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3.7 Diversity and Identification of Heterotrophs from Antarctic Rocks of the McMurdo Dry Valleys (Ross Desert)

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Zusammenfassung: Die Diversität endolithischer Gesteinsmikroorganismen aus den Dry Valleys wurde am Auftreten von Morphotypen in Anreicherungen gemessen. Die Lagerung von Gesteinsproben auf Trockeneis über 16 h veränderte die Diversität endolithischer Organismen, insbesondere die von Algen und Pilzen. Die Diversität in den einzelnen Proben hing von der Gesteinslage und -exposition, vom Gesteinstyp, und in gewissem Grade auch vom pH-Wert der pulverisierten Gesteinsproben ab. Sandstein enthielt meist mehr Morphotypen als Dolerit oder Granit. Das Auftreten vieler verschiedener Phototropher in Anreicherungen führte auch zu einer erhöhten Diversität bei Heterotrophen. Proben von Linnaeus Terrace und Battleship Promontory enthielten mehr Morphotypen (MT) als solche von exponierten Standorten, wie New Mountain, University Valley, Dais oder Mt. Fleming. Beacon Sandstein von Linnaeus Terrace (13 Proben) enthielt sehr unterschiedlich viele MT, obwohl die pH-Werte nur im Bereich von 4,2—5,3 variierten. Die höchste MT-Anzahl pro Probe (24) wurde auf der Oberfläche eines flachen Felsens mit Nordneigung beobachtet. Eine harte Sandsteinprobe von der windexponierten und schattigen Ostseite von Linnaeus Terrace enthielt nur 2 MT. In 15 Sandsteinproben von Battleship Promontory fanden wir eine höhere Diversität: es traten insgesamt 131 verschiedene MT auf, verglichen mit nur 68 in den 13 Proben von Linnaeus Terrace. Einige Battleship Promontory Proben enthielten Cysten farbloser Flagellaten. Die meisten dieser Proben beherbergten eine Vielzahl verschiedener Cyanobakterien. Untersuchungen zur Verteilung von Actinomyceten-MT in Linnaeus Terrace Sandstein ergaben große Unterschiede zwischen einzelnen Felsblöcken. Mit repräsentativen Stämmen der 1500 isolierten Reinkulturen wurden Identifizierungstests und Lipidanalysen durchgeführt. Dies führte zu Gattungsnamen wie *Caulobacter, Blastobacter, Hyphomicrobium, Micrococcus, Arthrobacter, Brevibacterium, Corynebacterium, Bifidobacterium, Mycobacterium, Nocardia (Amycolata), Micromonospora, Streptomyces, Blastococcus* und *Deinococcus*. Unsere Untersuchungen zeigen die große Diversität antarktischer endolithischer Mikroben-Populationen.

Summary: Diversity of endolithic Dry Valley rock microorganisms was studied by evaluating the presence of morphotypes in enrichments. Storage of rock samples for 16 h over dry ice affected the diversity of endolithic organisms, especially that of algae and fungi. Diversity in various samples depended on rock location and exposure, on the rock type, and to some extent on the pH of the pulverized rock samples. In most cases sandstone contained more morphotypes than dolerite or granite. Presence of many different phototrophs resulted in greater diversity of the heterotrophs in the enrichments. Samples from Linnaeus Terrace and Battleship Promontory had higher morphotype (MT) numbers than those from more exposed sites such as New Mountain, University Valley, Dais, or Mt. Fleming. Beacon sandstone (13 samples) from Linnaeus Terrace varied greatly with respect to MT numbers, although the pH values ranged only from 4.2—5.3. The highest MT number of 24 per sample was obtained from the upper surface of a flat boulder tilted to the North. Only two MT's were found in a hard sandstone sample from the wind-exposed and more shaded east side of the Terrace. 15 sandstone samples from Battleship Promontory contained more diverse populations: there occurred a total of 131 different MT's in these samples as compared to only 68 in Linnaeus Terrace samples. Cysts of colorless flagellates were found in some Battleship Promontory samples; most samples were populated with a wealth of different cyanobacteria. Studies on the distribution of actinomycete morphotypes in Linnaeus Terrace sandstone revealed great differences between individual boulders. Identification tests and lipid analyses made with representative strains of the isolated 1500 pure cultures led to genus names such as *Caulobacter, Blastobacter, Hyphomicrobium, Micrococcus, Arthrobacter, Brevibacterium, Corynebacterium, Bifidobacterium, Mycobacterium, Nocardia (Amycolata), Micromonospora, Streptomyces, Blastococcus*, and *Deinococcus*. Our data demonstrate the great diversity of Antarctic endolithic microbial populations.

