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Chitinolytic bacteria and chitin mineralization in the marine waters and sediments along the Antarctic Peninsula

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1. SUMMARY

Chitinolytic bacteria were enumerated and isolated from marine waters and sediments along the highly productive Antarctic Peninsula. Chitinolytic bacteria were found in low concentrations (approximately 1 cell per ml) in the water column and at much higher levels in marine surface sediments (10^4–10^5 per g). The predominant chitinolytic bacteria isolated from the water column were identified as psychrophilic *Vibrio* spp. Rates of chitin mineralization were measured by collection of $^{14}CO_2$ respired from ^{14}C-labeled chitin synthesized from chitosan and [1-^{14}C]acetic anhydride. Chitin mineralization rates were extremely low in the marine waters analyzed (0.00085–0.0019% of the added label respired in 48 h) and appreciably higher in the marine sediments (0.0039–0.01% per 48 h), suggesting that the sediments are much more important in chitin degradation. Such low mineralization rates suggest that chitin may be accumulating in Antarctic marine sediments, though animals may also play an important role in chitin degradation.

2. INTRODUCTION

Chitin, a biopolymer composed of β 1 → 4 linked units of N-acetyl-D-glucosamine, is produced in prodigious amounts in the marine environment. Annual production in the aquatic biosphere is estimated to be 10^{11} metric tons [1]. Much of the production is in the form of the carapaces of zooplankton. The dominant zooplankton species in Antarctic marine waters is the krill species *Euphausia superba*. 4–10% of the dry mass of krill is composed of chitin [2,3] and the total biomass of krill is estimated at 0.5–3 billion metric tons [4]. Therefore, approximately 20–300 billion metric tons of krill chitin exist in Antarctic waters.

Microorganisms or higher organisms having chitinases could degrade chitin and utilize it as a source of carbon, nitrogen and energy. The presence of chitinolytic bacteria and the rates of chitin degradation in the Antarctic marine ecosystem have been poorly studied. The objective of our research was to enumerate and characterize the

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chitinolytic bacteria and to assess the mineralization rates of chitin for water column and sediment samples collected along the biologically productive Antarctic Peninsula.

3. MATERIALS AND METHODS

3.1. Sampling locations

Using the R.V. Polar Duke and the U.S. Coast Guard Arctic Survey Boat, water and sediment samples were collected from various sites along the Antarctic Peninsula. Table 1 lists the positions of the sampling sites, sampling dates, and the laboratory sampling codes used throughout the study and Fig. 1 shows representative charts of the sampling sites. During the late austral spring period (November 1986) sampling sites at Wilhelmina Bay and Penola Strait were located in open water on the edge of fast ice. Stations A1 and C1 were located at the ice edge and Stations A2, A3, C2 and C3 were within a kilometer of the ice edge. A small number of water samples were collected during the late austral summer (February–March 1986) from the Palmer vicinity and were used for heterotrophic potential studies [6] and the isolation of chitinolytic bacteria for taxonomic studies.

Table 1

Station locations and laboratory codes used for the sites

Station positions at Sites A, B, C, and E were determined on the R.V. Polar Duke by a satellite navigation system. Station positions at Site H and L were determined on the Arctic Survey Boat using radar, visual sightings, and a navigational chart.

Site	Station	Location	Latitude	Longitude
A	1	Wilhelmina Bay	64° 40.9′ S	62° 10.3′ W
	2		64° 40.8′ S	62° 09.7′ W
	3		64° 40.6′ S	62° 09.6′ W
B	1	Gerlache Strait	64° 36.5′ S	62° 43.6′ W
C	1	Penola Strait	65° 17.9′ S	64° 16.9′ W
	2		65° 15.5′ S	64° 12.9′ W
	3		65° 13.9′ S	64° 11.2′ W
E	1	Bismark Strait	64° 53.1′ S	63° 05.8′ W
H	1	Palmer Station	64° 46.2′ S	64° 05.2′ W
	4A	Vicinity	64° 46.6′ S	64° 05.1′ W
	6		64° 46.9′ S	64° 07.4′ W
	7		64° 47.1′ S	64° 09.3′ W
L	1	Palmer Station	64° 46.2′ S	64° 05.2′ W
	4A	Vicinity	64° 46.6′ S	64° 05.1′ W
	6		64° 46.9′ S	64° 07.4′ W

3.2. Collection and processing of samples

3.2.1. Water samples. Water samples were collected from aboard the research vessels using Niskin water bottle samplers (General Oceanics) that were rinsed and brushed out with 70% ethanol before sampling. Samples were collected from 10, 25 and 100 m from the sites located in Wilhelmina Bay, Gerlache Strait, Penola Strait, and the Bismark Strait. Additional deeper water samples were collected from the Gerlache Strait at 200 and 400 m, and from the Bismark Strait at 200 and 500 m. For the relatively shallow water stations near Palmer Station, samples were collected from 10 and 25 m for sites H1 and H4A, and at 10, 25 and 75 m for sites H6 and H7. All water samples were passed through a 100 μm Nitex netting to eliminate the larger zooplankton and particles and then stored in autoclaved polypropylene bottles, placed on ice until they were processed within 6 h of collection aboard the Polar Duke or at Palmer Station, located on Anvers Island (64° 46′S, 64° 3′W).

