Polar Biol (1988) 9:37–44

Characterization of 15 Selected Coccal Bacteria Isolated from Antarctic Rock and Soil Samples from the McMurdo-Dry Valleys (South-Victoria Land)

J. Siebert and P. Hirsch

Institut für Allgemeine Mikrobiologie, Christian-Albrechts-Universität Kiel, Olshausenstraße 40/60, D-2300 Kiel, Federal Republic of Germany

Received 10 December 1987; accepted 21 January 1988

Summary. Approximately 1500 cultures of microorganisms were isolated from rocks and soils of the Ross Desert (McMurdo-Dry Valleys). From these, 15 coccoid strains were chosen for more detailed investigation. They were characterized by morphological, physiological and chemotaxonomical properties. All isolates were Gram-positive, catalase-positive and nonmotile. Six strains showed red pigmentation and could be identified as members of the genera *Micrococcus* (*M. roseus*, *M. agilis*) or *Deinococcus*. In spite of their coccoid morphology, the remaining nine strains had to be associated with coryneform bacteria (*Arthrobacter*, *Brevibacterium*), because of their cell wall composition and G+C ratios. Most of the strains were psychrotrophic, but one strain was even obligately psychrophilic, with a temperature maximum below 20 °C. Red cocci had in vitro pH optima above 9.0 although they generally originated from acid samples. Most isolates showed a preference for sugar alcohols and organic acids, compounds which are commonly known to be released by lichens, molds and algae, the other components of the cryptoendolithic ecosystem. These properties indicate that our strains are autochthonous members of the natural Antarctic microbial population.

Introduction

The "Ross Desert" (McMurdo Dry Valleys) of South-Victoria Land lacks visible life forms on the surface of soils and rocks, perhaps with the exception of a few lichens in protected locations (Friedmann 1982). Dry and cold katabatic winds warm up by descending from the Antarctic ice plateau and create true desert conditions in the valleys. Life is restricted to a narrow zone under the surface of rocks, especially of beacon sandstone, which is colonized by endolithic microorganisms. Friedmann and Ocampo (1976) first reported on this kind of cryptoendolithic life. Meanwhile a considerable number of different microorganisms (algae, yeasts, filamentous fungi, cyanobacteria and eubacteria) have been isolated from these habitats. Some taxonomical and physiological work has already been reported on Antarctic eucaryotic microorganisms and cyanobacteria (Friedmann and Ocampo 1976; Friedmann et al. 1980; Vishniac 1982; Kappen 1983; Kappen and Friedmann 1983; Hale and Ocampo-Friedmann 1984). However, little is known so far about heterotrophic bacteria and their part in the cryptoendolithic microbial ecosystem.

Coccal-shaped heterotrophic bacteria formed the majority of 1500 cultures which were isolated in our recent attempts to characterize the natural microflora of Dry Valley rocks and soils (Hirsch et al. 1985). Therefore, we selected 15 representative coccoid strains for identification by classical and chemotaxonomic methods. Also, some ecophysiological experiments were performed to show which properties would enable these bacteria to survive in such an extreme environment as the Ross Desert. Our data indicate that the coccal isolates could be placed with 6 different taxa, although morphologically they were quite similar.

Materials and Methods

The origin of the bacterial strains and method of their enrichment are shown in Table 1. With the exception of AA36 and AA63, which came from samples taken by E. I. Friedmann during the 1979/1980 season, all other strains came from samples collected 1984/1985 by one of us (P.H.) on Linnaeus Terrace (McMurdo Dry Valleys). A detailed description of the aseptic soil sampling procedures has been published previously (Hirsch et al. 1985). Aseptic rock samples were obtained with ethanol-treated hammer and chisel and transported in double, sterile Whirl-Pack plastic bags over dry ice. For the enrichment the rock samples were fragmented after wrapping in 4 layers of sterile gauze and 2 layers of sterile aluminium foil. To prevent losses during purification procedures the organisms were always cultivated as during their initial isolation. Purification was achieved after 3 to 5 subcultures on solid media. The following media were employed: PYGV (Staley 1968); PYGV/10 was a tenfold dilution of PYGV; 66a/5 was a fivefold dilution of Bacto Nutrient Broth (Difco, Detroit) with 2 g/l of glucose. Solid media contained 1.8% Bacto-Agar. Growth was evaluated after 2–3 weeks of incubation. For measurements of bacterial growth, whole cell protein was determined by the Bio-Rad protein assay (Bio-Rad, München). Biochemical and enzymatic tests (starch hydrolysis, gelatine liquefaction, esculin hydrolysis, phosphatase, β-galactosidase, nitrate reduction, NaCl tolerance and resistance against 100 μg/ml penicillin G) as well as the Gram-reaction were carried out following methods of Smibert and Krieg (1981). The oxidation or fermentation of glucose were tested according to Baird-Parker (1965). For testing the ability to tolerate UV radiation, 0.1 ml of young cultures were plated on PYGV and kept for 10 min under UV light of 254 nm (MinUVis, Desaga) at a distance of 8 cm. To differentiate Gram-positive bacteria, modern taxonomy relies heavily on the structure and composition of the bacterial cell wall, especially the amino acid and sugar composition of the rigid part of the cell wall (murein or peptidoglycan). To identify the peptidoglycan type, we followed the procedures of Schleifer and Kandler (1972), with qualitative as well as quantitative analyses by two-dimensional thin-layer chromatography (Harper and Davis 1979), and employing an aminoacid analyzer (Multichrom B, Beckman, München). DNA base compositions were determined by the melting point T_m-method (Marmur and Doty 1962; Mandel et al. 1970). Cell lysis was achieved enzymatically by lysozyme and SDS treatment. The purification of DNA followed the method of Marmur (1961). Fatty acids were extracted, methylated and identified by gas chromatography as described by Eckardt et al. (1979). The pigments of red strains were isolated according to Cooney et al. (1966). Absorption spectra (250 to 600 nm) were obtained with a Unicam SP-1800 UV spectrophotometer. Plasmid DNA isolation and separation on 0.7% agarose by gel electrophoresis were done according to Anderson and MacKay (1983).