1. INTRODUCTION

The major ice-free regions of South Victoria Land are the "Dry Valleys" along the Ross Sea. In these "oases" katabatic winds from the polar ice plateau remove the rarely occurring snow and create true desert conditions. Due to the harsh climate (McKAY & FRIEDMANN 1985) these areas lack completely any visible higher plant or animal life, except perhaps for a few mosses in protected places, and except for epilithic lichens (FRIEDMANN 1982). The discovery of cryptoendolithic lichens and algal growth by the American microbiologist, W. Vishniac, led to closer investigations of these organisms' ecology within the sandstone rocks (FRIEDMANN & OCAMPO 1976, FRIEDMANN 1982).

In these studies it was soon realized that the endolithic growth was not restricted to the lichen phyco- and mycobionts, but that a variety of heterotrophic bacteria and fungi, other than the lichen partners, shared the protected locations within the rocks.

The objectives of our present research were to study the qualitative diversity and distribution, within different rock samples, of the heterotrophic components of the cryptoendolithic ecosystem, to understand their dependence on the phototrophs present, and to identify if possible to the genus level, the bacteria that could be obtained in

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pure culture. Such a study can be conducted in three different ways: (1) by studying the organisms' morphology (by their classification as "morphotypes"), (2) by investigating their physiology (and identifying them as "physiotypes"), or (3) by analyzing rock samples for marker components of these microorganisms, such as ATP, storage granules, RNA, cell wall or membrane components. As the physiology of these heterotrophic endoliths was initially unknown, it was realized that specific enrichment conditions would select for only a fraction of the total population present (HIRSCH & HOFFMANN 1988). For these reasons we chose to initially describe the endolithic populations by morphological features. We were able to differentiate, in some rock samples, up to 66 morphotypes, while other samples contained only a few, perhaps indicating recent origin of this small endolithic population or, conversely, old age and extremely unfavorable conditions which selected for only a few survivors. The morphotype diversity, together with information on the pure culture isolates available, allowed us to preliminarily describe these endolithic populations.

2. MATERIALS AND METHODS

2.1 *Study Area and Sampling Conditions*

The samples came from the Beacon Heights area (University Valley, New Mountain), from Asgard Range (Mt. Fleming, Linnaeus Terrace, Dais), or from the Convoy Range (Mt. Gran: Battleship Promontory). Rock samples were collected in the years 1984/85, 1985/86 and 1986/87, as indicated by the numbering: 845/x, 856/x, or 867/x, respectively. All samples were collected aseptically, i. e. broken off with 70% ethanol-treated hammer and chisel, and transported in double and sterile Whirl-Pack® bags. Storage and transportation were always carried out under freezing conditions, initially with dry ice. But when it was later discovered that dry ice (CO_2) changed the organism's diversity, the samples were stored and transported to Germany only under refrigeration with commercially packaged frozen mixtures, i. e., under normal air. In Kiel the samples were stored frozen at –20° C or refrigerated at +4° C.

2.2 *pH Measurements*

In order to measure pH, samples were homogenized by grinding in a mortar and suspended (1:1, v/v) in 0.1 N KCl, or in some cases in double distilled water. Measurements were taken immediately after wetting and as long as 60 h afterwards to observe possible changes. Many of the samples stabilized after 30—60 min.

2.3 *Direct Microscopy of Sample Extract*

For direct phase contrast microscopy of fresh or stored samples we followed procedures of WAID (1973). A pea-sized amount of ground up rock sample was placed in a 10 cm watch glass and distilled water was allowed to flow to the bottom from a Pasteur pipette until the sample was completely covered. While rising, the water removed a thin polymer film from the rock particle surfaces and this film was then collected as a whole on an agar-coated glass slide by using a wire loop (PFENNIG & WAGENER 1986). After covering with a glass slip the preparation was viewed with 1000x phase objective lenses.

2.4 *Enrichments, Media and Growth Conditions*

For the enrichments we used oligotrophic medium PYGV containing 0.25 g/l of peptone, yeast extract and glucose as well as a vitamin mixture and mineral salts; the total organic carbon content was 4.8 mg/l (STALEY 1968). This medium has been found to allow growth and observation of the largest number of different morphotypes (HIRSCH & HOFFMANN 1988). Enrichments were set up with 50 ml medium in 125 ml Erlenmeyer flasks covered with cotton stoppers and loose aluminum foil. The inoculum consisted of 3—6 g rock sample containing the colonized zone. Also pulverized rock (approx. 0.35 g) was sprinkled directly onto PYGV agar plates, pH 7.2. Incubation was always at 9° C and under dim light of 100—300 lux. Cultures were scanned for morphotypes ("MT") after 4, 8 and 12 weeks and again after 6 and 12 months. Morphotypes were recognized by comparison with a table of 170 MT which was prepared in previous studies (HIRSCH & HOFFMANN 1988) and later extended. Significance of MT diversity was checked by studying parallel cultures; here the total MT numbers differed by 10—30%. Long term incubation (1 year) of agar plates occurred in sterile plastic bags at 3° C.

