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3.8 Psychrophilic Myxobacteria from Antarctic Soils

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Summary: 24 soil samples from the ice-free McMurdo Dry Valleys, South Victoria Land, were investigated for the presence of myxobacteria. Sampling occurred during an expedition in 1985/86. Three different types of myxobacteria were found on agar plates seeded with food bacteria, after dark incubation for 10 months at 4° C. The three types differed in vegetative cell size, growth rate, shape, and in the size of the swarms formed. They were bacteriolytic, agarolytic, and psychrophilic myxobacteria that did not grow at 18 or 30° C. Sporadically myxospores occurred, while sporangioles or fruiting bodies have not been found so far. On the basis of cell shape and swarming behaviour the three types appeared to belong to the suborder Sorangineae. All isolates came from one sample; the other 23 samples were negative for these myxobacterial types. When rabbit fecal pellets were used for bait, or when agar plates with food bacteria were incubated at 30° C, myxobacteria could not be detected. A fourth type of bacteria occurred in 10 of the samples but not in the sample positive for the three other types. This type was agarolytic but not bacteriolytic and its colonies were sunken into the agar in a terrace-like fashion. The enrichments contained, besides the myxobacteria, also pigmented bacteria and *Bacillus* spp. as well as (in one sample) an ameba.

Zusammenfassung: 24 Bodenproben aus den eisfreien McMurdo Trockentälern, Südviktorialand, wurden auf Myxobacterien getestet. Die Probennahme erfolgte während einer Expedition im Südsommer 1985/86. Nach 10 Monaten Inkubation (dunkel, 4° C) traten auf den Agarplatten mit Futterbakterien drei verschiedene Myxobakterientypen auf. Sie unterschieden sich in der Größe ihrer vegetativen Zellen, der Wachstumsrate, der Form und der Größe der gebildeten Schwärme. Es handelte sich um bakteriolytische, agarolytische und psychrophile Myxobakterien, die nicht bei 18 und 30° C wachsen. Gelegentlich traten Myxosporen auf, während Sporangiolen oder Fruchtkörper bisher nicht beobachtet wurden. Nach Zellform und Schwärmverhalten schienen die drei Typen zur Unterordnung Sorangineae zu gehören. Alle Isolate kamen aus einer Probe, die anderen 23 Proben enthielten diese Myxobakterientypen nicht. Mit Kaninchenmistköder oder auf Agarplatten mit Futterbakterien, die bei 30° C bebrütet wurden, konnten keine Myxobakterien beobachtet werden. Ein vierter Myxobakterientyp trat in 10 Proben auf, aber nicht in der Probe, die die drei anderen Typen enthielt. Dieser Typ war agarolytisch, aber nicht bakteriolytisch, und die Kolonien waren terrassenartig im Agar eingesenkt. Neben den Myxobakterien enthielten die Anreicherungen auch pigmentierte Bakterien und *Bacillus* spp. sowie eine Amöbe (in einer Probe).

1. INTRODUCTION

We know little about the occurrence and distribution of myxobacteria in extreme biotopes, although there are two publications about myxobacteria in extremely cold environments. BROCKMANN & BOYD (1963) examined 17 soil samples from the Alascan and Canadian artic. Myxobacteria wee not found in 13 samples, but of the remaining four samples, two yielded *Myxococcus xanthus* and three contained *Polyangium sorediatum*. None of the strains developed at low temperatures. RÜCKERT (1985) investigated five crude top-soil samples from Antarctica (Fildes Peninsula, King George Island in South Shetland Islands). He found myxobacteria in two of the samples. Five strains of *Myxococcus virescens* were isolated from water agar plates with food organisms in the form of baker's yeast. One strain of *Myxococcus stipitatus* was found on rabbit dung pellets. The samples were not incubated under psychrophilic conditions.

Occasionally reference is made in the literature to the presence of myxobacteria in extremely cold biotopes, although closer studies have not been made. But it is not known whether psychrophilic or mesophilic myxobacteria exist in Antarctic soils which have not been contaminated by man.

2. MATERIALS AND METHODS

2.1 *Soil samples*

During an expedition in December/January 1985/86 to the ice-free McMurdo Dry Valleys (Ross Desert) in South Victoria Land, 24 soil samples were collected under sterile conditions as described by HIRSCH et al. (1985) and transported to Kiel over dry ice. Table 1 indicates sampling locations. The ice-free area is located between 160 and 164° E, and 76° 30' and 78° 30' S; it was formerly known as the "Ross Desert" (Geographic Names of the Antarctic, 1980—81).

#Dedicated to Prof. Dr. Karl-Ernst Wohlfahrt-Bottermann (Bonn) on the occasion of his 65th birthday.

