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1.4 Preliminary Characterization and Identification of 1984/85 Continental Antarctic Soil Microorganisms of Linnaeus Terrace (Altitude 1600 m; McMurdo Dry Valleys)

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Summary: During the 1984/1985 Antarctic summer we isolated 229 pure cultures of oligotrophic microorganisms from 13 Linnaeus Terrace soils (1600 m altitude, Asgard Range; South Victoria Land). Among these were heterotrophic bacteria, yeasts and filamentous fungi as well as phototrophic green algae. Some of the soil samples yielded up to 36 different morphotypes, others were nearly sterile. Among the bacteria were cocci, short rods, rods, vibrios, club-shaped coryneforms, spore-formers, a few prosthecate organisms, and actinomycetes. Extracellular polymer formation was common among these isolates. About one-half of the strains were studied biochemically in greater detail. For this we employed especially miniaturized techniques, as for example the microtiter plates. 98 of 144 bacteria tested were Gram-positive. Most isolates grew at 4°C, and 70% were capable of growth at 30°C, which classifies them only as psychotrophic. Oligocarbophilic growth on mineral salts medium was observed with 57%, growth without added nitrogen sources with 47%. About half of the test strains precipitated iron oxides, but manganese oxidation or reduction was carried out by only a few strains. One-third of all cultures hydrolyzed starch, degraded DNA, or tolerated 10% NaCl. About 25% of the strains cleaved Na-protocatechuate after induction with Na-hydroxybenzoate. Preliminary identification was possible in many cases. Apart from members of the genera *Micrococcus, Deinococcus, Corynebacterium, Arthrobacter,* or *Brevibacterium* there were also *Bacillus* and *Pseudomonas* spp. Except for a few strains from a urine-contaminated site with good growth at 30° or even 37°C, most strains appeared to be indigenous to this hostile environment.

Zusammenfassung: Aus 13 Bodenproben, die im Südsommer 1984/85 auf Linnaeus Terrace (1600 m hoch gelegen; Asgard Range, Süd Victoria Land) gesammelt wurden, isolierten wir 229 Reinkulturen oligotropher Mikroorganismen. Darunter befanden sich heterotrophe Bakterien, Hefen, fädige Pilze und auch Grünalgen. Während aus einigen Bodenproben bis zu 36 verschiedene Morphotypen isoliert werden konnten, erwiesen sich andere als nahezu steril. Bei den Bakterien handelte es sich um Kokken, Kurzstäbchen, Stäbchen, Vibrionen, Sporenbildner, Coryneforme, einige prosthekate Bakterien sowie Actinomyceten. Viele dieser Isolate bildeten extrazelluläre Polymere. Etwa die Hälfte dieser Stämme untersuchten wir genauer hinsichtlich ihrer physiologischen Eigenschaften in einem miniaturisierten Testsystem (Mikrotiterplatten). 98 von 144 Bakterien erwiesen sich als Grampositiv. Die meisten der Isolate wuchsen bei 4°C, und 70% von ihnen waren psychrotroph, da sie sowohl bei 4°C als auch bei 30° Wachstum zeigten. 57% wuchsen oligocarbophil auf Mineralsalzmedium 337 ohne zugesetzte C-Quellen, 47% wuchsen ohne zugegebene Stickstoffquelle. Ungefähr die Hälfte der untersuchten Stämme konnte Eisenoxide präzipitieren, während Manganoxidation oder -reduktion nur bei wenigen Isolaten nachgewiesen werden konnten. Ein Drittel der Kulturen hydrolysierte Stärke oder baute DNA ab, oder tolerierte bis zu 10% NaCl. Ungefähr 25% der isolate spalteten Na-protocatechuat nach Induktion mit Na-Hydroxybenzoat. In vielen Fällen war eine vorläufige Identifizierung möglich; neben Gattungen wie *Micrococcus, Deinococcus, Corynebacterium, Arthrobacter* oder *Brevibacterium* traten auch *Bacillus* und *Pseudomonas* spp. auf. Abgesehen von einigen Stämmen, die von einer mit Urin kontaminierten Stelle kamen und gutes Wachstum bei 30°—37°C zeigten, schienen die meisten Stämme in diese feindliche Umwelt zu gehören.

