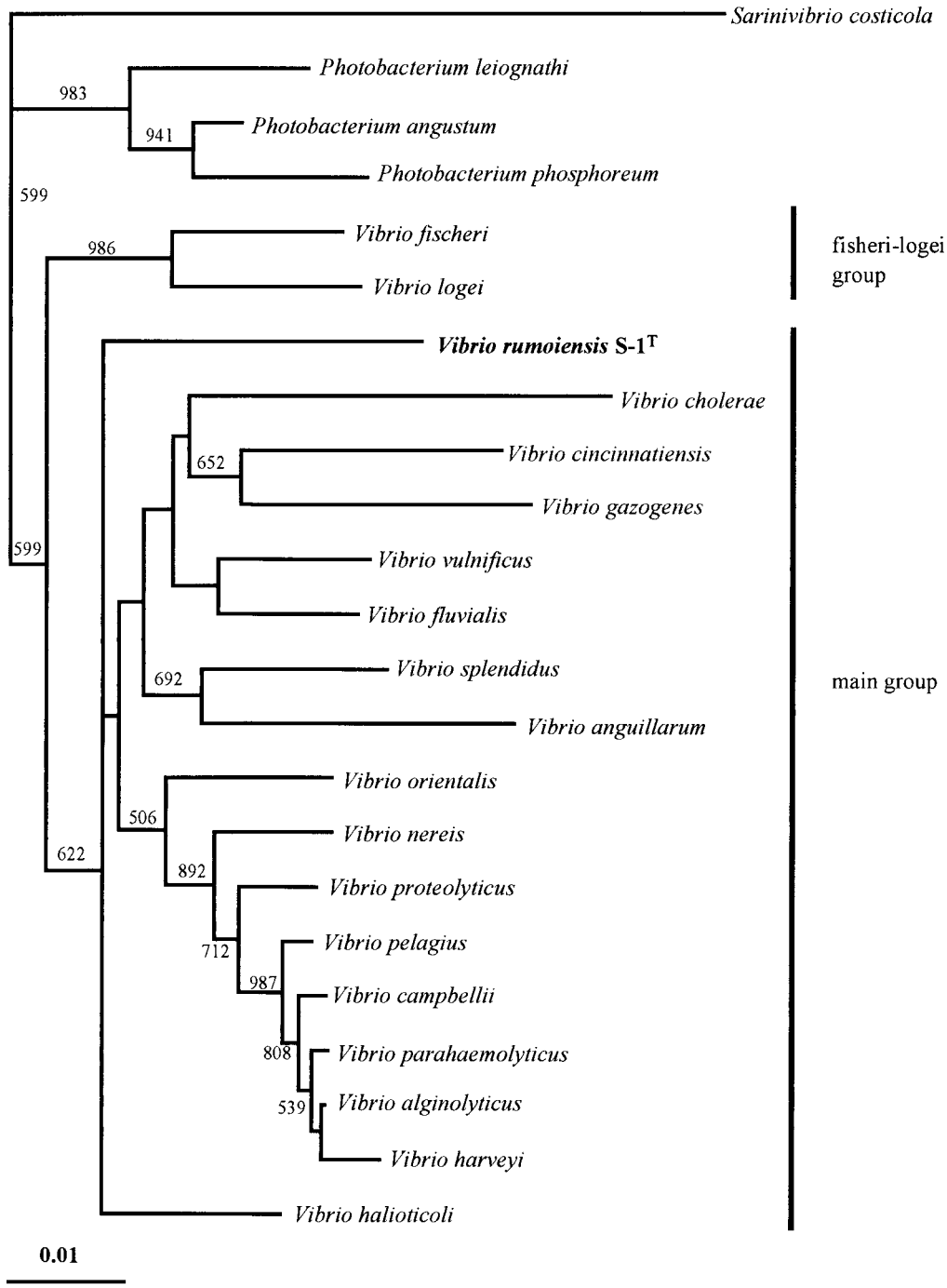

FIG. 2. Phylogenetic tree of *V. rumoiensis* S-1^T, other *Vibrio* strains, and other related strains derived from 16S rRNA sequence data, using the neighbor-joining method for calculation. Numbers indicate bootstrap values of greater than 500. Bar, 0.01 K_{nuc} unit.

