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Factors Affecting High-Oxygen Survival of Heterotrophic Microorganisms from an Antarctic Lake

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We sought to determine factors relating to the survival of heterotrophic microorganisms from the high-dissolved-oxygen (HDO) waters of Lake Hoare, Antarctica. This lake contains perpetual HDO about three times that of normal saturation (40 to 50 mg liter^{-1}). Five isolates, one yeast and four bacteria, were selected from Lake Hoare waters by growth with the membrane filter technique with oxygen added to yield dissolved concentrations 14 times that in situ, 175 mg liter^{-1}. One bacterial isolate was obtained from the microbial mat beneath the HDO waters. This organism was isolated at normal atmospheric oxygen saturation. The bacteria were gram-negative rods, motile, oxidase positive, catalase positive, and superoxide dismutase positive; they contained carotenoids. The planktonic isolates grew in media containing 10 mg of Trypticase soy (BBL Microbiology Systems)-peptone (2:1) liter^{-1} but not at 10 g liter^{-1}. Under low-nutrient levels simulating Lake Hoare waters (10 mg liter^{-1}), two of the planktonic isolates tested were not inhibited by HDO. Growth inhibition by HDO increased as nutrient concentration was increased. A carotenoid-negative mutant of one isolate demonstrated a decreased growth rate, maximal cell density, and increased cell lysis in the death phase under HDO compared with the parent strain. The specific activity of superoxide dismutase was increased by HDO in four of the five bacterial isolates. The superoxide dismutase was of the manganese type on the basis of inhibition and electrophoretic studies. The bacterial isolates from Lake Hoare possess several adaptations which may aid their survival in the HDO waters, as well as protection due to the oligotrophic nature of the lake.

We previously demonstrated that high dissolved oxygen (HDO; 42 mg liter^{-1}) inhibited D-[U-^{14}C]glucose assimilation, respiration, and production of CFUs compared with normal atmospheric saturated values (ADO; 10 to 14 mg liter^{-1}) in plankton samples from oligotrophic Mountain Lake, Giles County, Va. (24). Subsequently, we reported lack of inhibition or stimulation of D-[U-^{14}C]glucose assimilation-respiration by HDO in plankton samples from Lake Hoare, Antarctica. Only CFU formation was inhibited by HDO in Lake Hoare samples relative to ADO (25). Lake Hoare is oligotrophic, and its perennial ice cover isolates the water from the atmosphere, allowing the development of HDO (27).

Intra- and extracellular reactions involving oxygen can create by-products more reactive and hazardous to cells than molecular oxygen. These include superoxide, hydrogen peroxide, singlet oxygen, and the hydroxyl radical (4, 11, 12, 26). Against these toxic oxygen species, aerobic organisms possess defense mechanisms such as catalase, peroxidase, superoxide dismutase (SOD), and carotenoids (3, 5, 11, 12).

No previous study had disclosed a natural environment with perpetual HDO and with a microbial community so resistant to oxygen toxicity. We therefore sought to select Lake Hoare microbial isolates resistant to high concentrations of oxygen to determine their physiological adaptations.

MATERIALS AND METHODS

Sampling. Plankton samples from Lake Hoare, Antarctica (77° 38′ S, 162° 53′ E), were collected during the Austral summer of the 1982 to 1983 field season at 2 m below the lake ice with sterile collection bottles in a Wildco sewage sampler (Wildlife Supply Co.). Benthic microbial mat underlying HDO waters was collected in sterile Whirl Pack bags by workers wearing self-contained underwater breathing apparatus (SCUBA). Samples were held on ice and prevented from freezing during transport by helicopter to the Eklund Laboratory.