1. INTRODUCTION

Linnaeus Terrace is located in the continental Antarctic McMurdo Dry Valley region ("Ross Desert"), an area which is one of the harshest environments on earth (VISHNIAC & MAINZER 1973). This region is practically devoid of all higher terrestrial life forms, except for occasional overflights of skuas (VISHNIAC & HEMPFLING 1979). Some authors such as HOROWITZ et al. (1972) considered the Dry Valleys (especially the soils) to be "abiotic". But closer investigations of some valley soils in this region (BENOIT & HALL 1970, BOYD 1967, CAMERON 1971) showed that nearly all samples investigated there contained numerous types of microorganisms, some of which could be identified.

Taxonomic work has been performed on Antarctic filamentous fungi and yeasts (ATLAS et al. 1978, SUGIYAMA 1969, VISHNIAC & HEMPFLING 1979), on algae (TSCHERMAK-WOESS & FRIEDMANN 1984), and on actinomycetes (TSYGANOV et al. 1970). Among the bacteria the taxonomic work concentrated mainly on *Bacillus* spp. and coryneform bacteria (JOHNSON & BELLINOFF 1978, MADDEN et al. 1978). In a detailed study of stressed Antarctic soils from several McMurdo Dry Valley sites, GALLIKOWSKI (1985) and GALLIKOWSKI & HIRSCH (1989) observed high microbial diversity in nine samples. It was possible to isolate 86 strains, of these there were 68 bacteria, 3 yeasts and 15 filamentous fungi. The question needs to be asked if these microorganisms were indigenous to Antarctic soils or contaminants that arrived recently from elsewhere. They could have been transferred from weathering rocks, where a rich cryptoendolithic microbiota has been observed

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(FRIEDMANN 1982). They could also be airborne "contaminants" which have simply survived in these soils. UYDESS & VISHNIAC (1976) demonstrated in situ growth of bacteria in some Antarctic valley soils which indicated that at least some of the viable forms were indigenous. Similar conclusions were reached by VISHNIAC & HEMPFLING (1979) in their ecophysiological studies with Antarctic yeasts (*Cryptococcus* spp., *Sporobolomyces* spp., and *Tilletiopsis* spp.). Recently, more than 200 pure cultures of soil microorganisms were isolated from Linnaeus Terrace 1984/85 samples (HIRSCH et al. 1985). The purpose of this present ivestigation was to characterize and possibly identify several of these recent isolates in order to eventually compare them to other soil isolates from more favourable locations and especially to organisms from the cryptoendolithic ecosystem. It could be expected, indeed, that several of the organisms from soil resembled those from rocks, which would indicate that there does exist an indigenous soil microflora in these exposed locations of the McMurdo Dry Valleys.

2. MATERIALS AND METHODS

Linnaeus Terrace is located in the Asgard Range (South Victoria Land; 77°63'S, 161° 0.5'E; 1600—1650 m altitude). The Beacon Sandstone boulders there are particularly rich in cryptoendolithic life (FRIEDMANN 1982). In December of 1984 a total of 13 soil samples were taken aseptically (Table 1, HIRSCH et al. 1985) and transported to Kiel over dry ice. The samples came from seemingly undisturbed areas, as well as from a dry surface depression which was heavily contaminated with human urine ("Kidney Pond"). The samples' pH ranged from 5.2 to 6.7 (Table 1). We employed several enrichment methods (HIRSCH et al. 1985) in order to obtain as

Sample	Location	pH[1]	Number of pure cultures observed[2]
845/201	„Kidney Pond", site A, 0–2 cm	5.9	18
202	„ „ 2–5 cm	6.2	13
203	„ site B, 0–2 cm	6.5	11
204	„ „ 2–5 cm	6.7	21
205	Stream bed of snow melt water	6.6	10
213	Powder inside of sphere	6.7	12
224	North of boulder I	6.4	29
225	Northeast of boulder I	6.3	25
226	Under east end of boulder I	6.4	17
227	South of boulder I	6.3	36
228	Under west end of boulder I	6.3	4
246	Under boulder III, 0–5 cm	5.2	11
247	„ 5–7 cm	5.6	12
			total: 229