16S rRNA sequence analysis. The almost-complete 16S rRNA sequence of strain S-1^T, which consists of 1,494 nucleotides, was found to have 92.4 to 95.5% similarity to the 16S rRNA sequences of *Vibrio* and *Photobacterium* strains. In contrast, its similarity to the 16S rRNA sequences of *Pseudoalteromonas*, *Shewanella*, *Moritella*, *Alteromonas*, *Escherichia*, *Pasteurella*, *Aeromonas*, and *Colwellia* strains was determined to be 83.6 to 90.1%. A phylogenetic tree constructed by the neighbor-joining method showed that strain S-1^T was part of the cluster of the genus *Vibrio*. However, strain S-1^T existed as an independent branch between the *V. fischeri-V. logei* group and the main *Vibrio* group (Fig. 2). Strain S-1^T showed similarities of 89.5, 93.0 to 94.3, 93.3 to 93.8, and 92.4 to 95.5% to *S. costicola*, *Photobacterium* strains, the *V. fischeri-V. logei* group, and the main *Vibrio* group (27), respectively. Recently, a nonmotile *Vibrio* strain, *V. halioticoli*, was isolated from abalone gut. Strain S-1^T showed a similarity of 94.8% to *V. halioticoli.*

DNA-DNA hybridization. According to 16S rRNA sequence analysis, strain S-1^T is closely related to the genus *Vibrio*. The levels of DNA-DNA relatedness were estimated by using strain

TABLE 1. Levels of relatedness of *V. rumoiensis* S-1^T DNA and DNAs of other *Vibrio* strains and related strains

Strain	% Homology
V. rumoiensis S-1^T	100
V. halioticoli IAM 14596^T	3.2
V. alginolyticus V477	8.9
V. pelagius biovar I ATCC 25916^T	4.0
V. campbellii biovar I ATCC 25920^T	8.2
V. fischeri ATCC 7744^T	8.2
V. splendidus biovar I ATCC 33125^T	7.1
V. parahaemolyticus JCM 2147	9.0
V. harveyi NCMB 1280^T	8.8
P. leiognathi NCMB 391	5.8
P. phosphoreum IAM 12085	6.4

S-1^T and representative strains from the genus *Photobacterium*, the *V. fischeri-V. logei* group, and the main *Vibrio* group (27). The levels of DNA-DNA relatedness between strain S-1^T and type or reference strains of the 10 species tested were significantly low (Table 1). Although the 16S rRNA sequence similarity between strain S-1^T and *V. campbellii* was 95.5%, which was the highest similarity value among the strains shown in Fig. 2, the level of DNA-DNA relatedness was only 8.2%.

Characterization of catalase activity. To compare the catalase activity of strain S-1^T with those of other strains, we estimated the catalase activities in cell extracts of bacterial strains that were incubated under the culture conditions described in Materials and Methods. It was found that the catalase activity of strain S-1^T was 1 or 2 orders of magnitude higher than those of the other tested strains (Table 2).

To determine whether the catalase of strain S-1^T is an intracellular or an extracellular enzyme, we estimated the catalase activity of strain S-1^T in both culture medium and cell extract. Only 6 U/mg of protein was detected in the 29-h culture medium. Based on this value, it was determined that the total amount of extracellular catalase activity is 1.8% of the sum of the total intracellular and extracellular catalase activities (data not shown).

It was reported that several kinds of catalase exist in cells of several microorganisms and that they are induced in different fashions (7, 10, 15, 17, 19, 23). *E. coli*, for example, has two kinds of catalase, hydroperoxidase I (HP I) and HP II, which are encoded by two separate genes, *katG* and *katE*, respectively. HP I exists in the periplasmic space, and its level increases gradually to about twofold that of HP II during the logarithmic growth phase but ceases to increase during the stationary phase. On the other hand, HP II exists in the cytoplasmic space, and its level, which is initially lower than that of HP I, increases 10-fold during growth to the stationary phase (7, 23). Therefore, we investigated whether strain S-1^T has more than one kind of catalase. To this end, the cell extract was ultracentrifuged, and the supernatant was subjected to polyacrylamide gel electrophoresis. The results showed only one band representing catalase activity (Fig. 3). During the first purification step, anion-exchange chromatography, in which a crude soluble fraction was loaded into the column, only one peak representing catalase was detected (data not shown).

TABLE 2. Catalase activities in cell extracts of *V. rumoiensis* S-1^T and other strains[a]

Strain or species	Catalase activity (U/mg of protein)[b]
V. rumoiensis S-1^T	4,092.4 ± 408.2
A. faecalis	500.7 ± 121.2
C. glutamicum	368.8 ± 46.2
S. aureus	347.1 ± 85.0
A. hydrophila	144.9 ± 8.3
E. coli	65.8 ± 7.4
P. fluorescens	44.5 ± 25.0
B. subtilis	16.4 ± 1.1
V. parahaemolyticus	14.2 ± 0.8

[a] The catalase activities in cell extracts prepared from cells obtained under the same culture conditions were assayed. The catalase activity was measured as described in the text.

[b] Results are averages and standard deviations from at least nine experiments with at least two independent preparations of cell extracts. Statistical analysis was done by Student's *t* test at $P = 0.05$.