2.5 *Isolation of Pure Cultures*

For purification purposes the enrichments or single colonies from agar plates were streaked onto PYGV containing 1.8% Difco agar. Single colonies were transferred to master plates using sterile tooth picks. Purity was checked

by repeatedly streaking out as well as by phase contrast microscopy. Pure cultures were maintained on agar slants, or frozen in Eppendorf caps, or in the lyophilized state.

2.6 *Tests for the Characterization of Pure Cultures.*
Actinomycetes were identified by morphology, by cell wall analyses (U. MEVS unpubl.), and by various other tests listed in Table 6. Degradation of xylan, tyrosine, esculin and arbutin were studied by following procedures of HORAN & BRODSKY (1986). Hyrolysis of casein and utilization of adenine, hypoxanthin and xanthin were studied according to GORDON et al. (1974). The presence of active sialidase was found by using a fluorometric method suggested by POTIER et al. (1979). Tolerance of NaCl was assayed for in PYGV to which 1—10% of NaCl had been added; growth was estimated by protein determination according to the Bio-Rad method.

2.7 *Fatty Acid Analyses*
Selected isolates with a recognizeable morphology were subjected to fatty acid analyses for comparative identification purposes. Lyophilized cells (50—100 mg) were hydrolyzed with methanolic KOH and esterified with methanol — 2% H_2SO_4 for 2 h at 70° C. Fatty acid methyl esters were analyzed by gas liquid chromatography with a Packard 419 Becker Gas Chromatograph equipped with a flame ionization detector and Autolab Digital Integrator 6300. Samples were chromatographed either isothermally at 200° C or with a temperature gradient from 160° to 200° C, with a rise of 1° C/min. A glass column (4 m x 2 mm) packed with Chromosorb G, AW/DMCS, 70—80 mesh, coated with 5% DEGS was used. Peaks were identified by comparison with analytical grade standard fatty acid methyl esters.

Sample number	856/110	856/110	856/111	856/111
Sample treatment	air	dry ice	air	dry ice
		Total number of colonies observed		
Medium:				
PYGV	176	235	498	309
Sabouraud-dextrose agar	697	425	n.t.	n.t.
Nutrient agar w.glucose	229	n.t.	225	263
Organism:		Percentage of total colony number		
filamentous fungus 1	28	0	27	15
,, ,, 2	0	31	8	8
,, ,, 3	0	0	4	11
,, ,, 4	0	0	1	0
,, ,, 5	0	0	0	1
bacterium I	49	44	44	55
,, II	22	17	14	4
,, III	1	0	1	6
green alga 1	1	8	1	0

Tab. 1: Effects of storing Antarctic rock samples over dry ice before investigating the sample's morphotype diversity. The samples were kept naturally frozen until transported to the Ecklund Biological Laboratory in McMurdo (Ross Island). There they were treated at ambient minus temperatures for 16 h with dry ice or not. n.t. = not tested.

3. RESULTS

3.1 *Effects of Dry Ice (CO_2) on Morphotype Diversity in the Samples*
Two freshly collected rock samples from Linnaeus Terrace were divided and one-half of each stored frozen for 16 h in rock boxes with or without dry ice. After that about 0.4 g of the fractioned rock samples were inoculated into or onto various media (PYGV, Sabouraud-Dextrose Agar, Bacto Nutrient Agar with 1% glucose). The cultures were incubated for four weeks at 10° C, thereafter for one year at 3° C; agar plates were kept in sterile plastic bags to avoid drying out. Evaluation after one year showed that the total number of colonies of some MT could be recognized and counted; identity was checked by microscopy. Among those organisms that were detected on plates, there were some fungi that showed strong inhibition by the dry ice treatment, while others (algae) were actually stimulated. Although these observations may have been preliminary, the fact remains that the relative proportions of individual MT in these two rock samples changed, that is: the dry ice (most likely the higher CO_2 concentration) influenced the MT diversity in these samples. Consequently, later samples could only be stored and transported under refrigeration with commercially packaged frozen mixtures, i. e., in air.

3.2 *Growth of Rock Microorganisms in Oligotrophic Medium PYGV*
As preliminary experiments had shown that MT diversity in enrichments was greatest with low nutrient (oligotrophic) media, PYGV was used throughout for all experiments (Tables 2 and 3; HIRSCH & HOFFMANN

1988). Microorganisms were able to develop in all of the PYGV containing enrichment cultures and pure cultures originated from all samples tested, albeit in different numbers and with different MT diversity. Thus all organisms delt with in this study could be considered to be oligotrophic. Growth occurred even in a ten-fold dilution of this medium (PYGV/10, HIRSCH & HOFFMANN 1988).