Experiments with special ecological relevance such as the determination of temperature- and pH-ranges as well as growth stimulation by specific carbon sources (sugars, sugar alcohols, organic acids) were carried out in oligotrophic medium PYGV. For testing pH ranges, medium PYGV was supplemented with 0.05 g per liter each of KH_2PO_4 and $Na_2HPO_4 \cdot 2H_2O$, and the pH adjusted accordingly. Carbon sources were added as sterile solutions to medium PYV (i.e., PYGV without glucose) at a final concentration of 2 mM. All solutions were sterilized by filtration and inoculated.

Table 1. Origin and enrichment conditions of the coccoid bacterial strains tested. The pH was determined 30 min after suspension in 0.1 N KCl

Strain (AA-)	Sample		pH	Enrichment	
	No.	Type[a]		Medium	Temp. (°C)
From rock samples:					
36	A 790/23	G	n.d.[b]	PYGV	4
63	A 790/44b	M	n.d.	PYGV	4
306	845/207	S	4.2	PYGV	9
318	845/210	S	3.8	PYGV	9
370	845/232	S	4.4	PYGV/10	20
384	845/235	S	4.3	PYGV	9
412	845/238	S	4.9	PYGV	20
428	845/239	S	4.9	PYGV	20
455	845/249	S	4.8	PYGV	20
467	845/250	S	4.6	PYGV	20
532	845/256	S	5.3	PYGV	9
From soil samples:					
663	845/205	S	6.4	66a/5	9
692	845/224	S	6.5	PYGV	9
753	845/226	S	6.2	PYGV	9
761	845/227	S	6.2	PYGV	9

[a]G = granite, M = marble, S = Beacon sandstone
[b]Not determined

Results

Growth

Most strains grew well on oligotrophic medium PYGV. Growth on "richer" media, like 66a, was more slowly and

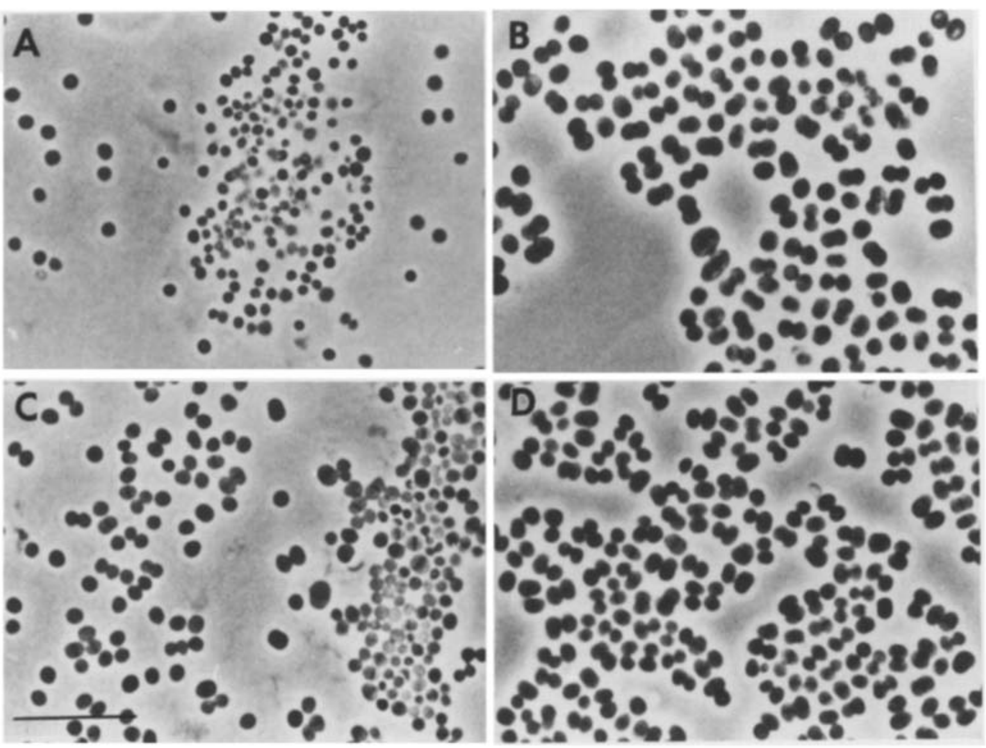

Fig. 1A–D. Phase contrast micrographs of coccal Antarctic bacteria from soil grown on medium PYGV at 9 °C. **A** strain AA663; **B** AA692; **C** AA753; **D** AA761. Wet mounts on agar slides; bar represents 10 μm