Tab. 1: Pure cultures isolated from 1984/85 Linnaeus Terrace soil samples. 1) measured in 1.0 N KCl for 2 hours, 2) Pure cultures of a given sample were all morphologically different.

many different pure cultures as possible. For closer description and identification we selected 184 strains of bacteria and yeasts showing good growth. We tested these strains for a great number of morphological and physiological properties with the microtiter plate technique, a miniaturized test system (DOTT & THOFERN 1980, MOALEDJ 1984). All experiments were carried out in triplicate. The standard medium was PYGV (STALEY 1968), which contained 0.25 g/l each of peptone, yeast extract, and glucose as well as mineral salts and some vitamines. Incubation was normally at 9°C in the dark for 4 weeks. For anaerobic cultivation we sealed the wells of the microtiter plates with paraffin (1 part solid plus 5 parts liquid) and incubated in a nitrogen atmosphere. The tests carried out were mostly done as described by MOALEDJ (1984), except that medium PYGV was used as the basal medium. Growth on basal inorganic medium and the test for ring cleavage of protocatechuic acid were as described by GERHARDT et al. (1981). Manganese oxidation was tested for according to SCHWEISFURTH (1972). Base composition (Mol% G+C) was determined after MANDEL & MARMUR (1968). The strains were also tested for sialidase activity. This was done according to POTIER et al. (1979).

3. RESULTS

From the 13 soil samples we could isolate a total of 229 different pure cultures of heterotrophic bacteria, yeasts,

filamentous fungi, and green algae. But the numbers and diversity of microorganisms varied in these samples. While enrichments from one soil yielded only four different organisms, other soil samples with for example 36 morphotypes showed a significantly higher diversity (Table 1).

The cell morphology of the bacterial isolates ranged from cocci to short rods, vibrios, and rod-shaped forms (Figs. 1 and 2). Appendaged or budding bacteria were rarely found among the isolates, but actinomycetes resembling *Geodermatophilus* spp. were quite common. Many strains had club-shaped cells and showed "snapping division" and thus were similar to coryneforms. Twelve strains formed endospores; one of these bacilli had short rods or cocci (Fig. 2.1). Many bacteria produced extracellular polymers, some forming large aggregations surrounded by slime capsules (Fig. 2.4). 98 of 144 bacteria tested (68.1%) were Gram-positive.

The effect of temperature on growth in PYGV was tested with 146 soil bacteria and yeasts (Table 2). Nearly all strains (86.7%) grew well at 9°C; there were thick pellets in the wells of these organisms. The remaining 13.3% showed only weak growth; there were small pellets or slight turbidity in the wells of the test plates. While the majority of isolates grew at 4°C, a few grew better at 20°C, 59% developed well at 30°C, and about 20% grew well even at 37°C.

Growth reponse	4°C	9°C	20°C	25°C	30°C	37°C	43°C
Good growth[1])	73.7	86.7	89.6	65.2	59.0	19.3	2.7
Weak growth[2])	11.2	13.3	3.3	11.0	10.9	16.0	9.3
No growth	15.1	0	7.1	23.8	30.1	64.7	88.0

Tab. 2: Effect of temperature on growth of 146 Antarctic soil bacteria and yeats in medium PYGV. Incubation: 4 weeks. Data expressed as percentage of total number of strains. [1]) distinct turbidity and/or pellet visible, [2]) turbidity and/or pellet barely visible

Growth on five different media is shown for 146 strains in Table 3. As could be expected, all strains grew on PYGV, the isolation medium. A basal inorganic medium (BIM) without any added organic carbon sources supported growth of 56.9% of the strains. As this difference to PYGV could have been caused by the missing substrates or by a specific effect of the basal salts of BIM, we also tested BIM with the addition of PYGV substrates except for Hutner's basal salts. The result was that 9.3% of the organisms were inhibited by the BIM mineral salts.