3.3 *Diversity of Morphotypes in Different Rocks from Various Locations*

A comparison was made of the total MT populations that could be observed in different rocks from various locations (Tab. 2). In PYGV the total MT numbers ranged from 1—39 per sample, with the highest number present in sandstone. There was no significant correlation with the sample pH. Cocci and rod-shaped bacteria were most numerous. Spore-formers, iron-oxidizers, and filamentous bacteria were seen only rarely. One sample (867/228) contained colorless flagellates. Five of the samples completely lacked phototrophs, but still these had up to 13 different heterotrophs present. One sample contained 14 different cyanobacteria MT. None of the samples was completely sterile, and the highest numbers of different MT were found in samples from Linnaeus Terrace and Battleship Promontory.

Location type a.sample		pH (0.1 N KCl; 30 min)	Prokaryotic												Eukaryotic				Total MT's		
			CO*	RD PR	FB	BB PB	SR	BR	SF	IO	CF	AM	CY		AL	YE	FF	FL	MT no.	photo-trophs	hetero-trophs
University Valley:																					
sandstone	867/209	5.2	4	5	–	1	2	–	–	–	1	1	2		5	3	4	–	28	7	21
granite	856/127	4.6	–	1	–	–	–	–	–	–	–	–	–		–	–	3	–	4	0	4
dolerite	856/154	5.0	–	–	–	–	–	–	–	–	–	–	–		–	–	1	–	1	0	1
New Mountain:																					
sandstone	856/129	4.3	3	3	1	3	–	2	–	–	1	1	1		–	1	1	–	17	1	16
yellow rock	856/130	3.2	1	–	–	–	–	–	–	–	–	–	–		–	–	–	–	1	0	1
Mount Fleming:																					
sandstone	867/210	3.7	1	1	–	–	–	1	–	–	1	–	1		–	2	2	–	9	1	8
sandstone	867/211	4.7	1	2	–	–	–	–	–	–	1	–	–		3	–	4	–	11	3	8
Dais (Up.Wright Valley):																					
granite	856/125	n.d.	5	5	–	2	–	1	–	–	1	–	3		–	–	2	–	19	3	16
dolerite	856/122	7.3	3	2	–	–	–	–	–	–	–	1	–		–	–	–	–	6	0	6
dolerite	856/123	6.5	4	4	1	–	1	1	2	–	–	1	–		1	–	–	–	15	1	14
Battleship Promontory:																					
dolerite	856/150	5.2	4	2	–	–	1	1	–	–	2	1	–		–	–	2	–	13	0	13
dolerite	867/201	9.2	8	8	–	2	3	3	1	–	1	3	7		–	1	–	–	37	7	30
sandstone	854/147	4.8**	6	6	–	3	1	1	–	–	1	1	–		2	1	5	–	27	2	25
Linnaeus Terrace:																					
sandstone	867/228	3.8	3	8	–	1	1	3	–	1	1	1	14		3	1	1	1	39	17	22
dolerite	867/247	5.7	7	3	–	–	2	2	–	–	1	–	1		–	5	–	–	21	1	20
sandstone	856/131	4.4	5	5	–	1	2	1	–	–	–	1	1		2	–	6	–	24	3	21

Tab. 2: Diversity of microbial morphotypes in various rock types from the McMurdo-Dry Valleys. Enrichments with medium PYGV, pH 7.2; incubation at 9° C at 100–300 lux. Evaluation after 4, 8, 12, 24, and 52 weeks. pH measurements after suspending the ground sample 1:1 (v/v) in 0.1 N KCl for 30 min. * CO–cocci, RD – rods, PR – pointed rods, FB – filamentous bacteria, BB – budding bacteria, PB – prosthecate bacteria, SR – short rods, BR – bent rods, SF – spore formers, IO – iron (or manganese) oxidizers, CF – coryneform bacteria, AM – actinomycetes, CY cyanobacteria, AL – algae, YE – yeasts, FF – filamentous fungi, FL – flagellates (colorless), MT – morphotypes. ** pH measured in (1:1, v/v) double distilled water; 30 min.

Sample no.	Sample location	pH	Inoculum (g)*	No. of different morphtypes BA**	AC	CY	YE	FF	AL	total
856/105	bouldeer II slope of Oliver Peak	5.0	5.64	13	0	5	2	1	0	21
856/106	boulder A, slope of Oliver Peak, exp. north	5.0	4.79	11	0	5	1	0	0	17
856/117	boulder B, top, exp. north-east	4.2	3.07	2	0	0	0	2	1	5
856/118	boulder B, top, exp. north-west	4.3	3.01	1	0	0	0	2	0	3
856/119	boulder C, flat top	5.2	5.26	6	1	1	0	3	2	13
856/131	boulder III, top, exp. north	4.4	5.01	14	1	1	0	6	2	24
856/132	boulder III, exp. east	4.8	3.95	10	0	1	0	3	2	16
856/133	boulder III, exposure north-east	4.8	4.57	14	0	4	0	1	4	23
856/136	boulder III, exposure south	4.8	5.89	14	1	1	2	2	1	21
856/137	boulder III, exp. south-southeast, very hard	5.3	11.36	3	0	0	0	0	0	3
856/155*	boulder D, very hard	4.7	2.68	5	0	0	0	1	1	7
856/162	Campbell Ledge, white, colonization spot, hard	4.7	3.26	0	0	0	1	1	0	2
856/163*	Campbell ledge, colon. spot, rather hard	5.0	5.64	1	1	2	3	4	2	13