VHO isolates, culturing, and transport. Samples were processed at the Eklund Laboratory within 4 h of collection. Measured aliquots of lake water were filtered onto Nuclepore filters (pore size, 0.2 μm) in prechilled filter apparatus. These filters were placed onto pads containing media as previously described (24). Some plates were incubated under ADO conditions. Replicate plates were placed in a modified pressure cooker to which oxygen was added (unpurged) at 55 lb in^{-2} (379 kPa). This approximated 19 times the normal atmospheric partial pressure of oxygen and resulted in dissolved oxygen 14 times the in situ concentration at sea level with an incubation temperature of 12°C (very high oxygen [VHO]). In a control experiment, an equal pressure of helium had no effect on CFU formation from Mountain Lake water (24). Plates were incubated for 2 weeks at 12°C. Colonies selected from VHO- and ADO-incubated plates were purified by being streaked several times onto medium containing 12 g of agar, 2 g of Trypticase soy (BBL Microbiology Systems), 1 g of peptone, and 200 mg of filter-sterilized yeast extract liter of Lake Hoare water^{-1}. Unless otherwise stated, this is the standard growth and maintenance medium. Isolates on agar slants were transported to Virginia Polytechnic Institute packed in ice but protected from freezing.

Isolate characterization. The six isolates were characterized with respect to Gram reaction, cell shape, motility, production of catalase and oxidase, pigmentation, and ability to grow at higher temperatures and different concentrations of nutrients. In addition, the DNA of the mat isolate and one planktonic isolate were characterized for their moles percent

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guanine plus cytosine (mol% G+C). DNA was extracted, and the thermal melting point of the DNA was determined by the method of Johnson (15). The mol% G+C was calculated by the equation of Mandel et al. (19). The reference strain was *Escherichia coli* B. Other details of methods used in these characterizations will be outlined subsequently.

Nutrient concentration and ADO:HDO growth yields. The standard growth medium was diluted with tap water after yielding final nutrient concentrations (Trypticase soy plus peptone [2:1]) of 1,000, 500, 100, 50, and 10 mg liter^{-1}. The media were prefiltered to remove suspended particulates (GF/F; Whatman Paper, Ltd.), and 60-ml aliquots were dispensed into 125-ml serum bottles. ADO conditions were achieved by using loose foil caps. HDO conditions were maintained with Bellco butyl rubber serum stoppers as previously described (24). HDO equals the average maximum dissolved oxygen concentration of Lake Hoare (42 mg liter^{-1}). Bottles were stored at the incubation temperature, allowing dissolved oxygen equilibration (12 to 24 h). Inocula grown in standard growth medium to late log phase were washed twice in the diluted (10 mg liter^{-1}) standard growth medium. Inoculated serum bottles were incubated at 12°C on a reciprocal shaker (110 oscillations min^{-1}). Optical density at 550 nm was determined at regular intervals by using a Perkin-Elmer 55B spectrophotometer. The spectrophotometer had been modified to accept the serum bottles, allowing a light path of 5 cm. Growth yield was interpreted from maximal optical density. Cultures were monitored microscopically for flocculation which would void the linear relationship between cell numbers and absorbance.

SOD. Cells were grown in standard growth medium to late log phase under ADO and HDO conditions. They were harvested by centrifugation (10,000 × *g* for 15 min), washed twice, and suspended in 50 mM potassium phosphate–1 mM EDTA, pH 7.8. Cells were broken by sonication (Fisher sonicator [3/4-in. tip, 60% power]) for 6 min. Cell extracts were clarified by centrifugation (20,000 × *g* for 25 min). Protein content of the supernatant fluid was determined with the Bio-Rad protein assay. Bovine serum albumin was used as the protein standard. SOD activity was assayed by the procedure of McCord and Fridovich (20). Sodium azide inhibition of SOD was determined by adding sodium azide to the standard assay mixture to final concentrations of 1 and 5 mM. Hydrogen peroxide inactivation of the enzyme sample was obtained by incubation of the sample at room temperature with 5 mM hydrogen peroxide plus 1 mM sodium cyanide as a catalase inhibitor. Subsamples were removed at 5-min intervals for up to 1 h to determine SOD activity. Disc gel electrophoresis of clarified cell extracts was performed in 7.5% acrylamide gels (10) with a 5 mM Tris–39 mM glycine buffer (pH 8.3) at a constant current of 2.5 mA. SOD activity was localized on the gels by the method of Beauchamp and Fridovich (6).