Growth reponse	PYGV[1])	BIM[2])	BPYGV[3])	NTM[4])	NTM[4]) +0.1%$(NH_4)_2SO_4$
good growth	86.7	37.3	85.4	13.3	61.3
weak growth	13.3	19.6	5.3	33.3	19.3
no growth	0	43.1	9.3	53.3	19.3

Tab 3: Effects of various media on the growth of 146 selected Antarctic soil bacteria and yeasts. Data expressed as percentage of total number of strains investigated.

1) PYGV (isolation medium): peptone (0.025%), yeast extract (0.025%), glucose (0.025%), vitamin solution, basal salts; 2) BIM: basal inorganic medium; 3) BPYGV: basal inorganic medium + PYGV (without Hutner's basal salts); 4) NTM: Nitrogen test medium (Rosswall & Persson 1982).

To investigate the utilization of $(NH_4)_2SO_4$ as a nitrogen source, we compared N-free test medium with and without ammonium sulfate (Table 3). Surprisingly, many strains (46.6%) grew without a nitrogen source (i. e. oligonitrophilically). It was unlikely that the inoculum contained much nitrogen. Dinitrogen fixation by these isolates still remains to be tested.

Table 4 shows some biochemical properties of the test strains. Nitrate reduction under aerobic conditions was carried out by only 17 strains (formation of nitrite). All isolates had been enriched for under aerobic conditions; therefore, the high percentage of catalase-positive strains could be expected. Only 7 cultures were cytochrome oxidase-positive. While manganese oxidation was carried out by only 5 out of 151 strains (in the presence of organic carbon), iron precipitation was a more common property. Manganese reduction was also tested with 5 g/l of glucose and 0.75 g/l of meat extract as carbon, energy and nitrogen sources. One-fifth of all strains reduced