3.2.2. Sediment samples. Sediment samples were collected aboard the Polar Duke using a Smith-MacIntyre grab and from the Arctic Survey Boat using a small grab. Samples were removed from the Smith-Macintyre grab using plastic syringes that had been modified into a 'mini-corer' by removal of the tapered tip. Sediment samples were held on ice until processed within 6 h of collection.

3.3. Measurement of temperature and salinity

Water sample temperatures were recorded using a hand-held ASTM thermometer placed in the collected seawater or by reversing thermometers attached to the Niskin bottles. Salinity was determined with a Beckman Induction Salinometer using standardized seawater as a control.

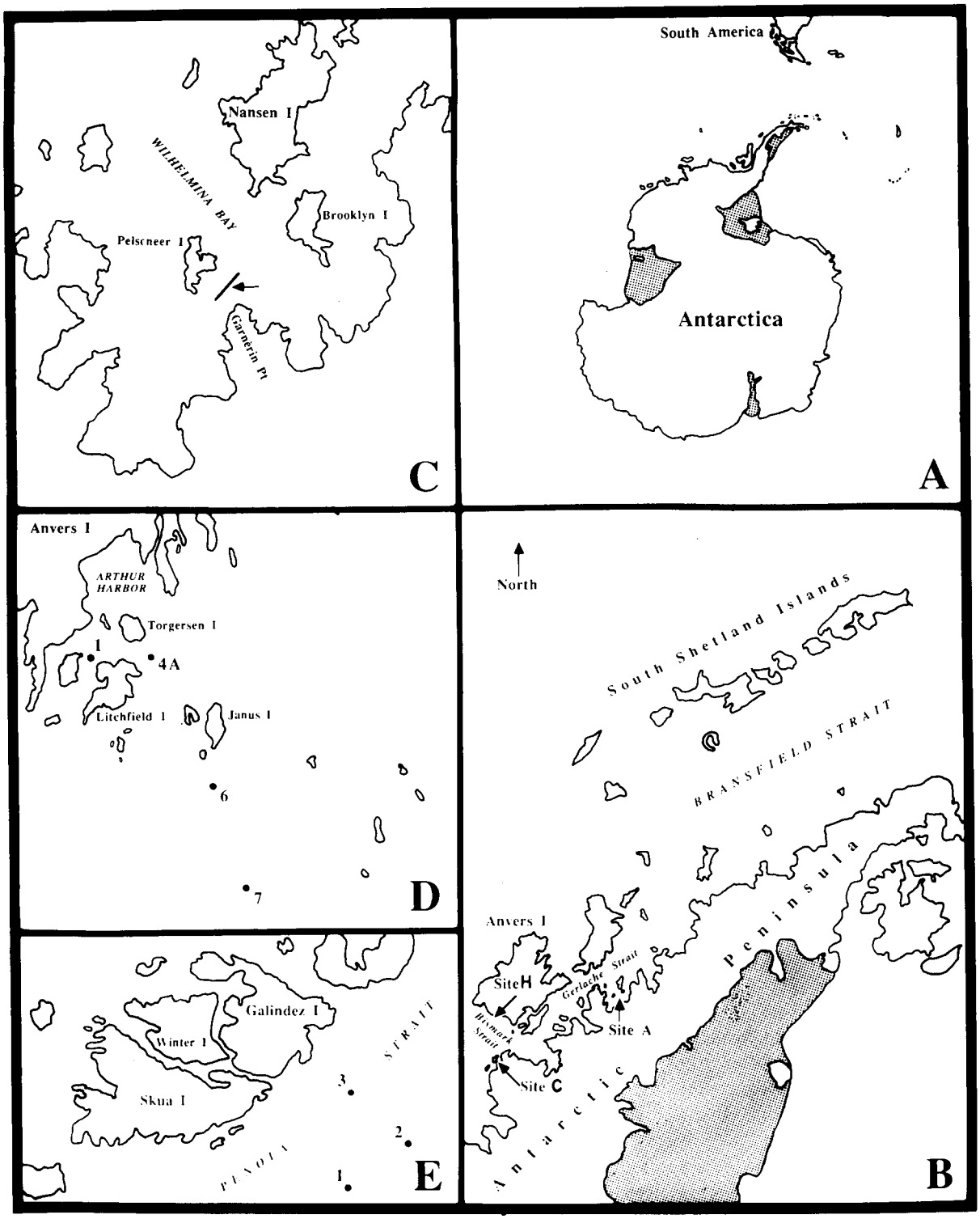

Fig. 1. Water and sediment sampling sites along the Antarctic Peninsula (B). Samples were collected from transects in Wilhelmina Bay (Site A, C); Palmer Station Vicinity (Sites H and L, D); Penola Strait (Site C, E). Three stations (Wilhelmina Bay, Site A) were located along the transect line indicated by the arrow in C. Single station sites were located in the Gerlache Strait and Bismark Strait (B). Site L was in the same location as Site H, but sampling was conducted on a different day.