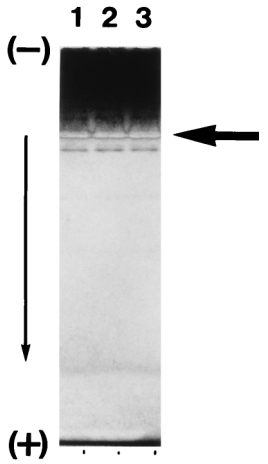

FIG. 3. Identification of catalase activity of *V. rumoiensis* S-1^T at different growth phases. The arrow indicates staining parts of catalase activities. Cell extracts were obtained from cells grown for 24 h (late exponential growth phase) (lane 1), 48 h (mid-stationary phase) (lane 2), or 72 h (late stationary phase) (lane 3).

The temperature dependence of the catalase activity of the cell extract, which was obtained from cells grown at 27°C, was estimated and is shown in Fig. 4. Compared with that of most enzymes, the temperature dependence of the catalase activity was not great. In the case of bovine liver catalase also, the activity was weakly dependent on the temperature (data not shown). Recently, a thermostable catalase from the culture broth of the thermophilic fungus *Thermoascus aurantiacus* was purified and characterized. The catalase activity of the fungus was weakly dependent on temperature (32). From the facts described above, it is considered that the weak temperature dependence of the activity is a common feature among the catalases. We estimated that the optimum temperature for enzyme activity was about 30°C. The stability of strain S-1^T catalase activity was examined by incubating a cell extract at a predetermined temperature for 15 min or 1 h (data not shown). Catalase activity was stable at a 40°C; however, it was almost completely eliminated at 60°C (in the case of bovine catalase, activity was almost completely eliminated at 65°C). After incubation at 50°C for 1 h, the remaining catalase activity of strain S-1^T was only 29.2% whereas the remaining bovine *Aspergillus niger*, and *T. aurantiacus* catalase activities were 80, 100, and 100%, respectively (32).

DISCUSSION

Strain S-1^T was isolated from the drain pool of a fish product processing plant that uses H_2O_2 as a bleaching agent, and its preliminary characteristics were studied (33). In the present

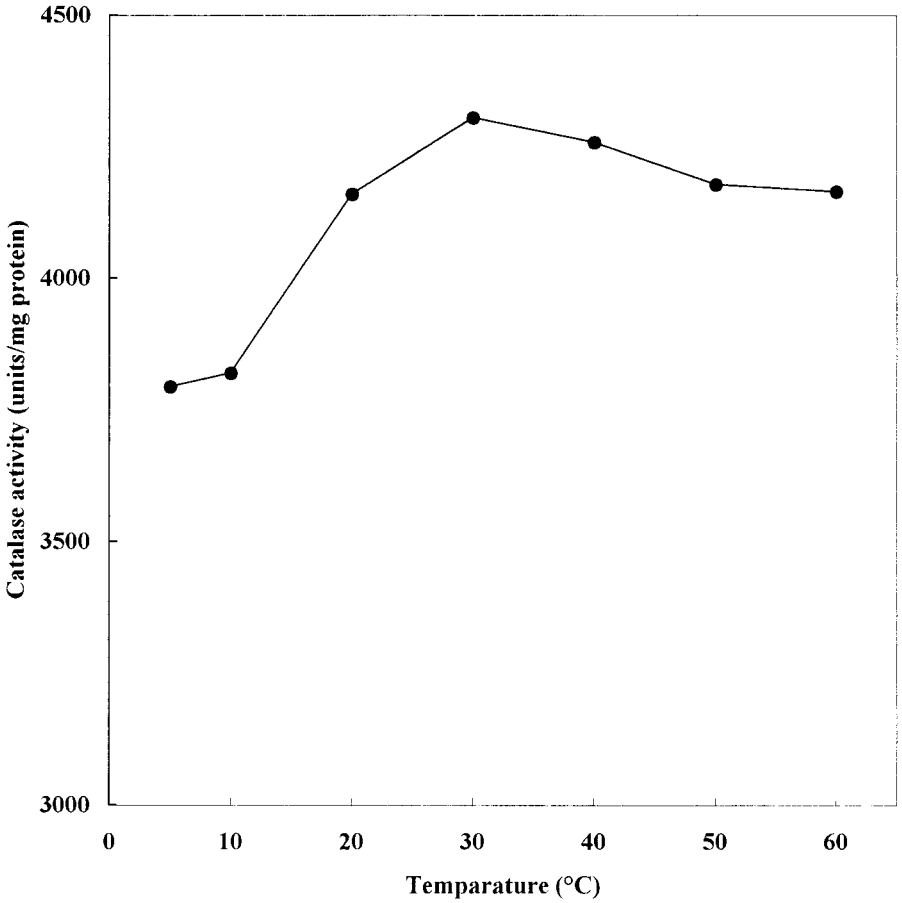

FIG. 4. Catalase activities in cell extracts of *V. rumoiensis* S-1^T at different temperatures. The microorganism was grown at 27°C.

study, we attempted to identify the microorganism to the species level and to characterize its crude catalase in the cell extract.