Carotenoid-negative mutants. Mid-log-phase cultures of isolates (10 ml) were centrifuged, and the cells were resuspended in 5 ml of the standard growth medium. Ethyl methanesulfonate (50 μl) (Eastman Kodak Co.) was added, and the cell suspension was mixed and then incubated for 2 h in a tube roller at 12°C. After incubation, cells were washed twice in 20 mM phosphate buffer, serially diluted, and plated onto the standard growth medium. After 2 weeks of incubation at 12°C, colonies showing change or loss of color were picked.

HDO growth rates of carotenoid-producing and -nonproducing strains. A VHO-selected isolate (PH2A) and a carotenoid-negative mutant of this isolate (PH2AM) were inoculated into the standard growth medium. Five replicate tubes of each organism were grown under 11 lb in^{-2} (76 kPa) of added oxygen. Tubes were closed with butyl rubber stoppers designed for anaerobe cultures (Bellco Glass, Inc.) to maintain pressure. ADO tubes were loosely fitted with screw caps. The medium in the HDO replicates was allowed to equilibrate for 24 h with oxygen before inoculation. Tube headspace was recharged with oxygen at 48-h intervals to maintain dissolved oxygen levels of >38 mg liter^{-1} throughout the incubation period. Tubes were incubated in a tube roller (30 rpm) at 12°C. Dissolved oxygen was measured by the modified Winkler procedure (1). Growth rates were determined with a Klett colorimeter by monitoring change in optical density at 640 to 700 nm. This is beyond the range of carotenoid absorbance.

Carotenoid extraction. Cells were grown on standard growth medium in the dark under HDO conditions (11 lb in^{-2}) in centrifuge bottles placed in modified pressure cookers; parallel cultures were prepared under ADO conditions. Cultures were incubated on a reciprocal shaker table (110 oscillations min^{-1}). Cells were harvested by centrifugation (10,000 × *g* for 15 min), washed once, and frozen in 20 mM phosphate buffer with 1 mM EDTA. After thawing, cells were lysed with lysozyme (0.5 mg ml^{-1}), and the carotenoids were extracted under N_2 with methanol at 4°C overnight. Cell debris was removed by centrifugation (20,000 × *g* for 15 min). The general precautions outlined by Britton and Goodwin (8) for handling carotenoids in all operations were followed, including working in a glove box containing a nitrogen atmosphere.

Carotenoid separation. An equal volume of chloroform was added to the methanol extract. Water containing NaCl (10%, wt/vol) was added to prevent emulsion formation, until two phases were formed. The chloroform phase containing the carotenoids was removed, washed with water to remove methanol and salt, and dried with Na_2SO_4, and the extract was concentrated under N_2. This crude carotenoid preparation was stored under N_2 at −15°C. More polar carotenoids (including the xanthophylls) were separated by partitioning the pigment mixtures between petroleum ether and 95% methanol as directed by Britton and Goodwin (8). The relative proportions in each phase were evaluated by their visible absorbance maxima in chloroform.

Thin-layer chromatography and absorbance spectra of carotenoids. The relative mobilities of constituent carotenoids in each of the five bacterial isolates were determined by thin-layer chromatography, using Kieselgel 60 plates (EM Reagents) and the following solvent systems: petroleum ether (bp 35 to 60°C)-chloroform-acetone (60:20:20 vol/vol), acetone-chloroform (50:50, vol/vol), and methanol-chloroform (7:93, vol/vol). Maximally separated bands were eluted into chloroform, and their absorbance spectra (220 to 600 nm) were determined by using a Perkin-Elmer 55B or Bausch and Lomb 2000 spectrophotometer. All solvents were Photrex grade (J. T. Baker Chemical Co.).