3.4. Chlorophyll a analyses

Particulate chlorophyll *a* was obtained from surface water samples (10–100 m) that were filtered through Whatman GF/F glass fiber filters and frozen at −70 °C. Frozen filter samples were extracted with methanol for 24 h in the dark at −20 °C. Chlorophyll *a* concentrations, corrected for the presence of phaeophytin *a*, were determined by measuring fluorescence using a Turner Designs 10-005R Fluorometer [5].

3.5. Acridine orange direct counts

Total counts of bacteria were determined using the acridine orange direct counts procedure as modified by Herwig et al. [6]. Water samples were fixed with 2% formaldehyde, final concentration, and refrigerated until counted. 6 ml of the fixed sample were passed through a 0.2 μm, 25 mm black Nucleopore polycarbonate membrane filter. After the filters were allowed to air dry, a drop of the acridine orange stain was placed on the filter, and the filter was covered with a cover slip. A Zeiss epifluoresence microscope was used to count the filters. Approximately 24 separate microscopic fields were counted for each water sample.

3.6. Microbiological media and inoculation

3.6.1. Seawater cytophaga (SWC) agar. Total heterotrophic counts of microorganisms were enumerated on seawater cytophaga (SWC) agar [7] modified by using artificial seawater ($ASW\frac{1}{2}$) prepared at half-strength, and agar at 15 g per l concentration. The final pH was 7.6. The formula of the $ASW\frac{1}{2}$ was: 205 mM NaCl/14 mM $MgSO_4$/13 mM $MgCl_2$/5.0 mM $CaCl_2$/4.7 mM KCl.

3.6.2. Chitin overlay (CO) agar. For the enumeration of chitinolytic microorganisms a bilayer agar medium was prepared using SWC agar as the base layer which was overlaid with chitin overlay (CO) agar. The formula for CO agar was: 30.0 g reprecipitated chitin/15.0 g agar/1000 ml $ASW\frac{1}{2}$; final pH 7.6. The reprecipitated chitin was prepared as follows: practical grade chitin (Sigma) was ground to a fine powder using a hammer mill and 50 g of the chitin powder were dissolved with constant mixing for 30 min in 1.5 l of a 50% solution of H_2SO_4 held in an ice-water bath. With constant mixing, the syrup solution was reprecipitated with the addition of 1.5 l of chilled distilled water. The chitin was concentrated by centrifugation, and the acidic pellet was rinsed with distilled water and neutralized with NaOH. The chitin was stored in the refrigerator and had a dry weight of approximately 0.1 g per 1.0 g of wet chitin.

3.6.3. Diluent. The formula for the diluent used for the dilution blanks was: 0.1 g peptone/1000 ml $ASW\frac{1}{2}$. The pH was adjusted to 7.6.

3.6.4. Enumeration procedures for viable heterotrophic and chitinolytic bacteria. Agar media were prepared in advance, poured into sterile petri dishes and allowed to 'dry' so that the surface contained little condensation. Before inoculation all media were prechilled in a refrigerator and then placed on ice during transport and holding in the laboratory. Dilution blanks and Waring blender jars were also chilled. Water and sediment samples were inoculated onto SWC and CO agar media. For enumeration of bacteria from the water column, 1.0 ml of water was pipetted onto agar media prepared in large (150 × 15 mm) petri dishes. 10 ml of wet sediment were added to 90 ml of diluent and mixed for 30 s in a sterile Waring blender. Serial dilutions were prepared from the blended sample, and 0.2 ml of the diluted samples was inoculated onto the media contained in 100 × 15 mm petri dishes. Samples were spread onto the surface of agar media with sterile glass 'hockey sticks' [8] and were incubated at 5 °C. Counts of the inoculated media were made over a period of at least 1 month to allow sufficient time for colonies to appear and to permit observations of the length of time required for their appearance.

3.7. ^{14}C-labeled chitin mineralization studies

3.7.1. Preparation of radioactive chitin. ^{14}C-labeled chitin was prepared by a method similar to the procedure used by Molano et al. [9] for the preparation of ^{3}H-labeled chitin. Acetic anhydride [1-^{14}C] (500 μCi, specific activity of 10 mCi/mmol, purchased from ICN Radiochemicals) was mixed with 5.0 g of chitosan (Sigma). The synthesized chitin was washed free of unreacted [^{14}C]acetic anhydride and other soluble radioactive compounds by repeatedly rinsing the gel on a scintered glass filter holder (Millipore) with solu-

tions of methanol and distilled water. The radioactive chitin was ground to a fine mesh using a Ten Broeck 2 ml glass tissue grinder (Wheaton). For long-term storage, the chitin was maintained in a 70% methanol solution. The experimental substrate was prepared by suspending the chitin in autoclaved 0.2 μm filtered distilled water at an activity of approximately 3 μCi per ml.