Tab. 3: Taxonomic affiliation of microbial morphotypes observed in enrichments which were inoculated with Linnaeus Terrace rock samples. *weight inoculated into 50 ml of medium PYGV; incubation at 9° C and 100–300 lux of incandescent light. **BA – bacteria, AC – actinomycetes, CY – cyanobacteria, YE – yeasts, FF – filamentous fungi, AL – green algae; *these samples were moist.

There was considerable variation in the MT numbers of samples even from one location, such as Linnaeus Terrace. It is thought that the exposure toward the sun may be responsible for these differences (MCKAY & FRIEDMANN 1985). This can be seen best with samples taken from boulder III: here the MT numbers varied from 3 to 24 per sample, with 3 MT's found in the hardest sample from the coldest side (856/137, exposure SSE). Among the Linnaeus Terrace samples were again three which completely lacked photosynthetic organisms.

The MT diversity in sandstone samples collected from Battleship Promontory was much greater than that observed in Linnaeus Terrace samples (Tables 3 and 4). The highest MT numbers were observed in samples 867/206 and 867/202. Both of these also contained the highest number of cyanobacteria morphotypes. There was a tendency of higher pH samples to contain more different MT's. Many of the Battleship Promontory samples were surprisingly rich in cyanobacteria. Next to these, cocci and rod-shaped bacteria were most numerous. Three different samples contained colorless flagellates and/or cysts of these. Two of these samples lacked phototrophic primary producers entirely.

Sample		pH*	Prokaryotic											Eukaryotic				Total		
			CO**	RD PR	FB	BB PB	SR	BR	SF	IO	CF	AM	CY	AL	YE	FF	FL	MT	photo-trophs	hetero-trophs
867/202	white	7.4	10	14	–	2	5	3	–	1	1	3	16	3	–	2	–	60	19	41
867/203	surface pink	5.1	7	8	–	1	3	4	–	–	–	1	5	7	7	6	–	49	12	37
867/204	near dolerite	7.3	5	8	–	3	4	3	3	2	–	2	15	3	1	1	–	50	18	32
867/205	w. black layers	7.0	10	8	1	1	3	1	1	–	1	2	9	3	1	5	1	47	12	32
867/206	grayish	9.3	8	9	1	4	6	2	–	1	–	5	17	6	2	2	3	66	23	43
867/221	reddish	8.5	11	12	–	1	3	2	2	–	1	1	12	2	–	1	–	48	14	34
867/213	olive green	8.9	9	9	2	4	4	2	–	–	–	2	10	1	1	1	–	45	11	34
867/215	greenish	9.2	10	12	2	1	6	3	2	–	–	3	11	2	1	–	1	54	13	41
856/146	from Camp Road	4.8	6	5	–	–	1	1	–	–	1	1	–	2	–	3	–	20	2	18
856/147	moist, Camp Road	4.8	5	5	–	3	1	1	–	–	1	1	–	2	1	5	–	25	2	23
867/232*	5 cm above dolerite	6.6	6	1	–	–	1	–	–	–	–	1	–	–	1	–	–	10	–	10
867/233*	35 cm above dolerite	6.9	3	2	–	–	–	2	1	–	–	–	–	–	–	–	–	8	–	8
867/234*	55 cm above dolerite	5.0	9	9	–	2	3	3	–	–	2	4	4	4	4	3	–	47	8	39
867/235*	90 cm above dolerite	4.7	8	10	1	–	5	2	–	–	1	2	9	4	6	4	–	52	13	39
867/236*	135 cm above dolerite	4.6	3	3	–	1	1	–	–	–	1	2	4	1	3	5	–	24	5	19

Tab. 4: Diversity of morphotypes in sandstone samples from Battleship Promontory. Enrichments: medium PYGV, pH 7.2; incubation at 9° C under dim light (100–300 lux). *pH deterrmination after 30 min suspension of the ground sample in double distilled water. **for abbreviation of MT groups see legend to Table 3. *samples 867/232 to 867/236 were part of a sandstone profile which was investigated more closely.