Electron microscopic observation revealed the presence of blebs on the cell surface. Similar membrane structures were also reported for other gram-positive and gram-negative bacteria (1, 8, 9, 11). Although the function of the blebs of strain S-1^T is not known, these structures could be associated with its ability to grow in an H_2O_2-containing environment. For example, the surface vesicles of strain S-1^T may contain hydrogen peroxide-decomposing enzymes such as catalase and peroxidase.

Based on our phenotypic and phylogenetic characterization, strain S-1^T was identified as a member of the genus *Vibrio*, although it has no flagella. Recently, a nonflagellated *Vibrio* strain, *Vibrio halioticoli*, was isolated from the gut of abalone (29). Strain S-1^T was isolated from the drain pool of a herring egg processing plant. It requires NaCl for growth and is able to decompose chitin. Therefore, it is considered that the origin of strain S-1^T is probably the intestine of herring. Although at present we know little about the intestinal microflora of marine organisms, other unknown nonflagellated *Vibrio* strains may exist in the intestines of marine organisms. Interestingly, the phylogenetic 16S rRNA sequence analysis demonstrated that strain S-1^T occupies a distinct position, similar to the case for nonmotile *V. halioticoli*. However, their phylogenetic positions differed, and the level of DNA homology between strain S-1^T and *V. halioticoli* was only 3.2%. Although the phylogenetic positions of these nonmotile strains are unique, bootstrap analysis indicates that they are in the main *Vibrio* group. In conclusion, on the basis of phenotypic characteristics, phylogenetic analysis, and DNA-DNA hybridization experiments, strain S-1^T is confirmed to be a new species, and the name *Vibrio rumoiensis* is proposed.

In our previous study, we compared the catalase activity of strain S-1^T with those of *E. coli*, *B. subtilis*, *V. parahaemolyticus*, and *Micrococcus luteus* and found that the catalase activity of strain S-1^T was as high as that of *M. luteus*, which is well known for its high catalase activity, and 1 or 2 orders of magnitude higher than those of *E. coli*, *B. subtilis*, and *V. parahaemolyticus* (33). In the present experiments, for a more comprehensive comparative study, we added five species for comparison and performed experiments on a total of eight reference species. Our results confirm that the catalase activity of strain S-1^T is much higher than those of other bacterial species.

The optimum temperature for strain S-1^T catalase activity in the cell extract was around 30°C (Fig. 4), which was 20°C lower than that for the activity of purified catalase from bovine liver (data not shown). In the case of *T. aurantiacus*, the optimum temperature for catalase activity was 70°C. From comparison of optimum temperatures for catalases from another origins, it is considered that the catalase of strain S-1^T is more adaptable to cold environments than other known catalases.

Description of *Vibrio rumoiensis* sp. nov. *Vibrio rumoiensis* (ru.moi.en′sis. L. adj. *rumoiensis*, from Rumoi, the place where the microorganism was isolated). Cells are rod shaped (0.5 to 0.9 by 0.7 to 2.1 μm), gram negative, and nonflagellated, and numerous blebs exist on the cell surface. Colonies are white.

Catalase and oxidase reactions are positive. The organism ferments D-glucose, L-arabinose, D-fructose, D-maltose, D-mannose, sucrose, D-xylose, and D-mannitol. No growth is observed in the absence of NaCl in the culture medium; however, growth is prolific in medium supplemented with 3, 4, or 6% NaCl. Susceptibility to vibriostatic compound O/129 (10 and 150 μg) is observed. Growth occurs between 2 and 34°C. The organism is positive for methyl red, citrate utilization, and reduction of NO_3 to NO_2 but negative for the Voges-Proskauer test, arginine dihydrolase, and indole and H_2S production. It hydrolyzes chitin, starch, DNA, and Tweens 20, 40, 60, and 80 but not casein, gelatin, or alginic acid. The organism utilizes L-arabinose, D-fructose, D-glucose, glycerol, lactose, and D-gluconate as the sole carbon and energy source for growth but not melibiose, raffinose, and D-sorbitol. The G+C content of the DNA is 43.2 mol% (determined by HPLC). The type strain *V. rumoiensis* S-1 has been deposited in the Patent Microorganism Depository, National Institute of Bioscience and Human Technology (Tsukuba, Japan), as strain FERM P-14531.

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