3.7.2. Sample preparation and incubation flasks. Water and sediment samples were collected on 10 January 1987 from the Palmer Vicinity (Site L) transect for chitin mineralization studies. Radioactive chitin, approximately 0.27 μCi, was injected into each 125 ml Erlenmeyer flask containing 50 ml of a seawater sample or a sediment-overlying seawater mixture. The sediment mix was prepared by mixing 10 g of freshly collected sediment with 5 ml of overlying seawater collected from the same site. Rubber stoppers, used to close the flasks, were modified by removing a core out of the center to permit easier penetration of the stopper by a syringe needle, and a small hole was drilled on the bottom so a plastic collection bucket (Wheaton) could be attached. A 2.5 × 6.0 cm piece of Whatman No. 1 filter paper was folded to fit inside the bucket. The rubber stoppers were coated with a thin layer of silicone grease and secured firmly by tape. 0.2 ml of base (0.1 N NaOH) was added to the filter paper before the start of the experiment and again after completion of the experiment for the collection of respired $^{14}CO_2$. The sealed flasks were placed on a shaking water bath (New Brunswick), containing a bath mixture of 50% ethylene glycol/water, and maintained at 1.0 ± 0.5 °C with a refrigerated circulator (Neslab). Experimental flasks were prepared in triplicate for each sample and a formaldehyde-fixed, 2% (final volume), control was used. At the end of a 48 h incubation, samples were acidified with 0.2 ml of 6 N H_2SO_4 to drive the CO_2 from solution and to stop the experiment. The flasks were opened to remove the filter paper 2 h after acidification.

3.7.3. Radioactivity measurements. Filter paper used to collect the $^{14}CO_2$ from the ^{14}C-labeled chitin mineralization experiments were placed in 10 ml of Aquasol-2 (New England Nuclear) and counted on the LKB 1217 Rackbeta Scintillation Counter located at Palmer Station. Samples were corrected for quench using an external standard ratio.

3.8. Taxonomic evaluation of selected isolates

Chitinolytic bacteria from the water column in the Palmer Station Vicinity during the 1986 late austral summer (February–March 1986) were isolated by restreaking a minimum of two times on CO agar until only colonies showing clearing of the chitin were present. Isolates were maintained on SWC agar slants at 5 °C and characterized using the following taxonomic tests: Gram stain, oxidase, catalase, sensitivity to the vibriostat 0/129, growth in medium containing 0 and 1% NaCl, motility, cell morphology of wet mount, growth in an oxidative-fermentation agar containing 1% glucose under aerobic and anaerobic conditions. All test media were incubated at 5 °C. Details of media and procedures for the taxonomic procedure are outlined in Herwig and Staley [10].

3.9. Growth studies on temperature gradient incubator

The temperature range of growth of the Antarctic chitinolytic isolates was examined in inoculated SWC broth using the temperature gradient incubator as described by Matches and Liston [11]. The incubator held a temperature range of −3 to 35 °C and allowed for the examination of growth of the isolates at increments of approximately 1 Cdeg. The incubator held the culture tubes at a particular temperature ±0.5 °C. The absorbance measured at 660 nm, of cultures incubated at the various temperatures was followed for a period of approximately 1 month. Initially the cultures were examined daily for the first 5 days, then at 2–3 day intervals. When the absorbance of a particular culture tube began to decline, then it was removed from the incubator. The temperature optimum for growth (based upon growth rate) and temperature range of growth (based upon culture turbidity) were then determined.

Table 2

Location, date of sampling, station numbers, depth of water samples and sediment surface, water temperature, salinities, chlorophyll *a* concentrations in water samples, acridine orange direct counts (AODC), total viable counts and chitinolytic bacteria counts

In several sampling locations more than one station was sampled at various depths. Chlorophyll *a* analyses were performed only on water samples collected from the 10–100 m depths.