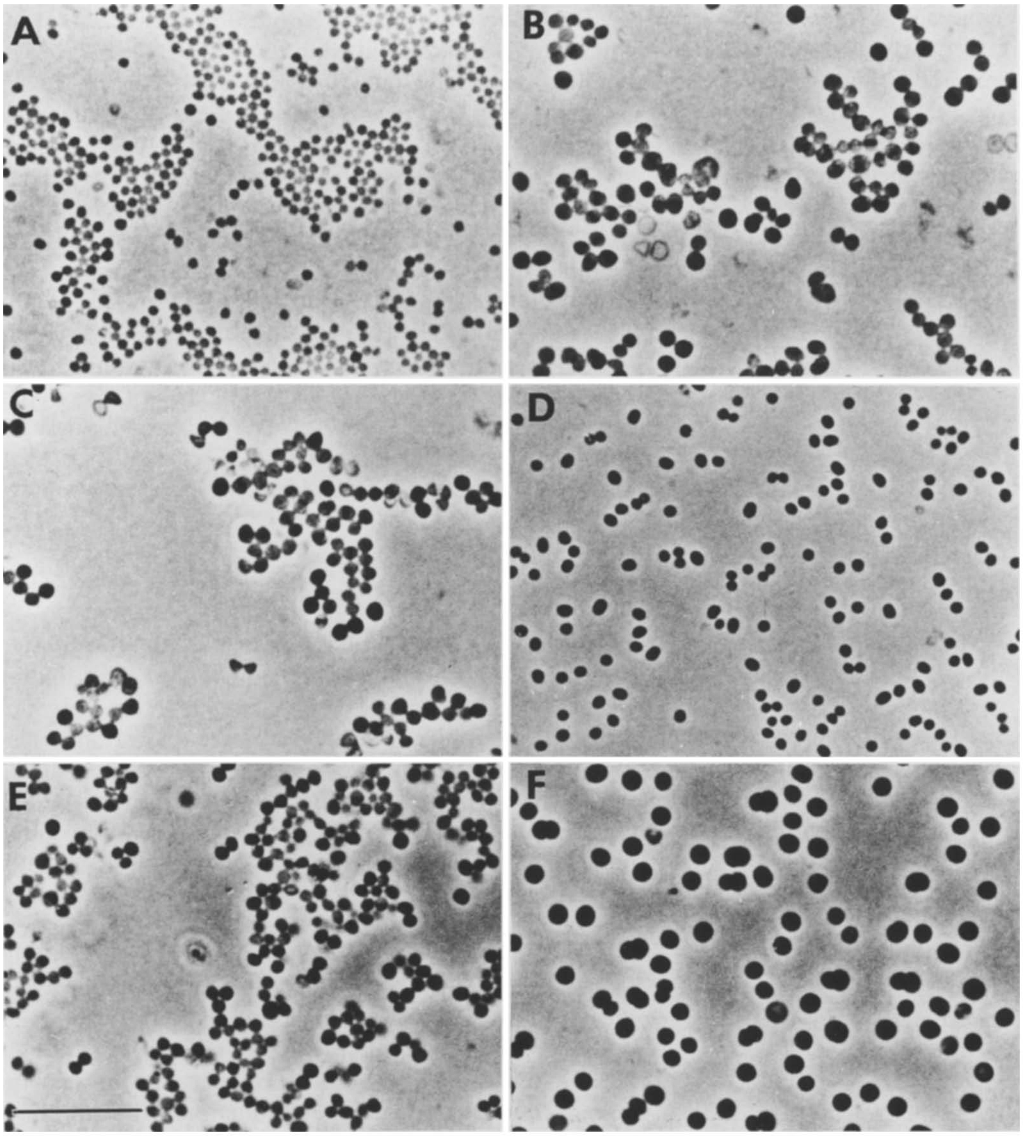

Fig. 2A–F. Phase contrast micrographs of coccal Antarctic bacteria from rocks grown on PYGV at 9 °C. **A** strain AA306; **B** AA318; **C** AA384; **D** AA532; **E** AA36; **F** AA63. Wet mounts on agar slides; bar respresents 10 µm

the cells often showed microscopically aberrant forms. Strains isolated at 4° or 9 °C grew faster on medium PYGV than those obtained on 20 °C.

Morphology

Most strains were true cocci except for AA428 and AA532, which sometimes resembled short rods. All were Gram-positive, nonmotile, and had their cell diameter between 0.4 and 2.0 µm (Figs. 1 and 2). Six strains, which represented the majority of all coccal isolates had red pigmentation. Table 2 describes the colony morphology and pigmentation of the strains on PYGV.

Biochemical Properties

All strains were catalase- and cytochrome positive and did not show acid or gas formation with glucose, aerobically or anaerobically. Selected physiological and biochemical properties (Smibert and Krieg 1981) including resistance against penicillin G and UV radiation are presented in Table 3. Two strains (AA63 and AA692) still showed growth after 10 min of UV radiation.

Cell Constituents

The cell wall compositions of our Antarctic coccoid strains with qualitative (diamino acid) and quantitative data (molar ratios) as well as their G+C base compositions are described in Table 4. The G+C content ranged from 61.3 to 69.4 mol%.

Fatty acid compositions of three selected, red pigmented cocci are shown in Table 5. The predominant components detected in strain AA761 were branched-chain esters, escpecially C15:0Br (for nomenclature see

Table 5). These branched-chain acids were absent in AA63 and AA692; here the C16:1 fatty acid dominated. In addition there were oddnumbered straight-chain fatty acids (C15:0, C17:0).