RESULTS

Table 1 lists the physiological and morphological characteristics of the six VHO-selected Lake Hoare isolates. Although <35% of planktonic ADO-selected CFU survived HDO conditions, <1% of ADO-selected CFU formed colonies with VHO incubation (25). All of the bacterial isolates in this study appeared similar on the basis of morphology, motility, gram-negative reaction, possession of oxidase, and pigmentation. The benthic mat isolate (MH1C) had a larger

TABLE 1. Characteristics of Lake Hoare isolates

Isolate	Oxygen selection	Source[a]	Gram reaction (morphology)	Cell dimensions (μm)[b]	Motility	Catalase	Oxidase	Growth[c] at:		Growth on TSPEP[d]:		Mol% G+C[e]	Color
								20°C	30°C	10 g liter^{-1}	10 mg liter^{-1}		
PH2ASO	VHO	P	− (rod)	2.0 by 0.2	+	+	+	+	−	(+)[f]	+	ND[g]	Orange-red
HB	VHO	P	− (rod)	1.1 by 0.3	+	+	+	+	−	(+)	+	ND	Pink
MH1C	ADO	M	− (rod)	2.0 by 0.6	+	+	+	+	+	+	−	64	Orange
PH2A	VHO	P	− (rod)	1.4 by 0.3	+	+	+	(−)[h]	−	(+)	+	66	Orange-red
PH2B	VHO	P	− (rod)	1.1 by 0.3	+	+	+	(−)	−	(+)	+	ND	Orange-red
PH2A (1227)	VHO	P	Yeast		−	+	ND	+	+	+	−	ND	Pink

[a] P, Plankton; M, benthic mat.
[b] Average cell dimensions of log-phase cells in standard broth medium.
[c] Measured as turbidity increase in standard medium.
[d] TSPEP, Trypticase soy-peptone (2:1).
[e] Standard agar medium, 2-week incubation at 12°C.
[f] Parentheses indicate reduction in growth compared with that in 3-g liter^{-1} standard medium.
[g] ND, Not determined.
[h] Parentheses indicate slightly detectable growth.

cell diameter and demonstrated greater tolerance to increased temperature and nutrients than the planktonic bacterial isolates did. Isolates PH2A and PH2B demonstrated very little growth at 20°C. All these isolates from Lake Hoare had catalase, including those which we will term facultative oligotrophs (i.e., grew at 10 mg liter^{-1} but were suppressed at 10 g of total substrate liter^{-1}). The mat isolate (MH1C) did not produce measurable turbidity with the 10-mg liter^{-1} concentration of our standard medium.

Isolates PH2A and MH1C were subjected to DNA melting-point analysis (T_m for PH2A was 94.1°C and for MH1C was 93.6°C). Both values were in the range (G+C = 55 to 70%) of carotenoid-containing gram-negative rods suggested by McMeekin and Shewan to compose the genus "*Empedobacter*" (22). Flagellar patterns were not established for these isolates; however, all bacterial isolates exhibited swimming, not gliding motility.

Only 3 of 32 isolates from Lakes Hoare, Vanda, Fryxell, and Bonney from the 1981–1982 and 1982–1983 Antarctic field seasons failed to grow on the standard medium after initial isolation on our CFU medium, despite the >10-fold nutrient concentration increase (0.22 to 3.0 g liter^{-1}). Nevertheless, 10 g liter^{-1} (identical ratio of peptone-Trypticase soy-yeast extract as standard medium) inhibited the growth of the four planktonic bacterial isolates (Table 1). A study of three of the bacterial isolates revealed that as nutrient concentrations were decreased, the HDO repression of maximal cell density decreased (Fig. 1). This response is illustrated conveniently as the ratio of optical density maxima, HDO/ADO. The two planktonic bacteria, HB and PH2A, showed no HDO inhibition at 10 mg liter^{-1}. In fact, a higher cell density was established by PH2A with HDO. Although the mat isolate (MH1C) showed reduced inhibition of cell yields with HDO at reduced nutrient levels, we could not measure its growth (increase in optical density) below 50 mg liter^{-1}.

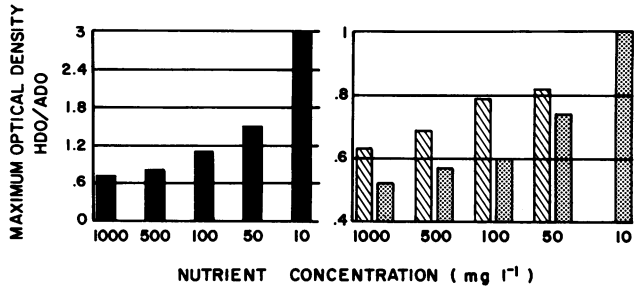

FIG. 1. HDO/ADO ratio of maximal cell densities (optical density) of isolates PH2A (|||||), HB (▒), and MH1C (▨) grown in dilutions of standard growth media.