Location	Date	Station	Depth (m)	Temperature (C) [a]	Salinity (‰)	Chlorophyll *a* (mg/m^3)	AODC (cells/ml)	Total count (cells/ml) (cells/g wet) [b]	Chitinolytic (cells/ml) (cells/g wet) [b]
Wilhelminia Bay (A) [c]	11-18-86	1	10	n.d. [e]	34.30	3.89	n.d.	$8.6 \cdot 10^1$	< 0.5
			25	n.d.	34.47	1.26	$1.36 \cdot 10^5$	$4.7 \cdot 10^1$	< 0.5
			100	n.d.	34.57	0.324	$1.92 \cdot 10^5$	$2.7 \cdot 10^1$	2
			sed 275 [d]					$6.0 \cdot 10^5$	$8 \cdot 10^3$
		2	10	n.d.	34.32	3.60	$1.37 \cdot 10^5$	$5.5 \cdot 10^1$	3
			25	n.d.	34.49	2.35	$9.81 \cdot 10^5$	$1.2 \cdot 10^2$	< 0.5
			100	n.d.	34.62	0.277	$9.15 \cdot 10^4$	$8.9 \cdot 10^1$	0.5
			sed 263					$4.4 \cdot 10^5$	$3 \cdot 10^4$
		3	10	n.d.	34.40	3.04	$1.44 \cdot 10^5$	$7.0 \cdot 10^1$	1
			25	n.d.	34.40	2.96	$1.43 \cdot 10^5$	n.d.	1
			100	n.d.	34.61	0.455	$9.51 \cdot 10^4$	$8.2 \cdot 10^1$	1
			sed 225					$2.9 \cdot 10^5$	$1 \cdot 10^4$
Gerlache Strait (B)	11-19-86		10	0.2	34.32	3.37	$1.13 \cdot 10^5$	$1.2 \cdot 10^2$	< 0.5
			25	0.6	34.38	0.549	$8.40 \cdot 10^4$	$2.5 \cdot 10^2$	< 0.5
			100	0.1	34.56	1.15	$6.40 \cdot 10^4$	$6.7 \cdot 10^1$	1
			200	−0.3	34.57		$6.12 \cdot 10^4$	$3.7 \cdot 10^1$	0.5
			400	−0.6	34.63		$5.49 \cdot 10^4$	$1.0 \cdot 10^1$	6
			sed 504					$3 \cdot 10^4$	$8 \cdot 10^3$
Penola Strait (C)	11-21-86	1	10	n.d.	33.78	0.134	$6.02 \cdot 10^4$	$3.3 \cdot 10^2$	1
			25	n.d.	34.12	0.0521	$5.93 \cdot 10^4$	$1.4 \cdot 10^2$	2
			100	n.d.	34.54	0.00697	$6.31 \cdot 10^4$	$6.8 \cdot 10^1$	1
			sed 130					$1.6 \cdot 10^5$	$5.5 \cdot 10^4$
		2	10	−1.2	33.88	0.159	$6.25 \cdot 10^4$	$2.8 \cdot 10^2$	< 0.5
			25	−0.6	34.16	0.168	$5.18 \cdot 10^4$	$1.5 \cdot 10^2$	1
			100	0.3	34.53	0.00926	$5.47 \cdot 10^4$	8	< 0.5
			sed 158					$3.8 \cdot 10^5$	$1.5 \cdot 10^4$
		3	10	−1.2	33.86	0.136	$6.19 \cdot 10^4$	$2.1 \cdot 10^2$	0.5
			25	−0.5	34.03	0.0442	$5.26 \cdot 10^4$	$8.8 \cdot 10^1$	1
			100	0.3	34.52	0.00782	$4.59 \cdot 10^4$	$1.5 \cdot 10^1$	1
			sed 180					$3.9 \cdot 10^5$	$2.5 \cdot 10^4$
Bismark Strait (E)	11-24-86		10	0.0	34.17	1.08	$8.88 \cdot 10^4$	$6.3 \cdot 10^2$	1
			25	0.0	34.27	0.548	$9.81 \cdot 10^4$	$2.5 \cdot 10^2$	3
			100	0.4	34.46	0.0821	$4.99 \cdot 10^4$	$5.0 \cdot 10^1$	1
			200	0.5	34.60		$4.02 \cdot 10^4$	$3.6 \cdot 10^1$	1
			500	0.9	34.69		$5.96 \cdot 10^4$	8	< 1
			sed 720					$8.8 \cdot 10^4$	$6 \cdot 10^3$
Palmer Station Vicinity (H)	12-26-86	1	10	0.6	33.52	3.60	$6.65 \cdot 10^4$	$1.3 \cdot 10^2$	8
			25	0.4	33.77	1.45	$5.81 \cdot 10^4$	$1.3 \cdot 10^2$	$3 \cdot 10^1$
			sed 32					$4 \cdot 10^4$	$1 \cdot 10^4$
		4A	10	0.8	33.61	3.65	$4.54 \cdot 10^4$	$8.4 \cdot 10^1$	2
			25	0.6	33.93	2.06	$8.35 \cdot 10^4$	$1.7 \cdot 10^2$	$1.3 \cdot 10^1$
			sed 35					$1 \cdot 10^4$	$< 0.5 \cdot 10^4$
		6	10	0.7	33.67	2.72	$5.93 \cdot 10^4$	$1.4 \cdot 10^2$	0
			25	0.8	33.86	2.26	$7.32 \cdot 10^4$	$9.0 \cdot 10^1$	0.5
			75	0.7	34.36	0.871	$1.23 \cdot 10^5$	$7.2 \cdot 10^1$	0.5
			sed 120					$1.0 \cdot 10^5$	$5 \cdot 10^3$

Table 2 (continued)

Location	Date	Station	Depth (m)	Temperature (C) [a]	Salinity (‰)	Chlorophyll *a* (mg/m^3)	AODC (cells/ml)	Total count (cells/ml) (cells/g wet) [b]	Chitinolytic (cells/ml) (cells/g wet) [b]
		7	10	0.8	33.79	2.82	$6.38 \cdot 10^4$	$4.0 \cdot 10^1$	< 0.5
			25	0.6	33.89	2.93	$1.04 \cdot 10^5$	$1.3 \cdot 10^2$	< 0.5
			75	0.5	34.36	0.624	$8.75 \cdot 10^4$	$6.0 \cdot 10^1$	2
			sed 100					$7.7 \cdot 10^5$	$1 \cdot 10^4$

[a] For locations sampled in Wilhelmina Bay, Gerlache Strait, Penola Strait, and Bismark Strait reversing thermometers were used to measure water temperatures. For several of the samples retrieved during the early part of the study, the reversing thermometers were not found to be very reliable. At the Palmer Station Vicinity locations a mercury-filled hand-held thermometer was used.