Absorption spectra of the chloroform extract of the six red pigmented strains were determined. Strains AA36, AA663, AA753 and AA761 had three maxima at 477, 505 and 541 nm; the remaining two strains had only one peak at 490 nm. Seven of the 15 strains had plasmids of different size (Table 6).

Special Ecologically Relevant Properties

The temperature range for growth in PYGV was determined for all strains, with the exception of AA63 (Table 7). Only one strain had the optimum above 20 °C, and three strains had their maximum at 30 °C or above. Most cultures, especially those isolated at 4°C or 9 °C, still grew at 0.5 °C, the lowest temperature tested. There was no growth at 37 °C.

The strains were also tested for their pH range. There was definitely better growth in the alkaline range (Table 8). Especially the high pH optima of red pigmented cocci (Table 2) were remarkable as most of these strains came from rather acid rock- or soil-samples.

Table 9 summarizes the results with those eight strains which were stimulated by selected carbon sources. The other seven strains did not grow with any of these compounds. Glucose or mannose were utilized only in a few cases, but sugar alcohols (mannitol, ribitol) or organic acids evidently served as good carbon and energy sources.

Table 2. Colony morphology and pigmentation of coccal strains from rock and soil samples. Incubation 2 weeks on PYGV and at the original enrichment temperature

Strain (AA-)	Colony morphology	Pigmentation
From rock samples:		
36	circular, smooth	pink/red
63	circular, smooth, slimy	pink/red
306	circular, smooth, slimy	yellow
318	irregular, rough	bright yellow
370	circular, smooth	ochre/yellow
384	irregular, rough	bright yellow
412	circular, smooth	orange
428	circular, smooth, slimy	white
455	circular, smooth	white/brown
467	circular, smooth	white
532	circular, smooth, slimy	white
From soil samples:		
663	circular, smooth, slimy	pink/red
692	circular, smooth, slimy	pink/red
753	circular, smooth	pink/red
761	circular, smooth	pink/red

Discussion

Most of our strains are oligotrophic. This is so since they grew much better in oligotrophic medium PYGV than in Bacto Nutrient Agar ("66a"), a characteristic which could be the result of selection by their location, where low nutrient fluxes prevail and productivity is low due to the harsh environmental conditions (Friedmann 1982). Oligotrophic bacteria often use amino acids (i.e., glutamate) and certain organic acids, especially glycolate (Ishida and Kadota 1981), which indicates a close dependency on algal exsudated products. It could be expected that there exists a correlation between this special stimulation by exsudates and the location, from where these strains originated. The cryptoendolithic ecosystem is a very simple one with no higher predators (Friedmann 1982) and with only an autochthonous microflora having a small but crucial primary production. Allochthonous

Table 3. Selected physiological properties of coccal isolates from the McMurdo-Dry Valleys

Strain (AA-)	Starch hydrolysis	Gelatine liquefaction	Esculin hydrolysis	Phosphatase	β-galactosidase	Nitrate reduction	Salt tolerance		Penicillin-G resistance (100 ug/ml)	UV resistance (254 nm)
							5%	10%		
From rock samples:										
36	–	+	–	+	+	(+)[a]	+	–	–	–
63	(+)	–	–	+	+	–	–	–	–	+
306	–	+	–	+	–	–	+	–	–	–
318	–	–	+	–	(+)	(+)	–	–	+	–
370	–	–	–	+	–	+	–	–	–	–
384	–	–	+	–	(+)	(+)	–	–	+	–
412	+	+	+	+	+	–	+	–	–	–
455	–	–	–	+	–	+	–	–	–	–
467	–	–	–	–	–	–	–	–	–	–
532	–	+	–	+	–	+	+	+	–	–
From soil samples:										
663	–	–	+	+	+	–	+	–	–	–
692	(+)	–	–	–	+	–	–	–	–	+
753	+	–	–	+	–	–	+	–	–	–
761	+	–	–	+	–	–	+	–	–	–

[a] Weak reaction

Table 4. Cell wall composition and base ratio (T_m) of 14 Antarctic coccal isolates. The column "diamino acid type" refers to the presence of a distinct diamino acid in the peptidoglycan peptide side chain

Strain (AA-)	Quantitative data (molar ratios: muramic acid = 1.0)								Base ratio (mol% G+C)
	Diamino acid type[b]		$GlcNH_2$	$GalNH_2$	Glu	Ala	Gly	Thr	
From rock samples:									
36	lysine:	1.0	1.0	–	1.0	6.2	–	1.1	64.1
63	ornithine:	1.0	1.0	–	1.2	1.8	1.7	–	62.8
306	LL-DAP:	1.0	1.0	–	1.1	2.1	1.0	–	69.4
318	m/DD-DAP:	1.0	0.9	–	1.1	1.7	–	–	62.1
370	LL-DAP:	1.0	1.0	–	1.3	2.2	1.1	–	68.1
384	m/DD-DAP:	1.0	0.8	–	0.9	1.4	–	–	61.8
412	LL-DAP:	1.0	1.1	–	1.2	1.6	2.9	–	68.9
428	m/DD-DAP:	1.0	1.9	0.8	1.3	3.2	–	–	66.2
467	m/DD-DAP:	1.0	1.2	–	1.2	2.4	–	–	n.d.[a]
532	m/DD-DAP:	1.0	1.1	–	1.0	1.8	–	–	67.3
From soil samples:									
663	lysine:	1.0	2.2	–	1.1	5.7	–	1.0	62.8
692	ornithine:	1.0	1.0	–	1.0	1.9	1.6	–	61.8
753	lysine:	1.0	3.1	–	1.5	5.3	–	–	61.3
761	lysine:	1.0	2.4	–	1.3	5.7	–	–	62.0