Table 2 summarizes the results of the SOD induction experiments repeated a minimum of five times. All the bacterial isolates, except HB, exhibited higher specific activities of SOD when grown under HDO versus ADO conditions ($P < 0.05$). Table 2 also shows the SOD sodium azide inhibition and hydrogen peroxide inactivation studies and the electrophoretic mobility patterns of SOD activity in 7.5% acrylamide gels. SOD activity in all bacterial isolates was similarly inhibited upon addition of 5 mM sodium azide to the assay mixture, and the isolates had long half-lives of SOD activity in 5 mM hydrogen peroxide. These results are consistent with SOD containing manganese as the active site. There was no discernible difference in these inhibition patterns when comparing ADO- with HDO-induced SOD. MH1C resembled HB (0.80 to 0.81) and PH2A resembled PH2ASO (0.89 to 0.91) in relative mobility of their major bands of SOD. The relative mobility of the SOD band from PH2B differed from those of the other bacterial isolates (0.54 to 0.55). Only PH2ASO exhibited a secondary SOD activity band (relative mobility of 0.78). No real differences in SOD relative mobility patterns were noted for any isolate when grown under HDO or ADO conditions.

All of the VHO-selected isolates contained carotenoids. Each isolate produced pigmented colonies with visibly different colors. Maximal pigment concentration was produced by all isolates in early stationary-growth phase. Solvent extraction of the carotenoids in lysed cell preparations was in the following decreasing order of efficiency: methanol, acetone, and chloroform. Fracturing the cells was a prerequisite to total one-step extraction. Sonication resulted in a marked spectral change of the crude chloroform-soluble extract (7- to 12-fold increase in UV absorbance) compared with anaerobic lysozyme cell fractionation. Crude chloroform-soluble extracts of HB, PH2ASO, PH2B, and PH2A produced absorbance spectra classified as type 2 by McMeekin et al. (21). MH1C exhibited a type 1 spectrum. Most (>50%) of the carotenoids in MH1C apparently were polar (i.e., xanthophylls) on the basis of their partitioning behavior in aqueous methanol-petroleum-ether mixtures as described by Britton and Goodwin (8). The carotenoids of

TABLE 2. Specific activities, inactivation studies, and relative electrophoretic mobilities of SODs from Lake Hoare bacterial isolates

Isolate	Growth conditions	Sp act	% Inhibition at azide concn (mM):		Half-life (min) in 5 mM H_2O_2	Relative mobility
			1	5		
MH1C	ADO	22.7	<5	8	>120	0.81
	HDO	30.1[a]	<5	13	>120	0.80
PH2A	ADO	25.7	<5	8	>120	0.91
	HDO	38.2[a]	<5	22	>120	0.91
PH2B	ADO	30.9	<5	20	>120	0.54
	HDO	42.1[a]	<5	21	>120	0.55
HB	ADO	12.9	<5	20	72	0.80
	HDO	10.0	<5	7	112	0.81
PH2ASO	ADO	19.8	<5	14	>120	0.90, 0.79[b]
	HDO	37.7[a]	<5	16	>120	0.89, 0.77[b]

[a] HDO specific activity > ADO specific activity ($P < 0.05$).
[b] Minor SOD band.

HB, PH2ASO, PH2B, and PH2A were mostly nonpolar (i.e., carotenes). The polarity differences in constituent carotenoids of MH1C and the other bacterial isolates are illustrated by the R_f values (Table 3). Methanol-chloroform and acetone-chloroform solvent systems produced poor separation in the VHO isolates as the carotenoids migrated with the solvent front. The visual differences in pigmentation of the isolates were due to differences in carotenoid type (illustrated by R_f values and absorbance spectra) and the relative proportion of each, as seen by the intensity of thin-layer chromatography-separated carotenoids.