3.4 *Distribution of Actinobacteria and Actinomycetes in Linnaeus Terrace Sandstone Boulders or Samples*

Variation of the morphotype diversity in samples from different Linnaeus Terrace boulders was also observed with respect to actinomycete distribution (Tab. 5). While sandstone boulder I was rich in actinomycetes and actinobacteria, other boulders had only 1—2 different morphotypes of this group. Most wide-spread was "Mycococcus I" (MT 56), which was found in all rock samples studied so far. These actinomycetes resembled micrococci with often multiple and irregular cross wall formation (KRASIL'NIKOV 1938). Actinomycetes resembling *Geodermatophilus* spp. were only present in one of the samples; they typically formed black and crumbling colonies. Similar forms have been isolated from the surface of rocks in the Sonoran desert (USA) by D. E. CALDWELL (pers. comm.).

Morphotype Genus*	MT	Boulders or sample numbers: boulder I	boulder II	boulder III	boulder A	845/207	845/210
Corynebacterium P	81	+	+	+	–	–	–
Corynebacterium L	129	+	–	–	–	–	–
Mycococcus I**	56	+	+	+	+	+	+
Mycococcus II	116	+	+	–	–	–	–
Mycobacterium sp.	24	+	–	+	–	–	–
Bifidobacterium sp.	109	+	–	–	–	–	–
Nocardia/Amycolata	51	+	+	+	–	–	–
Streptomyces I	53	–	–	–	+	–	–
Streptomyces II	128	+	–	–	–	–	–
Micromonospora sp.	83	–	–	+	+	–	–
Blastococcus sp.	104	+	–	+	–	–	–
Geodermatophilus	106	–	–	–	–	–	+

Tab. 5: Presence of actinobacteria and actinomycetes in various Linnaeus Terrace rock boulders or samples.*identification preliminary. **genus Mycococcus sensu Krassil'nikov 19.

Property	Mycobac-terium		Nocardia		Micromo-nospora		Blastococcus					Bifido-bacter.	Streptomyces		
	458	379	101	319	459	321	682	802	826	803	542	702	300	632	348
Substrate mycelium	(+)	(+)	+	+	+	+	(+)	(+)	(+)	(+)	(+)	(+)	+	(+)	+
Aerial mycelium	–	–	(+)	+	+	+	–	–	–	–	–	–	+	–	+
Pigmentation*	yel	yel	–	por	yel	por	–	–	–	–	yel	ppi	–	yel	–
Degradation of:															
adenine	–	–	+	+	+	–	–	–	–	–	–	–	+	+	+
hypoxanthin	–	–	+	–	+	+	–	–	–	–	–	–	+	+	+
xanthin	–	–	+	+	+	+	–	–	–	–	–	–	+	+	+
tyrosin	–	–	+	+	+	+	–	–	–	–	–	–	+	+	–
xylan	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Hydrolysis of:															
casein	–	–	+	+	+	+	–	–	–	–	–	–	+	+	+
esculin	+	–	+	+	+	+	–	–	–	+	+	+	+	+	+
arbutin	+	–	+	+	+	+	–	–	+	–	+	+	+	+	+
Sialidase activity	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–
Salt tolerance (%NaCl):															
1 – 3 %	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4 %	n.t.**	–	n.t.	n.t.	+	+	+	+	+	(+)	+	n.t.	+	+	+
5 %	n.t.	–	n.t.	n.t.	+	–	+	+	+	–	+	n.t.	+	+	–
10 %	n.t.	–	n.t.	n.t.	–	–	–	–	–	–	+	n.t.	–	–	–

Tab. 6: Some morphological and physiological properties of Antarctic actinomycete isolates from the Dry Valleys. *yel = yellow, por = pale orange, ppi = pale pink; **n.t. = not tested.

3.5 *Characterization of Selected Pure Cultures*

All 1500 isolates grew well at 9° C, and a large proportion grew equally well at 4° C, with temperature maxima well below 20—25° C (SIEBERT & HIRSCH 1988). Eleven coccal isolates from 1979/80 and 1984/85 rock samples have already been characterized and identified to at least the genus level (SIEBERT & HIRSCH 1988). Among these were *Micrococcus agilis* (one strain), *Deinococcus* sp. (one strain), *Arthrobacter simplex/tumescens* group (4 strains) and *Brevibacterium linens* (5 strains).

The properties of 15 actinomycete isolates are shown in Table 6. The proposed genus names were confirmed by cell wall analyses carried out by one of us (U. MEVS). The presence of sialidase activity in one of the *Streptomyces* strains was most remarkable since sialidase (neuraminidase) activity is rare among bacteria (NEES et al. 1975, SCHAUER 1975).