[a]Not determined
[b]DAP = diaminopimelic acid

Table 5. Percentages of principal fatty acids of 3 red pigmented Antarctic cocci

Strain (AA-)	i14:0[a]	14:0	15:0	15:0br	16:0	i16:1	16:1	17:0	18:0	un[b]
63	–	2.65	2.78	–	27.94	–	52.44	1.98	1.08	11.13
692	–	0.40	0.98	–	10.19	–	58.65	14.40	1.00	14.38
761	3.09	1.99	–	24.36	10.26	6.56	33.31	1.39	0.39	18.65

[a]Number to the left of the colon refers to number of carbon atoms; number to right refers to numbers of double bonds; br denotes branched-chain acid; i denotes modus of branching
[b]Unidentified

Table 6. Number of plasmid bands and their sizes from plasmid-positive Antarctic cocci

Strain (AA-)	No. of plasmid bands	Size[a]	
		>RP4	<RP4
63	6	3	3
318	1	–	1
384	3	–	3
412	1	1	–
467	2	2	–
692	4	1	3
753	2	2	–

[a]Molecular weight standard plasmid RP4 = 36.3 Mdal

input of organic carbon is almost negligible. The bacteria are therefore restricted to primary products released by algae or cyanobacteria, or organic matter becomes available after lysis or cell death. Sugar alcohols (i.e., ribitol) play a major role as transport metabolites from algae to

Table 7. Temperature ranges for growth of Antarctic cocci

Strain (AA-)	Isolation Temp. (°C)	Temperature (°C)		
		Minima[a]	Optima	Maxima
From rock samples:				
36	4	0.5	17.0	26.0
63	4	n.d.[b]	n.d.	n.d.
306	9	2.0	17.0	30.0
318	9	0.5	12.5	24.0
370	20	11.5	17.5	22.0
384	9	0.5	10.0	24.0
412	20	0.5	14.0	32.0
428	20	0.5	17.0	31.0
455	20	12.0	19.0	23.0
467	20	14.0	21.0	24.0
532	9	0.5	17.0	26.0
From soil samples:				
663	9	0.5	12.5	27.0
692	9	0.5	18.0	25.0
753	9	0.5	11.0	23.5
761	9	0.5	10.0	18.0

[a]0.5 °C was the lowest temperature tested
[b]Not determined

Table 8. pH optima and ranges for growth of Antarctic cocci in PYGV in comparison to the original sample pH

Strain (AA-)	pH		
	Optimum	Range	Original sample
From rock samples:			
36	> = 9.0	6.0 to > = 9.0	n.d.[1]
63	n.d.	n.d.	n.d.
306	7.0	6.0 to > = 9.0	4.2
318	5.0	5.0 to 7.0	3.8
370	8.0	6.0 to > = 9.0	4.4
384	6.0	5.0 to 7.0	4.3
412	5.0	5.0 to > = 9.0	4.9
428	8.0	5.0 to > = 9.0	4.9
455	9.0	6.0 to > = 9.0	4.8
467	6.0	<4.0 to 7.0	4.6
532	8.0	5.0 to > = 9.0	5.3
From soil samples:			
663	> = 9.0	6.0 to > = 9.0	6.4
692	> = 9.0	6.0 to > = 9.0	6.5
753	> = 9.0	7.0 to > = 9.0	6.2
761	> = 9.0	7.0 to > = 9.0	6.2

[a]Not determined

Table 9. Selected carbon sources (2 mM) utilized by some coccoid Antarctic isolates. Growth of strains AA306, AA318, AA384 and AA412 (from rock samples) or AA692 (from soil) was not stimulated by any of these compounds. (glyc = glycolate; ac = acetate; mal = malate; cit = citrate)

Strain (AA-)	Carbon sources utilized			
	Sugars	Sugar alcohols	Organic acids	Amino acid
From rock samples:				
36	mannose	mannitol		
428		ribitol	glyc, ac	
455		ribitol	ac, mal, cit	
467				glutamate
532				glutamate
From soil samples:				
663		mannitol		glutamate
753			cit	glutamate
761	glucose	mannitol		

fungi (Richardson et al. 1968; Hill 1970). Mannitol is a well known storage product in lichens (Lewis and Smith 1967), and it occurs in many algae (Craigie 1974). Tearle (1987) reported on the release of arabitol by Antarctic lichen fungi. Glycolate is excreted into the environment by most algae (especially *Chlorophyceae*) and by cyanobacteria due to photorespiration (Hellebust 1974). This all explains the organism's stimulation by glutamate, mannitol, ribitol, glycolate and other organic acids over sugars. It indicates that there indeed are close interactions between the members of this ecosystem. In situ carbon metabolism experiments of Vestal and Friedmann (1982) with ^{14}C-labeled organic carbon sources suggested that the cryptoendolithic community actively metabolized dissolved carbon compounds.