ADO and HDO conditions influenced the growth of PH2A and the carotenoid-negative mutant PH2AM (Fig. 2). Under ADO, the carotenoid-negative mutant (PH2AM) demonstrated reduced growth rates and maximal cell density compared with the parent strain. Inhibition by HDO was greater in the carotenoid-negative mutant (Fig. 2). It exhibited a lengthened lag phase, a decreased growth rate, reduced maximal cell density, and relative increase in cell lysis upon entering stationary growth phase. This was confirmed by microscopic observation as lysis and not flocculation. PH2AM colony and cell morphology, Gram reaction, and growth inhibition at 20°C were identical to those of the parent strain. The specific activities of SOD in log-phase cells (90 h) of the parent and mutant strain were indistinguishable in this experiment (30 versus 32). PH2A and PH2AM were catalase positive.

DISCUSSION

HDO reduced the maximal cell density at higher nutrient concentrations in the three isolates examined. This inhibition could be from toxic oxygen by-products arising endogenously or from exogenous toxic by-products derived from interactions between oxygen and medium constituents as demonstrated by Hoffman et al. (14). Photochemical generation of superoxide radical, hydrogen peroxide, and singlet oxygen in media was minimized by dark storage and incubation. Even the most concentrated medium used (1,000 mg $liter^{-1}$) was dilute compared with standard clinical bacteriological media, thus minimizing toxic oxygen by-products. Nutrient reduction resulted in decreased HDO repression of maximum-culture optical density. Indeed, in the planktonic isolates examined, reduction of cell yield was alleviated (HB) or higher cell densities were produced (PH2A) with HDO than with ADO at <50 mg of nutrients $liter^{-1}$. These results support the theory of Kuznetsov et al. linking oligotrophy and oxygen toxicity (18). How HDO conditions contribute to acceleration of growth, as evidenced in PH2A,

TABLE 3. Relative mobilities of carotenoids from Lake Hoare bacterial isolates on thin-layer chromatography

Color[a]	R_f[b]	Absorbance maxima (nm) in chloroform	Bands present for:				
			HB	PH2ASO	PH2B	PH2A	MH1C
Yellow	0.06	468, 272, 265, 260, 238					+
Yellow	0.10	478, 273, 265, 260, 238					+
Yellow	0.27	480, 273, 265, 260, 238					+
Yellow	0.35	492, 272, 266, 260, 238					+
Yellow	0.65	487, 273, 266, 260, 238					+
Yellow	0.78	469, 272, 264, 260, 238					+
Yellow	0.77	488, 462, 432, 274	+	+	+	+	
Orange	0.87	516, 484, 458, 368, 296	+	+	+	+	
Pink	0.93	538, 504, 473, 392, 373, 322, 308	+		+	+	
Pink	0.93	518, 500, 476, 276		+			
Orange	0.94	468, 272, 265, 258, 238				+	+
Yellow	0.96	460, 271, 264, 259, 239		+	+	+	
Orange	0.97	515, 486, 454, 259, 239				+	

[a] Standard broth medium, 12°C incubation, stationary phase.
[b] Kieselgel 60, petroleum ether-chloroform-acetone (3:1:1, vol/vol/vol).

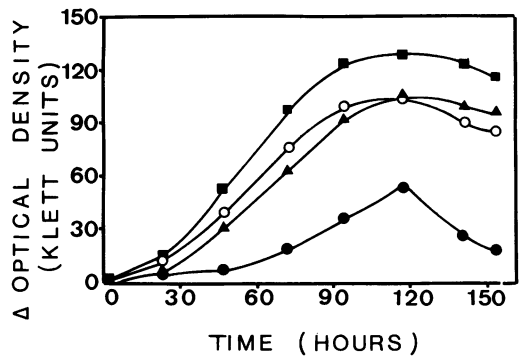

FIG. 2. Growth curves (change in optical density) of ADO-grown PH2A (■) and PH2AM (▲), and HDO-grown PH2A (○) and PH2AM (●).

is not clear. It seems unlikely that oxygen is limiting in a low-temperature oxygen-saturated environment even if these bacteria possess low-oxygen-affinity cytochrome oxidases. However, work by Andersen and von Meyenburg suggests that the respiration rate of *E. coli* may be the growth-limiting factor in static cultures (2). Increased respiration under HDO could result in increased growth rates (undetected by our technique at low nutrient concentrations) and decreased radical production by side reactions of the electron transport system, such as the production of superoxide and hydrogen peroxide by reduced quinones and flavoproteins (7, 13). The oligotrophic nature of Lake Hoare may be the most significant factor in high-oxygen tolerance of the bacteria in the lake waters.