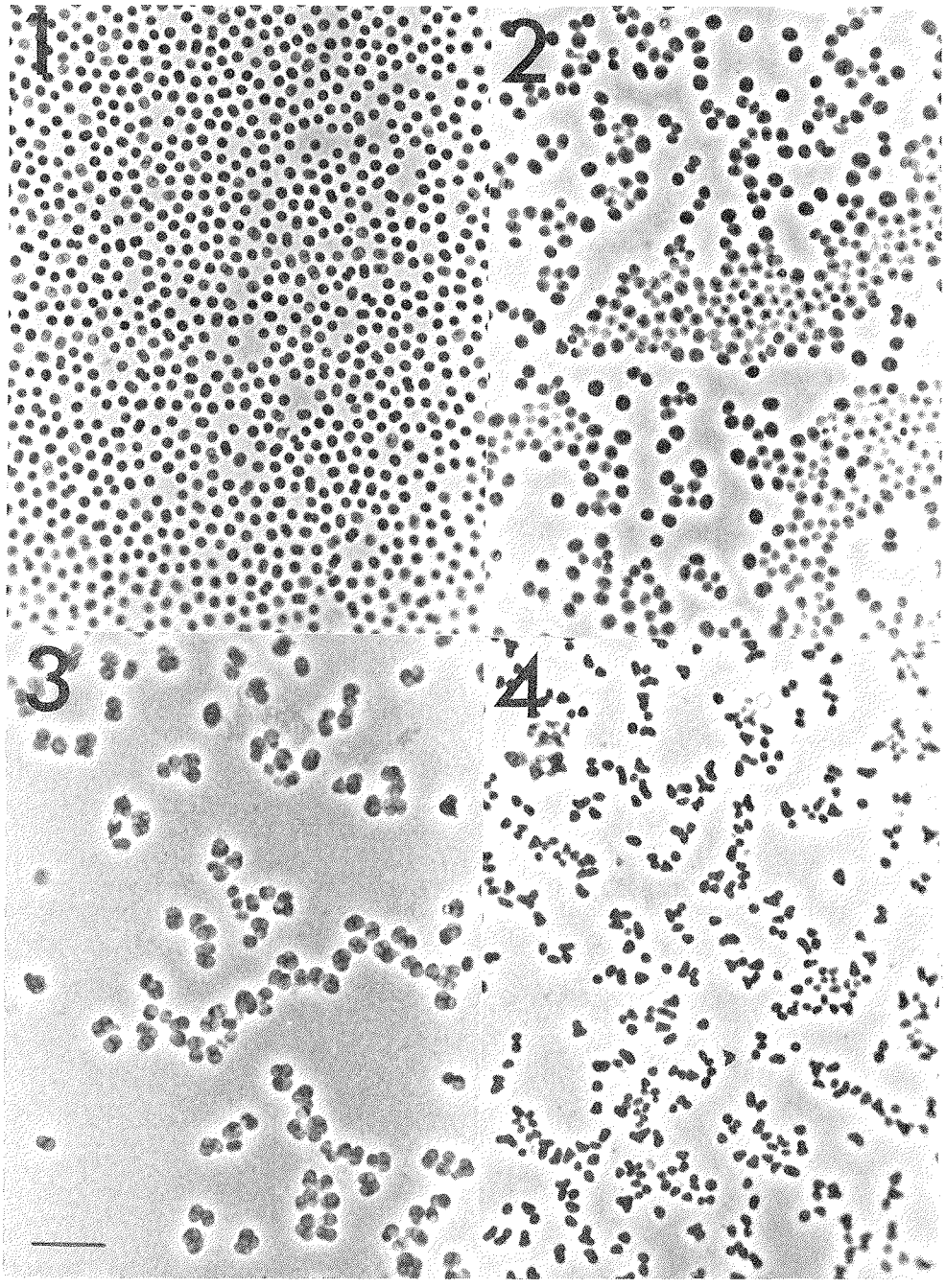

Fig. 1: Morphology of selected Antarctic soil isolates.
1) *Deinococcus* sp. (AA-829) from sample 845/224. 2) Large coccus (AA-758) from 845/227 (uncontaminated). 3) "Mycococcus" (AA-616) from 845/201 (Kidney Pond). 4) Coryneform bacterium (AA-616) from 845/227. Scale bar 10 microns.