In nature oligotrophic bacteria are often psychrophilic (Ishida and Kadota 1981), as low temperatures often prevail where there is also a lack of nutrients (i.e., in oceans and polar regions). Depending on the definition used (Stokes 1963; or Morita 1975), most of our coccal isolates would be psychrotrophs or even psychrophiles, which indicates that they are part of the indigenous cryptoendolithic ecosystem. The unusually high pH optima of red cocci are in contrast to the pH observed in rock or soil samples (Table 8). Presently, this discrepancy can not be explained unless one assumes special conditions in the organisms' microhabitats, as for example an increased pH existing within polymer capsules. Such pH ranges are not uncommon in Antarctic soil samples (Johnson and Bellinoff 1981; Johnson et al. 1981).

All 15 strains were Gram-positive, catalase-positive, aerobic, and nonmotile. Because of their morphology, pimentation, and cell wall composition, four of the six red pigmented strains could be placed in the genus *Micrococcus* (*M. roseus*, *M. agilis*), while the other two red strains belonged to the genus *Deinococcus* in the sense of Schleifer (1986). Table 10 summarizes those properties of the isolates, which lead to their identification. Strains belonging to the genus *Micrococcus* could be easily distinguished from *Deinococcus* spp. (Brooks et al. 1980) by their fatty acids (Table 5) and by less UV

Table 10. Properties of the red-pigmented *Micrococcus/Deinococcus* strains leading to their identification

Characteristic	*"Micrococcus roseus"*		*Micrococcus roseus*[a]	*"Micrococcus agilis"*		*Micrococcus agilis*[a]	*"Deinococcus sp."*		*Deinococcus ssp.*[a]
	AA753	AA761		AA36	AA663		AA63	AA692	
Peptidoglycan type	Lys-Ala_{3-4}		Lys-Ala_{3-4}	Lys-Thr-Ala_{3-4}		Lys-Thr-Ala_{3-4}	Orn-Gly_2		Orn-Gly_2
G+C (mol%)	61.3	62.0	66–75	64.1	62.8	67–69	62.8	61.8	62–70
Characteristic fatty acid	n.d.	15:0br	15:0br	n.d.	n.d.	15:0br	16:1	16:1	16:1
UV resistance	–	–	–	–	–	–	+	+	+
Pigments in $CHCl_3$ (nm)[d]	505	505	n.d.	505	505	n.d.	490	490	n.d.
Plasmid bands	2	–	+ (55%)[c]	–	–	+ (20%)[c]	6	4	2 or 3 size classes[b]

[a]Schleifer (1986); [b]MacKay et al. (1985); [c]Mathis and Kloos (1984); [d]Main absorption peak

resistance (Table 3). Additional differences were the occurrence of plasmids (Table 6; MacKay et al. 1985; Mathis and Kloos 1984) and the absorption spectra of their pigments. The G+C-ratios of deinococci were in the lower range of the data reported in the literature (Schleifer 1986). The G+C-ratios of our *Micrococcus* strains (Table 10) were also considerably lower than those published so far for micrococci (Schleifer 1986).

In spite of their more coccoid morphology, the remaining nine strains will have to be placed within the coryneform bacteria (*Arthrobacter*, *Brevibacterium*; Table 11) because of their cell wall composition and DNA base ratios (Goodfellow and Minnikin 1981; Keddie and Jones 1981). The properties of five strains were in accordance with *Brevibacterium linens* (Goodfellow and Minnikin 1981; Keddie and Jones 1981). This organism has been isolated previously only from habitats with low water activity (cheese, sea fish; Keddie and Jones 1981). None of our strains was orange pigmented. The remaining four strains had LL-DAP in their cell wall and had higher G+C-ratios than the *Brevibacterium* strains mentioned above. These strains could be associated with the "*Arthrobacter simplex/tumescens*" group (Keddie and Jones 1981). The unassigned species cited by Keddie and Jones (1981) (a heterogeneous assemblage of mainly unnamed strains of uncertain taxonomic position) show some of the characteristics of our nine strains. As we know today, the genus *Micrococcus* is closely related to *Arthrobacter* (Stackebrandt et al. 1980), and there are currently only practical reasons for separating these two genera (Jones and Collins 1986). Therefore, there may exist coryneform bacteria, which have a coccoid morphology during all of their life cycle. Schleifer and Kandler (1972) have already mentioned such strains.

Table 11. Properties of Antarctic coccoid strains lacking red pigments and tentative identification

Microorganism (AA-)	Characteristic		
	Peptidoglycan type	G+C mol%	Gelatine liquefaction
Brevibacterium linens[a]	m/DD-DAP, direct	60–69	+
428	m/DD-DAP, direct	66.2	+
532	m/DD-DAP, direct	67.3	+
318	m/DD-DAP, direct	62.1	–
384	m/DD-DAP, direct	61.8	–
467	m/DD-DAP, direct	n.d.	–
Arthrobacter simplex/tumescens group	LL-DAP-$Gly_{1 \text{ or } 3}$	70–76	slowly +
306	LL-DAP-Gly	69.4	+
370	LL-DAP-Gly	68.1	–
412	LL-DAP-Gly_3	68.9	+
455	LL-DAP[b]	n.d.	–