Catalase may be essential in the HDO Lake Hoare environment. Dubinina, according to Kuznetsov et al. (18), reported an acceleration in cell lysis of catalase-negative oligotrophic isolates when grown in rich medium. This was attributed to increased hydrogen peroxide accumulation in the medium, which could be alleviated by addition of catalase. In our previously reported Mountain Lake study, catalase addition to the CFU medium did not retard HDO inhibition of CFU (24).

It is interesting to note the accelerated lysis in the carotenoid-negative mutant (PH2AM) (Fig. 2). This seems to be independent of SOD activity or catalase production. Cell lysis resulting from membrane disruption by autocatalytic lipid peroxidation has been established. Carotenoids have been reported to quench intermediates of this process, preventing initiation and propagation of this reaction (4, 17). The carotenoids in these isolates appear membrane bound, as evidenced by the difficulty in whole-cell extractions as reported by Meckel and Kester (23). This fits readily into the membrane-bridging hypothesis suggested by Burton and Ingold (9) and Taylor (29). Bound carotenoids may increase membrane stability by physical reinforcement and by quenching oxygen radicals, preventing lipid peroxidation. Proteolytic pretreatment before extraction as outlined by Thirkell and Hunter (30) was not tried, and so the mechanism of binding remains undetermined. Although the majority of the carotenoid protection literature concerns photosensitized reactions, dark generation and activity of peroxides would affect cells by the same mechanisms. This would be the case in the low-light environment of Lake Hoare as well as in the aforementioned HDO growth rate experiment which was performed in the dark. The possession of carotenoids may be advantageous in low-light environments, where high oxygen may catalyze lipid peroxidation. In this connection, it is interesting that the number of pigmented planktobacteria (*Micrococcus* and *Flavobacterium*) increased during the Austral summer in Lake Bonney, another Taylor Valley antarctic lake with HDO waters (L. S. Lane, personal communication).

SOD-specific activities reported for these organisms are higher than those reported for *E. coli* (12), albeit lower than those reported for certain aerobes such as *Bdellovibrio* spp. (31) and *Azotobacter* spp. (16). Four of the five bacterial isolates examined demonstrated an increase in SOD on growth in HDO. This induced SOD appears to be of the manganese type and does not differ in respective isolates from the ADO constitutive SOD on the basis of its relative electrophoretic mobility. Since the only known source of organic matter to these lakes is the photosynthetic algae, it is reasonable to assume that heterotrophic microorganisms will require their maximum protection from toxic oxygen by-products during their most active metabolic and growth phase, namely the Austral summer, with its maximum solar input and photosynthesis (27). If seasonally increased organic nutrients from higher productivity in the Austral summer increase the toxicity of oxygen, then perhaps an inducible-enzyme system would be advantageous to survival.

The high G+C molar ratio of the two isolates examined is interesting, as McMeekin and Shewan used the UV resistance of high G+C molar ratio strains of *Flavobacterium* spp. to separate them from low G+C UV-sensitive strains (22). High G+C ratio bacteria may have a survival advantage in high-light environments (28). Also, such a high G+C ratio might confer a survival advantage when free radicals are generated by means other than UV light.

Conclusion. This study has disclosed that heterotrophic microorganisms can possess a number of adaptive mechanisms for protection from various toxic oxygen by-products. These include oxidase, catalase, SOD, and carotenoids. Also, reduced rates of metabolism associated with low nutrient levels of an oligotrophic environment constitute an environmental means of protection. The unusual perpetual high-oxygen environment of Lake Hoare, Antarctica, appears to have selected for microorganisms capable of exercising a number of these mechanisms to protect from toxic oxygen effect.

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