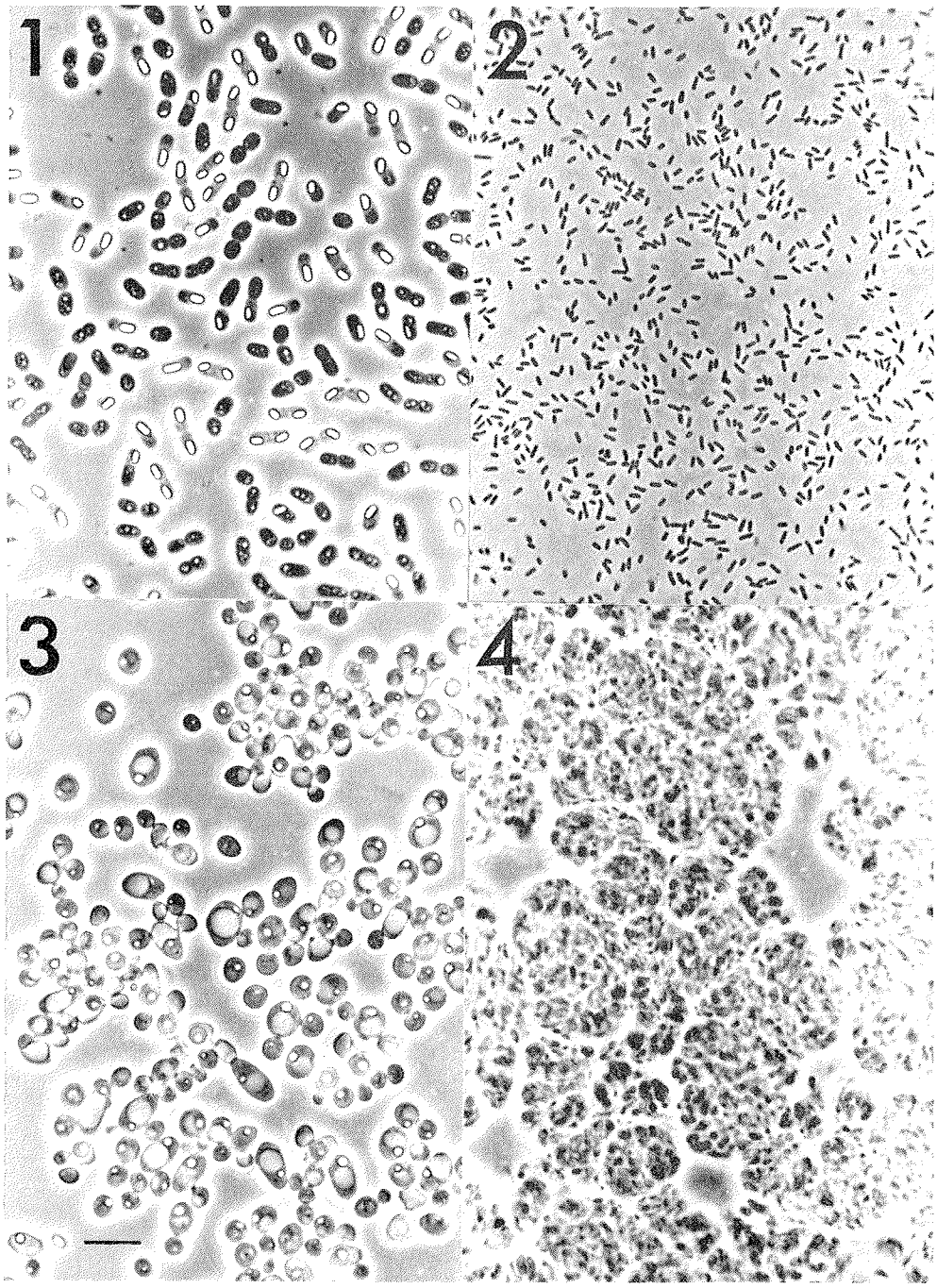

Fig. 2: Morphology of selected Antarctic soil isolates.
1) *Bacillus* sp. (AA-650) from 845/204 (Kidney Pond. 2) Small yellow rod (AA-679) from 845/213. 3) Yeast (AA 756) from 845/227 (uncontaminated).
4) Pink, aggregating rod in copious polymer masses, spreading on agar surfaces (AA-609) from 845/201 (Kidney Pond). Scale bar 10 micron.

manganese oxide.

Decomposition of selected carbohydrates was investigated with 90 strains of bacteria and yeasts under aerobic and anaerobic conditions (Table 5) according to HUGH & LEIFSON (1953) as modified by MOALEDJ (1984). Oxidative or fermentative decomposition (formation of acid) were evaluated every 2—3 days for 4 weeks for a

Charakteristic	Number of strains tested	Positive	Negetive
Catalase	154	135	19
Cytochrome oxidase	148	7	141
Nitrate reduction	124	17	107
Manganese oxidation	151	5	146
Manganese reduction	150	36	114
Iron precipitation	148	72	76

Tab. 4: Some selected biochemikal properties of Antarctic soil bacteria and yeasts.

color change of the added indicator. Aerobically, glucose, mannose or galactose utilization led to acid formation in only a few strains. Anaerobically, the fermentation of glucose was more widespread among the test strains (Table 5). All other sugars were less well metabolized.

Hydrolysis of starch was tested with 148 cultures, of which 32% had extracellular amylase. Other extracellular enzymes active in some strains were DNase (present in 34% of the isolates) and sialidase, which separates neuraminic acid from oligosaccharides, glycolipids or glycopeptides. The latter enzyme was tested for with 103 strains according to POTIER et al. (1979). Among the 14 sialidase-positive organisms were four isolates from the urine-contaminated "Kidney Pond" and six yellow, rod-shaped bacteria from various other locations.

The ability to cleave the aromatic ring of Na-protocatechuate was tested with 107 strains after induction with 0.1% Na-p-hydroxybenzoate. Ortho-cleavage was found in 8 cases, and cleavage in the meta-position occurred in 16 strains.