[a] Goodfellow and Minnikin (1981), Keddie and Jones (1981)
[b] Gly not tested

Previous studies of Antarctic soil bacteria were mainly concerned with the occurrence and distribution of psychrophiles and contaminants introduced by man (McLean 1918; Darling and Siple 1941; Straka and Stokes 1960; Boyd et al. 1966). Since then only few taxonomical investigations have been made (Johnson et al. 1978; Madden et al. 1978; Johnson and Bellinoff 1981; Johnson et al. 1981). The majority of strains isolated by Johnson et al. (1978) were Gram-positive, catalase-positive and nonmotile. They did not ferment glucose and were morphologically identified as *Corynebacterium*, *Arthrobacter*, *Brevibacterium*, and *Micrococcus* spp.; 71% of all strains investigated here belonged to these genera. Gram-negative bacteria (i.e., *Pseudomonas*) were nearly absent. Our results agree with these findings. Quite surprising was the discovery of *Deinococcus* strains among our isolates (AA63 and AA692). Counsell and Murray (1986) studied strain AA63 which came from a rock sample and related it to *D. radiopugnans*. This can now be confirmed. With respect to strain AA692 we can show for the first time, that deinococci also occur in soils of the Dry Valleys, sites which are not normally exposed to high radiation doses (E. I. Friedmann, personal communication) as all previous *Deinococcus* habitats were (Brooks and Murray 1981).

In addition to the coryneform bacteria, which seemed to dominate in Antarctic soils, Johnson et al. (1981) characterized *Micrococcus* strains in more detail. They were nonmotile, strictly aerobic, yellow or red pigmented and did not produce acid from glucose. The G+C-ratios of their DNA ranged from 58 to 72 mol percent. In all cases lysine occurred in the cell wall. The red pigmented strains were classified as *M. roseus*, but it is possible that some of them were misclassified and might have belonged to *M. agilis* since the cell walls were not studied quantitatively. The main difference between *M. roseus* and *M. agilis* is the lack of threonine in the cell wall.

Our data on the properties of the 15 coccal isolates show greater physiological diversity than would be expected from morphological considerations. At least six different taxa could be identified. The ecophysiological observations indicate that these isolates were well adapted to the local Antarctic conditions, a fact that excludes the possibility of their origin by human contamination.

Acknowledgements. The help of C. Gallikowski, W. Liesack, M. Sittig and C. Schuett was gratefully appreciated. These investigations would not have been possible without the stimulation by Prof. E. I. Friedmann of the Polar Desert Research Center, University of Florida, Tallahassee/Fl. The work was supported by Grant No. DPP83-14180 from the U.S. National Science Foundation to E. I. Friedmann and by grants from the Deutsche Forschungsgemeinschaft to P. Hirsch.

References

Anderson DG, McKay LL (1983) Simple and rapid method for isolating large plasmid DNA from lactic streptococci. Appl Environ Microbiol 46:549–552