[b] For sediment samples the numbers of bacteria that are listed are in units of cells per g of wet sediment.

[c] Letter within parentheses is the laboratory code letter for the site.

[d] sed, sediment sample for listed stations. The number next to sed is the depth of the water over the surface of the sediment, determined from R.V. Polar Duke and the Arctic Survey Boat using a depth finder.

[e] n.d., No data.

4. RESULTS

4.1. Microbiological counts, chlorophyll a *analyses, temperature and salinity data*

Data are presented in Table 2 for the heterotrophic and chitinolytic bacteria counts, chlorophyll *a* analyses, temperature, salinity, and the direct counts of microorganisms using the acridine orange direct counts procedure. All samples were collected from marine waters having temperatures near 0 °C and salinities between 33 and 34‰. Chitinolytic bacteria occurred in very low numbers in the water column at all sampling sites ranging from levels below detection (< 0.5 per ml) to 30 per ml. In the sediments chitinolytic bacteria were found at much higher concentrations, ranging from 10^3 to 10^4 per g of wet sediment. The counts of viable heterotrophic bacteria were much higher than the chitinolytic bacteria. Heterotrophic counts in the water column for the various stations ranged from $1.0 \cdot 10^1$ to $6.3 \cdot 10^2$ per ml. Trends for these enumerations were apparent. Generally the counts of viable heterotrophic bacteria were highest in the surface layers of the water column (10–25 m) and declined with depth, although in several cases the numbers of viable heterotrophic bacteria were greater at 25 m than at 10 m. In the sediments, the counts of heterotrophic bacteria ranged from $1 \cdot 10^4$ to $6.0 \cdot 10^5$ per g. The AODC counts, ranging from 10^4 to 10^5 per ml, were 100- to 1000-times greater than the viable counts for each water sample. These counts generally paralleled the viable counts, i.e., were greatest in the 10–25 m surface samples and declined with depth.

Chlorophyll *a* concentrations were the greatest

Table 3

Representative counts of chitinolytic and heterotrophic bacteria from samples incubated for an eight week period at 5 °C

Observations of the counts were made at the 2, 4, and 8 week incubation periods.

Sample	Media	Incubation period		
		2 weeks	4 weeks	8 weeks
A1 [a] sed	SWC [b]	$2.1 \cdot 10^5$ (13) [d]	$9.4 \cdot 10^5$ (59)	$1.6 \cdot 10^6$
	CO [c]	$2.5 \cdot 10^3$ (19)	$1.0 \cdot 10^4$ (77)	$1.3 \cdot 10^4$
A2 sed	SWC	$1.9 \cdot 10^5$ (68)	$2.6 \cdot 10^5$ (93)	$2.8 \cdot 10^5$
	CO	$5.0 \cdot 10^3$	$1.7 \cdot 10^4$	n.d. [e]
A3 sed	SWC	$1.9 \cdot 10^5$ (34)	$5.1 \cdot 10^5$ (91)	$5.6 \cdot 10^5$
	CO	$1.5 \cdot 10^4$ (75)	$1.7 \cdot 10^4$ (85)	$2.0 \cdot 10^4$
C1 sed	SWC	$2.2 \cdot 10^5$ (81)	$2.4 \cdot 10^5$ (89)	$2.7 \cdot 10^5$
	CO	$2.5 \cdot 10^4$ (27)	$6.8 \cdot 10^4$ (73)	$9.3 \cdot 10^4$
C2 sed	SWC	$2.2 \cdot 10^5$ (33)	$6.2 \cdot 10^5$ (95)	$6.5 \cdot 10^5$
	CO	$1.2 \cdot 10^4$ (48)	$2.3 \cdot 10^4$ (92)	$2.5 \cdot 10^4$
C3 sed	SWC	$2.9 \cdot 10^5$ (64)	$4.2 \cdot 10^5$ (93)	$4.5 \cdot 10^5$
	CO	$1.3 \cdot 10^4$ (39)	$3.0 \cdot 10^4$ (91)	$3.3 \cdot 10^4$

[a] Sampling code designates the site and station number.

[b] SWC, seawater cytophaga agar, used for the enumeration of total heterotrophic bacteria.

[c] CO, chitin overlay agar, used for the enumeration of chitinolytic bacteria.

[d] Number within parentheses is the percentage of colonies appearing at the 2 and 4 week incubation period compared to the 8 week incubation.

[e] n.d., no data, because of contamination of the media after the 4 week observation.

in surface waters and declined with depth. Samples collected in an area where the fast ice was just beginning to melt and break up, Penola Strait, showed the lowest surface chlorophyll *a* concentrations, i.e., less than 0.2 mg per m^3.