Substrate[1])	Aerobically			Anaerobically		
	acid formation	weak acid formation	no acid formation	acid formation	weak acid formation	no acid formation
Glucose	14	19	57	57	19	14
Mannose	18	6	66	4	6	80
Galactose	13	5	72	11	4	75
Ribose	5	8	77	5	2	83
Maltose	4	7	79	3	3	84
Saccharose	4	2	84	5	1	84
Fructose	0	1	89	0	4	86
N-acetyl-glucosamine	0	1	89	0	0	90

Tab. 5: Acid formation from selected sugars by 90 Antarctic soil bacteria and yeasts. Data expressed as number of strains in each case. [1]) sterile-filtered sugar solutions were added to an 0.05% casein hydrolysate medium to a final concentration of 0.1%

Growth response	NaCl addition to medium PYGV			
	0 %	1 %	5 %	10 %
good growth	86.7	80.7	66.7	21.3
weak growth	13.3	8.0	21.3	16.7
no growth	0	11.3	12.0	62.0

Tab. 6: Effect of sodium chloride on growth of 146 Antarctic soil bacteria and yeasts. Data expressed as percentage of total number of strains investigated.

The tolerance of 146 strains to increasing concentrations of NaCl was also investigated with medium PYGV at 9°C (Table 6). All strains grew without NaCl; the addition to PYGV of 5% NaCl still allowed growth of 88% of the isolates tested. Even 10% NaCl were tolerated by more than one-third of all cultures.

4. DISCUSSION

The miniaturized test system employed here enabled us to study many physiological properties of a large number of strains with a relatively small amount of material and within a short time. For most of the strains this method worked well. Strains which grew extremely slowly (i. e. those which needed more than two months to form visible colonies on agar) or cultures which grew faster and spread over a larger surface area (filamentous fungi, some bacilli) could not be tested together with other (normal) strains on the same microtiter plate. Generally the necessity of having all strains to be tested in one plate (96 cultures) at the same state of activity, caused problems. Therefore, we often had to exclude strains entirely because they did not grow in the positive contrals, i. e. on the PYGV medium.

Another problem was that some bacteria with extensive slime production did not suspend in liquid media but instead formed a viscous pellet. Also, onoculation by an atuomatic needle device was not always successful with these strains so that often only one of the parallels grew up. Such tests had to then be repeated. The high percentage of Gram-positive bacteria in Antarctic soils has been described by FLINT & STOUT (1960) and by GALLI-KOWSKI (1985). Gram-postive strains seemed to be better adapted to the dry and cold environment of the McMurdo Dry Valleys. Survival in a periodically dry soil would also be facilitated by the formation of copious amounts of extracellular polymer, which we so frequently observed in our cultures. The thick polymer layers could even protect the cells form an acidic environment. In enrichments it could be observed that other organisms (bacteria) grew within polymer capsules. Close proximity of cells increases the possibility of exchanging excretion products, of utilizing local dead cell material, or of taking advantage of partially degraded organic substrates. The extracellular polymer function could thus be looked upon as a means of furthering mutual relationships between organisms which are strongly dependent on each other in this hostile environment.

The growth response to various temperatures was somewhat unexpected: most cultures grew equally well at 9°C. SIEBERT (1986) and SIEBERT & HIRSCH (1988) determined the temperature optimum for 15 Antarctic cocci: it ranged from 10° to 21°C. STRAKA & STOKES (1960) found for most of their Antarctic soil bacteria isolates a temperature optimum of about 20°C. If one follows the definition of MORITA (1985), a psychrophilic organism should have its optimum below 15°C, which was not the case with most of our strains. Rather, these should be classified as "psychrotrophic" since their growth was possible near or slightly above 20°C as well as around 0°C.

The observed growth of 28 strains (19.3%) at 37°C indicated that possibly there were human contaminants among our strains. A few of these 37°C cultures actually came from the "Kidney Pond" and others were spore-forming bacilli. It is possible that those strains which did not grow at 4°C (15.1%) could have been such contaminants as well. Alternatively, the incubation time might have been too short for these organisms.

When we tested growth with different media, we included BIM, a mineral salts basal medium without added carbon sources. More than half of the Antarctic soil organisms grew on this medium, which can be taken as an indication for "oligocarbophily" (BEIJERINCK & VAN DELDEN 1903). Most likely, the carbon and energy sources came from impurities of the laboratory air (HIRSCH 1958, 1965). As we also observed a capability for "oligonitrophilic" growth, it could be assumed that within the Antarctic soil there could occur a nutrient exchange through volatile compounds released into the soil gas phase.