Baird-Parker AC (1965) The classification of *Staphylococci* and *Micrococci* from world-wide sources. J Gen Microbiol 38:363–387
Boyd WL, Staley JT, Boyd JW (1966) Ecology of soil microorganisms of Antarctica. In: Tedrow ICF (ed) Antarctic soils and soil forming processes, vol 8. Antarct Res Ser. Am Geophys Union, Washington, pp 125–159
Brooks BW, Murray RGE (1981) Nomenclature for "*Micrococcus radiodurans*" and other radiation-resistent cocci: *Deinococcaceae* fam. nov. and *Deinococcus* gen. nov., including five species. Int J Syst Bacteriol 31:353–360
Brooks BW, Murray RGE, Johnson JL, Stackebrandt E, Woese CR, Fox GE (1980) Red-pigmented micrococci: A basis of taxonomy. Int J Syst Bacteriol 30:627–646
Cooney JJ, Marks HW, Smith AM (1966) Isolation and identification of canthaxanthin from *Micrococcus roseus*. J Bacteriol 92: 342–345
Counsell TJ, Murray RGE (1986) Polar lipid profiles of the genus *Deinococcus*. Int J Syst Bacteriol 36:202–206
Craigie JS (1974) Storage products. In: Stewart WDP (ed) Algal physiology and biochemistry, vol 10. Blackwell, London, pp 206–235
Darling CA, Siple PA (1941) Bacteria of Antarctica. J Bacteriol 42:83–98
Eckardt FEW, Roggentin P, Hirsch P (1979) Fatty acid composition of various hyphal budding bacteria. Arch Microbiol 120:81–85
Friedmann EI (1982) Endolithic microorganisms in the Antarctic cold desert. Science 215:1045–1053
Friedmann EI, Ocampo R (1976) Endolithic blue-green algae in the dry valleys: primary producers and the Antarctic desert ecosystem. Science 193:1247–1249
Friedmann EI, Garty J, Kappen L (1980) Fertile stages of cryptoendolithic lichens in the dry valleys of southern Victoria Land. Antarct J US 15:166–167
Goodfellow M, Minnikin DE (1981) Introduction to the coryneform bacteria. In: Starr MP, Stolp H, Trüper HG, Balows A, Schlegel HG (eds) The Prokaryotes, vol II. Springer, Berlin Heidelberg New York, pp 1811–1826
Hale ME, Ocampo-Friedmann R (1984) Ascospore cultures of lichen phycobionts from the Antarctic desert. Antarct J US 19:170
Harper JJ, Davis GHG (1979) Two-dimensional thinlayer chromatography for amino acid analysis of bacterial cell walls. Int J Syst Bacteriol 29:56–58
Hellebust JA (1974) Extracellular products. In: Stewart WDP (ed) Algal physiology and biochemistry, vol 10. Blackwell, London, pp 838–863
Hill DJ (1970) The carbohydrate movement between the symbionts of lichens. D Phil thesis, University of Oxford
Hirsch P, Gallikowski C, Friedmann E (1985) Microorganisms in soil samples from Linneaus Terrace southern Victoria Land: Preliminary observations. Antarct J US 19:183–186
Ishida Y, Kadota H (1981) Growth patterns and substrate requirements of naturally occurring obligate oligotrophs. Microbiol Ecol 7:123–130
Johnson RM, Bellinoff RD (1981) Characteristics of cold desert Antarctic coryneform bacteria. In: Parker E (ed) Terrestrial biology, III. Antarct Res Ser, vol 30, pp 169–184
Johnson RM, Inai M, McCarthy S (1981) Characteristics of cold desert Antarctic coryneform bacteria. J Arizona-Nevada Acad Sci 16:51–60
Johnson RM, Madden JM, Swaford JR (1978) Taxonomy of Antarctic bacteria from soils and air primarily of the McMurdo station and Victoria Land dry valleys region. In: Parker E (ed) Terrestrial biology, III. Antarctic Res Ser, vol 30, pp 35–64
Jones D, Collins MD (1986) Irregular, nonsporing gram-positive rods. In: Krieg NR (ed) Bergey's manual of systematic bacteriology, vol 2, 9th edn. Williams and Wilkins, Baltimore, pp 1261–1434
Kappen L (1983) Ecology and physiology of the Antarctic fructicose lichen *Usnea sulphurea* (Koenig) Th. Fries. Polar Biol 1:249–255
Kappen L, Friedmann EI (1983) Ecophysiology of lichens in the dry valleys of southern Victoria Land, Antarctica. 2 CO_2 gas exchange in cryptoendolithic lichens. Polar Biol 1:227–232
Keddie RM, Jones D (1981) Saprophytic, aerobic coryneform bacteria. In: Starr MP, Stolp H, Trüper HG, Balows A, Schlegel HG (eds) The Prokaryotes, vol II. Springer, Berlin Heidelberg New York, pp 1838–1878
Lewis DH, Smith DC (1967) Sugar alcohols (polyols) in fungi and green plants. I. Distribution, physiology and metabolism. New Phytol 66:143–184
MacKay MW, Al-Bakri GH, Moseley BEB (1985) The plasmids of *Deinococcus* spp. and the cloning and restriction mapping of the *D. radiophilus* plasmid pUE1. Arch Microbiol 141:91–94
Madden JM, Siegel SK, Johnson RM (1978) Taxonomy of some Antarctic *Bacillus* and *Corynebacterium* species. In: Parker E (ed) Terrestrial biology, III. Antarct Res Ser, vol 30, pp 77–103
Mandel M, Igambi L, Bergendahl J, Donson ML, Scheltgen E (1970) Correlation of melting temperature and caesium chloride buoyant density of bacterial desoxyribonucleic acid. J Bacteriol 101:333–338
Marmur J (1961) A procedure for the isolation of desoxyribonucleic acid from microorganisms. J Mol Biol 3:208–218
Marmur J, Doty P (1962) Determination of the base composition of desoxyribonucleic acid from its thermal denaturation temperature. J Mol Biol 5:109–118
Mathis JN, Kloos WE (1984) Isolation and characterization of *Micrococcus* plasmids. Curr Microbiol 10:339–344
McLean AL (1918) Bacteria of ice and snow in Antarctica. Nature 102:35–39
Morita RY (1975) Psychrophilic bacteria. Bacteriol Rev 39:144–167
Richardson DHS, Hill DH, Smith DC (1968) Lichen physiology. XI. The role of the alga in determining the pattern of carbohydrate movement between lichen symbionts. New Phytol 67:469–486
Schleifer KH (1986) Gram-positive cocci. In: Krieg NR (ed) Bergey's manual of systematic bacteriology, 9th edn, vol 2. Williams and Wilkins, Baltimore, pp 999–1103
Schleifer KH, Kandler O (1972) Peptidoglycan types of bacterial cell walls and their taxonomic implications. Bacteriol Rev 36:407–477
Smibert RM, Krieg NR (1981) General characterization. In: Manual of methods for general microbiology. American Soc Microbiol Washington DC, pp 409–433
Stackebrandt E, Lewis BJ, Woese CR (1980) The phylogenetic structure of the coryneform group of bacteria. Zentralbl Bakteriol Mikrobiol Hyg Abt II Orig C 1:137–149
Staley JT (1968) *Prosthecomicrobium* and *Ancalomicrobium*, new prostecate fresh water bacteria. J Bacteriol 95:1922
Stokes JL (1963) Recent progress in microbiology. Gibbons NE (ed). University of Toronto Press, Toronto, pp 187
Straka RP, Stokes JL (1960) Psychrophilic bacteria from Antarctica. J Bacteriol 80:622–625
Tearle PV (1987) Cryptogamic carbohydrate release and microbial response during spring freeze-thaw cycles in Antarctic fellfield fines. Soil Biol Biochem 19:381–389
Vestal JR, Friedmann EI (1982) In situ carbon metabolism by the cryptoendolithic microbial community in the Antarctic cold desert. Antarct J US 17:190–191
Vishniac HS (1982) An enation system for the isolation of Antarctic yeasts inhibited by conventional media. Can J Microbiol 29:90–95