Characterization of Antarctic endolithic rock microorganisms has also been aided by fatty acid analyses. Micro-

Fatty acid	*Blastobacter* spp.				*Caulobacter* spp.			*Hyphom.*	*Amycolata* spp.	
	52	96	734	797	54	68	74	444	101	102
i 14:0	–	1.2	1.4	0.9	–	–	–	–	3.4	4.4
n 14:0	0.1	2.2	0.8	2.2	2.9	3.1	2.1	2.0	1.3	2.0
n 14:1	–	1.3	–	–	–	–	–	–	21.5	15.2
i 15:0	–	–	6.9	6.5	–	–	–	14.8	–	–
ai 15:0	–	–	–	–	–	–	0.6	–	–	–
n 15.0	–	1.8	0.6	6.0	1.0	1.0	0.6	–	n.t.	0.7
i 16:0	–	–	10.4	12.0	–	–	0.6	–	12.9	12.4
ai 16:0	–	–	–	–	0.9	1.0	–	–	–	–
n 16:0	2.8	5.3	4.0	9.2	7.4	7.1	6.5	8.9	11.4	16.9
n 16:1	0.5	34.1	11.1	8.0	22.4	21.1	31.2	46.9	28.2	28.6
ai 17:0	–	–	0.3	–	–	–	–	–	3.9	3.3
n 17:0	–	–	2.2	2.1	–	–	–	–	3.3	3.7
cy 17:0	–	–	15.5	11.9	2.2	2.3	1.6	–	–	–
n 18:0	3.6	2.6	2.2	1.6	1.5	1.3	–	1.7	0.8	0.8
n 18:1	75.5	42.3	42.5	31.3	52.6	52.9	53.3	3.6	0.8	1.4
n 19:0	5.6	–	–	–	–	–	–	–	–	–
cy 19:0	8.9	4.3	1.4	5.3	–	–	–	4.8	–	–
n 20:0	–	–	–	–	–	–	–	–	–	–
n 20:1	–	–	–	2.9	1.6	1.4	–	–	–	–
n 21:0	–	–	–	–	3.6	3.6	–	–	–	–
X–1	0.3	5.0	0.4	–	1.0	1.0	3.9	5.0	4.1	5.7
X–2	1.7	–	0.3	–	2.9	3.4	1.0	1.7	7.2	3.7
X–3	–	–	–	–	–	–	–	1.0	1.0	1.0
X–4	–	–	–	–	–	–	–	4.1	–	–

Tab. 7: Fatty acid composition (%) of some selected Antarctic bacterial isolates. The fatty acids were determined as methyl esters. Designation by total number of C atoms; after colon: number of double bonds. Prefixes i and ai refer to iso and anteiso branching; n = normal (straight chain), cy = cyclopropan ring within the molecule. X–1 to X–4 are unidentified components. *Hyphom.* = *Hyphomicrobium*-like; n.t. = not tested.

organisms growing at low temperatures would be expected to have , in their membranes, short chain and unsaturated fatty acids (BROCK et al. 1984). A comparison of the fatty acid methyl ester spectra revealed in many cases similarities between individual strains. The data collected by one of us (M. SITTIG) and presented in Table 7 indicate that there exist, generally, two different ways among Antarctic endolithic bacteria to maintain cell membrane fluidity: in the first group (one *Hyphomicrobium* and two *Nocardia* or *Amycolata* strains) the main components were shorter-chain fatty acids, especially straight chain n16:0 and n16:1. The second group (4 strains resembling *Blastobacter* spp. and 3 *Caulobacter* isolates) had n18:1 as the most prominent fatty acid. Within these groups there existed considerable similarities of the fatty acid spectra.

4. DISCUSSION

The effects of dry ice (CO_2) on morphotype diversity in our samples were rather unexpected, although stimulation of algal growth by increased pCO_2 in the atmosphere is common knowledge (GOLDMANN 1973).
Growth stimulation of filamentous fungi by CO_2 has also been described previously (KRITZMAN et al. 1977). However, our observations also indicated that the treatment of frozen samples for 16 h with dry ice must have actually decreased the viability of some of the endoliths that were initially present in the samples (e. g., filamentous fungus 1, bacterium II). Perhaps the poorly buffered sandstone rock became too acid for these organisms by the dry ice treatment. In any case, future ecological studies on the presence and numbers of viable microorganisms should not be made with dry-ice treated samples.

In the present investigation the microbial populations within these Antarctic rock samples were not characterized by standard counting procedures, but rather by determining types and numbers of morphotypes. The advantage of this procedure is, that it is less dependent of the rock volume to be studied as long as the sample is large enough. Preliminary studies have shown that an increase in sample size above 5 g did not result in higher MT numbers. This held true especially for samples with low numbers of morphotypes.

Cryptoendolithic growth in these rocks occurs in layers of variying depths, but the layers are often discontinuous and growth is patchy due to exfoliate weathering (FRIEDMANN 1982). All quantitative determination methods to describe the amount of biomass or total cell numbers suffer from this problem: ATP-, lipid-, muramic acid- or chlorophyll determinations vary in wide ranges in one rock sample.