In the Ross Desert the frequently changing water regime often causes the formation of salt accumulations and crusts (BEHLING 1971, CAMERON et al. 1970). Soil microorganisms would have to develop a certain degree of tolerance of higher salt concentrations. We tested 146 soil isolates for growth in the presence of various concentrations of NaCl (Table 6), although it was not clear if the salt crusts we observed on Linnaeus terrace were sulfates or chlorides. A total of 88% of all test strains tolerated 5% NaCl, and 21.3% still grew well in 10% NaCl. Halotolerant cocci seem to be quite common in the McMurdo Dry Valley soils; there are reports by MILLER & LESCHINE (1984) and the work by SIEBERT (1986) where halotolerance was mentioned.

The diversity of carbon sources in Antarctic soils is still largely unknown, exept for the presence of living or dead microbial cells. CAMERON (1972) found a wide range of organic carbon contents in Victoria Valley soils: 0.01—0.21 weight%. This 20-fold variation expressed the vast differences between adjacent locations, differences in microclimate, in microbial contens, and hence in microbial production. Such differences also were observed

by us when we studied the density and morphotypes of microorganisms in soil samples by direct microscopy methods (HIRSCH & GALLIKOWSKI, unpubl.). The main sources for organic carbon, therefore, would change from one place to another; the microbial soil population would have to be diverse with respect to utilizing specific compounds.

Several soil isolates were able to hydrolyze starch or degrade DNA, and a variety of sugars were utilized. Moreover, the ability to cleave aromatic ring systems was present in the populations we investigated. This shows that microbial matter can be recycled to a considerable extent. The occasional presence of viable green algae in Antarctic soil samples points to the possibility of a limited primary production, in soil, by photosynthesis. This point needs closer investigation.

Acyl neuraminic acids (sialic acids) occur in blood serum of animals, in sputum, in all animal cells, but they have rarely been found in microorganisms. Sialidases are produced by a few microorganisms, usually by those that live in close proximity to man or higher animals. SCHAUER (1975) mentions sialidases from *Clostridium perfringens*, *Vibrio cholerae* and a few other microorganisms. Sialidase activity has also been found in a few actinomycetes (U. MEVS, personal communication). Biologically, the effect of acyl-neuraminic acids lies in their strongly negative charge which results in the binding of many water molecules and gives a more slimy consistency to the polymer. One could speculate that sialidases serve the purpose of degrading the protective polymer layers which may cover many Antarctic soil organisms. An alternative explanation would have to postulate contamination of the Antarctic sampling sites with microorganisms derived from animals or man and which were also sialidase-positive.

The properties so far observed in our soil isolates, and supported by the work of SIEBERT & HIRSCH (1988) allow us to place many of the coccal strains with the genera *Micrococcus* or *Deinococcus*. Micrococcaceae were also found in Antarctic soils by BENOIT & HALL (1970), CAMERON et al. (1971), and by CAMERON (1974). The club-shaped, Gram-positive short rods common in soil samples were often identified as *Corynebacterium*, *Arthrobacter*, or *Brevibacterium* spp. (JOHNSON & BELLINOFF 1978, SIEBERT 1986). *Corynebacterium* spp. were found to dominate in many soils (CAMERON 1974). While HOROWITZ et al. (1972) and CAMERON et al. (1976) assumed that *Bacillus* spp. were not indigenous to the Dry Valley soils, other authors (BOYD & ROTHENBERG 1968, UYDESS & VISHNIAC 1976, MADDEN et al. 1978) have isolated several bacilli from Antarctic soils. MILLER et al. (1982) found that Antarctic spore-formers were well adapted to cold conditions and increased salt concentrations. In the present study we included 12 strains of bacilli among our isolates.

Members of the genus *Pseudomonas* were represented in our collection of pure cultures by yellow-pigmented, motile and slender rods which were Gram-negative and catalase- and cytochrome-oxidase-positive. *Pseudomonas* spp. were also described from Dry Valley areas by CAMERON et al. (1976). Further identification of our strains is in progress.

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