Plates for chitinolytic and total heterotrophic bacteria were incubated in the dark at 5 °C for a minimum of 4 weeks. Plates that were inoculated during the late austral spring (samples from Sites A, B, C, and E) were incubated up to 8 weeks. Samples collected afterward could not be incubated for the longer period, since our field team had to return to Seattle. The counts of bacteria were found to increase over the incubation period and the 4 week incubation period was found necessary for a large portion of the colonies to appear. The zones of clearing for the chitinolytic bacteria developed only after the colonies were visible on the plate. Table 3 lists representative colony counts from two separate sampling sites of total heterotrophic and chitinolytic bacteria found on the media incubated up to 8 weeks. Generally, over 85% of the colonies that were enumerated at 8 weeks appeared within 4 weeks of incubation.

4.2. ^{14}C-labeled chitin respiration studies

Chitin mineralization rates, as measured by the respiration of $^{14}CO_2$ from the ^{14}C-labeled chitin, are listed in Table 4. All samples were incubated for 48 h and rates of chitin degradation were much greater in the marine sediments than in the overlying waters. The highest rates (0.010%) were found in a sediment sample (L1) located near penguin rookeries at Torgersen Island and Humble Islands.

Table 4

Chitin mineralization rates for Antarctic marine waters and sediments collected on 10 January 1987 in the Palmer Station Vicinity. Water and sediment samples were incubated for 48 h at 1.0 °C with ^{14}C-labeled chitin

Site and Station	% ^{14}C-labeled chitin released as respired $^{14}CO_2$ per experimental flask
L1 water	0.00085
L1 sediment	0.010
L4A water	0.0019
L4A sediment	0.0039
L6 water	0.00048
L6 sediment	0.0053

Table 5

Typical results of the taxonomic screen of chitinolytic bacteria from the water column of Antarctica

Test	Result
Cell morphology	curved or straight rod
Motility	+
Gram stain	negative
Oxidase test	+
0/129 sensitivity	+
MOF medium, +1% glucose	Anaerobic acid production
Growth in CLED agar + 1% NaCl	+
Growth in CLED agar	−

MOF, marine oxidation-fermentation; CLED, Cysteine lactose electrolyte deficient.

4.3. Taxonomic evaluation and growth studies

Nine chitinolytic isolates collected during the 1986 late austral summer season (February–March) from the water column near Palmer Station were tentatively identified as *Vibrio* sp. based on the results of the taxonomic screen. Typical results of the tests are presented in Table 5.

Seven chitinolytic isolates were examined for temperature range of growth, growth rates and

Table 6

Temperature growth studies for chitinolytic bacteria isolated from the water column of the Antarctic Peninsula

Culture	Temperature range of growth		Temperatures of maximum growth rate (°C)
	Low	High	
C17	−1.5	21.0	5–15
C24	−1.5	23.0	10–15
C26	−3.0 [a]	22.0	5–15
C43	−1.5	20.5	10–15
C68	−1.5 [a]	23.0	10–20
C81	0.0 [a]	22.0	10–20
C83	−1.5	23.0	5–15

[a] Lowest temperature that was examined for culture, therefore lowest temperature for growth may be less.

optimum growth temperature using the gradient incubator (Table 6). Six of the cultures were able to grow at −1.5°C and the doubling time, as measured by absorbance, at this temperature was between 1 and 5 days. The upper temperature limit for growth was between 20.5 and 23.0°C. The temperature for optimum growth rate was between 5 and 20°C.

5. DISCUSSION

Millions of tons of chitin are produced in the marine environment each year and in the Antarctic ecosystem much of the chitin is in the form of krill exoskeletons. The figures reported for annual krill production do not, however, reveal the complete picture. For example, during the austral summer krill molt their exoskeleton and release the exuviae into the water column at intervals of approximately every 2 weeks [12,13]. Significant amounts of chitin are also present in the exoskeletons of other zooplankton species and in fecal pellets, which are coated with a chitinous membrane [14,15]. If chitin were not degraded, large quantities of carbon and nitrogen would be lost from the ecosystem.

This study was aimed at investigating chitin decomposition in the marine waters and sediments along the west coast of the Antarctic Peninsula. This area is known for high primary productivity, standing crop of algae [16], production of krill [17,18], and abundant populations of krill-feeding marine mammals and birds [17]. In addition, for United States scientists, the area is accessible for study because of the proximity of the U.S. base, Palmer Station, and the availability of research vessels. With the exception of the most southern sample site, Penola Strait, the chlorophyll *a* concentrations in the surface waters at sites investigated during this study were found to be generally comparable to the levels found by El-Sayed [16] in an earlier study. The 1986–1987 austral summer along the Antarctic Peninsula was somewhat delayed because of the heavy winter sea ice and the delay was more noticable at the southern sampling sites. For example, the chlorophyll *a* concentrations for comparable depths at Penola Strait (65°S latitude) were about 20-fold lower than Wilhelmina Bay (64°S latitude).

It is clear from this investigation that chitinolytic bacteria are present in the Antarctic marine waters and sediments of the Antarctic Peninsula, and furthermore that they are engaged in chitin decomposition. Chitinolytic bacteria were found in low concentrations in the water column (about 1 per ml) and in much higher concentrations in the sediments (10^5–10^6 per g). These concentrations are similar to those reported by other investigators for temperate and semi-tropical zone habitats. For example, early studies by ZoBell and Rittenburg [19], working off the coast of California, found the highest numbers in the surface sediment layers (10^3 per g). In the Woods Hole area, sediment samples collected from a depth of 878 m contained $1.3 \cdot 10^2$ chitinolytic bacteria per g [20]. Seki and Taga [21] reported that the chitinolytic bacterial populations of Sagami Bay were greatest in the surface waters (ranging from $0.001–5.0 \cdot 10^3$ per ml) and decreased with increasing depth.