Using morphotype diversity is also advantageous for another reason. The extent of MT diversity can be taken as an indication for the condition under which the ecosystem's population exists. An old, established, "normal" and adapted population would be expected to have a high MT diversity with rather low numbers of individuals, while a population that freshly colonizes a rock surface and consequently lives under stress would consist of much fewer species that can tolerate the prevailing hardships. However, these few colonizers may occur in relatively high cell numbers (HIRSCH & HOFFMANN 1988). Some of the samples investigated here were extremely hard. They came from colder, less sun-exposed sites and thus contained only a few morphotypes. Here it can not be decided if these few endolithic organisms were primary colonizers or remnants of a formerly more numerous and diverse population.

Several of the samples were found to lack photosynthetic primary producers. Here the question of their carbon and energy sources needs to be raised. So far we have no direct indication of the presence of chemolithoautotrophic bacteria. But the possibility exists that isolates of *Nocardia / Amycolata* spp. might be facultatively autotrophic. Such strains have been cultured on purely mineral salts media, with all care to omit possible organic contaminants from the culture vessel, medium or plug (P. HIRSCH, unpubl.). Members of this actinomycete group are known to be able to grow chemolithoautotrophically on hydrogen/oxygen/CO_2 or on $CO/O_2/CO_2$ (HIRSCH 1961, G. JOCHENS Ph D. thesis Kiel 1979). Another explanation for growth in the absence of photosynthetic organisms could be that some of the sandstone samples could have contained organic carbon from the time of their deposition or from previous colonization periods.

The higher numbers of morphotypes found in Battleship Promontory samples as compared to Linnaeus Terrace or other sampling sites could be explained by differences in local climate. Preliminary observations point to the fact that the Battleship Promontory site is wetter and it appears to be more wind-shielded. Also, these rocks may

have a different composition. Future work will have to clarify this point. Another possibility would be a higher rate of primary production in Battleship Promontory rocks. This could result in higher concentrations of organic carbon. Finally, diversity of phototrophs in Battleship rocks was found to be comparatively higher, which could lead to a larger spectrum of available carbon and energy sources. All of these possibilities would almost certainly result in an increase of MT diversity in these populations. DAWID et al. (1988) reported on the observation of myxobacteria in Battleship Promontory soil samples. The presence of these and other potential predators (colorless flagellates!) in Battleship samples could be further indications of an increased organic matter availability.

Finally, the question may be asked if the microorganisms discussed here were members of an indigenous microflora or if they could be considered to be recent contaminations. A number of observations indicate their indigenous origin: (1) Good growth at temperatures as low as +4° C to +9° C and the inability to grow above 30° C, in many cases even the failure to grow above 25° C (SIEBERT & HIRSCH 1988) indicates a psychrotrophic nature of many of the isolates.

This is supported by the observation of short chain and/or unsaturated fatty acids in their lipids. Similar observations were reported for fatty acids of *Mycococcus* spp. and *Micrococcus* spp. isolated from lithophilic lichens of alpine regions of Pamir (PINEVICH & ASEEVA 1972). Although psychrotrophs (and even psychrophiles) can be found in many locations, their predominance among the strains tested was remarkable.

(2) Most isolates were obtained (and grew well) under oligotrophic conditions, i. e., in the presence of only very low concentrations of nutrients (SIEBERT & HIRSCH 1988, HIRSCH & HOFFMANN 1988). Recent contaminants originating from humans or birds (the only possible vectors with access to the locations studied here) would be expected to prefer higher nutrient levels. Contamination through airplanes is unlikely but would need to be investigated.

(3) A high proportion of those strains tested were capable of growing with low concentrations of simple carbon compounds as would be excreted by primary producing phototrophs. Contaminations resulting from human or bird interference would again be expected to be more demanding and/or specialized with respect to the type and concentration of carbon source.

(4) Conversely, a number of soil isolates from Linnaeus Terrace are considered to be recent contaminants shed by humans during urination (GALLIKOWSKI & HIRSCH 1988). These latter isolates had much higher temperature maxima, they grew well at higher nutrient concentrations, and they were mostly unpigmented. Several of these "contaminants" were spore-formers. Such organisms could not be found in other, undisturbed soil samples from Linnaeus Terrace.

Note added in proof

Recent and more detailed investigations have revealed affiliation of some actinomycete strains to different genera (Tables 6 and 7): strains 458, 379, 542 and 632 ar *Arthrobacter* spp., strains 101, 102, 319, 300 and 348 are *Streptomyces* spp., strains 682, 802, 826, 803 and 702 are *Geodermatophilus* spp., strains 319, 459 and 321 are *Micromonospora* spp.

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