Thus, the numbers we have found in Antarctic marine sediments are as high or somewhat higher than those reported for temperate zone habitats. Furthermore, they range from about 1 to 25% of the total cultivable heterotrophic microflora of the sediments and therefore comprise a significant physiological group. Although we have not yet identified the predominant cultivable sediment chitinolytic bacterial populations, those from the water column have been characterized. The water column isolates are predominantly psychrophilic *Vibrio* spp. whose temperature optimum was between 5 and 20°C. The finding of *Vibrio* spp. is not surprising in view of the reports of others. For example, bacterial isolates retrieved from the water samples of Sagami Bay were classified as species of *Beneckea* [21], a genus since reclassified as *Vibrio* [22]. In the waters of Aburatsubo Inlet, Seki and Taga [23] found the chitinolytic organisms to be *Beneckea* (*Vibrio*) sp., having an optimum temperature for growth at 30°C. Again species of the genus *Beneckea* (*Vibrio*) were the predominant kinds of chitinolytic microorganisms found in a Louisiana salt marsh [24].

There are difficulties associated with the assess-

ment of rates of chitin degradation in natural environments. As a particulate substrate, typical procedures designed for soluble organic substrates are not directly applicable. Also, the experimental substrate may be supplied to the environment in various forms ranging from the crude natural state to purified forms, or even radiolabeled forms. Finally, the length of exposure of the substrate to the microbial community poses other problems. Should the exposure be short-term, assuming that the degrading enzymes are present and available for chitin decomposition, or should the exposure be longer term, to permit the colonization and natural development of degrading organisms on the particulate substrate? Most previous investigators working in temperate habitats have used gravimetric procedures for analyzing chitin degradation. In this procedure, natural carapaces of larger invertebrates such as shrimp or crab have been placed in the environment, recovered and weighed at subsequent time intervals. For example, high rates of degradation (87 mg/day per g of 'native' chitin) were reported from a Louisiana salt marsh using a gravimetric procedure [24]. In contrast, uniformly ^{14}C-labeled chitin, prepared by injecting molting blue crabs, *Callinectes sapidus,* with the chitin precursor N-acetyl-D-[1-^{14}C]glucosamine, was used as a substrate by Boyer and Kator [25]. They examined chitin degradation and mineralization in a southern Virginian estuary, the York River, for batch culture samples incubated at 20°C. The maximum rate of chitin degradation was 207 mg/day per g chitin, achieved after approximately 3 days when the numbers of chitinolytic bacteria within the flasks had increaed over 1000-fold to 10^7 cells per ml. However, before the sizeable increase in the chitinolytic population, little, if any, $^{14}CO_2$ was recovered.

In this investigation we used a purified ^{14}C-labeled chitin labeled in the N-acetyl moiety of the molecule, a radionucleotide that has not been used by others in measuring chitin degradation rates in natural environments. Thus, our data are not directly comparable to those of other investigators.

The rates of chitin degradation, as measured by the respiration of $^{14}CO_2$ from ^{14}C-labeled chitin, were found to be extremely low in the marine waters of Antarctica (0.00085–0.0019% of added label) and higher in the marine sediments (0.0039–0.010%) for samples incubated for 48 h at 1.0°C. The percentages would represent a minimum rate of chitin degradation, since the method depends upon two separate steps, the solubilization of chitin and respiration of the acetyl group of the molecule. All of the acetyl ^{14}C that was metabolized may not be respired, as some may be incorporated into cell material. In addition, some of the acetyl ^{14}C may accumulate as [^{14}C]acetate. Samples that were collected near Adelie penguin rookeries on Torgersen and Humble Islands showed the highest chitin mineralization rates. During the austral summer these islands contain over 9000 breeding pairs of Adelie penguins [26]. Adelie penguins feed on krill, and may be depositing large amounts of 'undigested' chitin in the rookery and the nearby marine environment. With such a low percentage of the chitin being mineralized, our results suggest that chitin may actually be accumulating in the marine sediments of Antarctica.

In studies of chitin degradation in the environment, animals that feed upon chitinous prey must also be considered. In Antarctica, several species of warm-blooded marine mammals and birds, and poikilothermic fish feed almost exclusively on krill [17]. The warm-blooded predators may be very important participants in chitin degradation, since their chitinases, if present, would be maintained 35–40 Cdeg higher than the environmental temperatures. Indeed, preliminary evidence from our laboratory indicates that fin whales, crabeater seals, and Adelie penguins are all able to degrade significant portions of krill chitin in their digestive tracts. In addition, a recent study of Antarctic fish indicates that several species produce chitinase [27]. These and other studies will undoubtedly lead to a better understanding of the significance of various micro- and macrobiological groups in chitin decomposition in polar regions.

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