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Location	Number of samples obtained	pH	Sample numbers
Battleship Promontory	2	5.8;6.5	856/148; 856/149
Dais (Upper Wright Valley)	1	7.8	856/121
Linnaeus Terrace	18	3.5–6.7	856/101, 102, 107, 108,114, 116, 120, 134, 140, 141, 142, 143, 144, 157, 158, 159, 164, 121
New Mountain	1	4.3	856/128
University Valley	2	5.8;6.8	856/126, 856/153

Tab. 1: Sampling sites, soil pH, and sample numbers.

Soil temperatures during the Antarctic summer may reach +15° C on "hot days" due to dark and black rock components such as dolerite; on normal summer days most soils have −1 to −11° C in the upper 10 cm zone. The soils studied here consisted of sandstone and dolerite fragments in various proportions; due to the lack of humic substances and clay minerals they should be called "regolith". The numbers and distributions of bacteria in these soils vary with the location (GALLIKOWSKI & HIRSCH, this volume).

2.2 *Bacterial strains*

Except for *Escherichia coli* K 12 (ATCC 9637), all food bacteria came from soils on Linnaeus Terrace (elevation about 1650 m, Asgard Range; Tab. 2). Four of them were Gram-positive, and the other two were orange, Gram-negative rods. The food bacteria were pregrown on medium PYGV (STALEY 1968) at 9° C.

Strain*	Isolation from sample	Morphology	Gram-reaction
AA–684	845/224	thin orange rods	negative
AA–689	845/224	thick orange short rods	negative
AA–723	845/225	pink pigmented cocci	positive
AA–730	845/225	whitish small cocci	positive
AA–742	845/226	yellow thin rods	positive
AA–819	845/247	grayisch small and thin rods	positive

Tab. 2: List of Antarctic soil bacterial cultures used as food bacteria for the enrichment of myxobacteria. *IFAM numbers, Inst. f. Allgemeine Mikrobiologie, Kiel.

2.3 *Enrichment methods*

Two methods were used for the enrichment and isolation of Antarctic myxobacteria; both techniques are well established in myxobacteria research. The "bacterial spot method" (DAWID 1984) was a variation of the "bacterial streak method" (SINGH 1947). Drops of a dense suspension of living *E. coli* K 12 or the mixture of 6 Antarctic soil bacteria (Tab. 2) were placed on the surface of water agar plates in the form of spots approx. 2—3 cm in diameter and these were allowed to dry. The water agar contained 1.5% agar and 0.1% (w/v) of $CaCl_2.2H_20$, pH 7.2 (REICHENBACH & DWORKIN 1981). Using a sterile spatula, aliquots of the samples were positioned in the center of the bacterial spots. The plates were then incubated at 4 and 30° C. The "bait method" of KRZEMIENIEWSKA & KRZEMIENIEWSKI (1926) was carried out as follows: soil samples were placed into sterile Petri dishes which contained sterile filter discs, and then all were moistened with sterile distilled water. The bait material, autoclaved dung pellets, was slightly pressed into the samples, and the closed Petri dishes were then incubated as moist chambers at 4 and 30° C in the dark.

From each of the 24 samples we inoculated 50 aliquots on bacterial spots, and two Petri dishes with soil samples

Number of samples tested	Food bacteria or substrate offered*	Incubation (°C)	Number of aliquots
24	*E.coli* K 12	30	1.200
24	Antarctic soil bacteria**	30	1.200
24	*E.coli* K 12	4	1.200
24	Antarctic soil bacteria**	4	1.200
24	rabbit dung pellets***	30	480
24	rabbit dung pellets***	4	480

Tab. 3: Enrichment experiments performed for obtaining myxobacteria. *Experimental design see Materials and Methods; **mixture of 6 strains, see Table 2; ***20 pellets per sample.

and 25 sterile dung pellets were prepared. Altogether 4,800 bacterial spots were inoculated, and 960 bait pellets were used. Table 3 is an overview of the experimental design.

3. RESULTS

After 10 months of incubation the following results were obtained: (1) agar plates with food bacteria incubated at 30° C did not show any myxobacteria on the *E. coli* spots or on the 1200 spots of the Antarctic bacterial mixture; (2) none of the 960 rabbit dung pellets incubated at 4 or 30° C showed growth of fruiting body formation of myxobacteria; (3) bacterial spot plates which were incubated at 4° C yielded three different myxobacterial types that appeared first after 8 weeks; (4) a fourth type, probably also a myxobacterium, was observed only after nine months of incubation.

The three main types were studied in greater detail and have the following properties:

The P-type (*Polyangium*-like type). The swarm formed an elevted rim that was reminescent of *Polyangium* spp. (Fig. 1). Cell aggregates were found in bacteriolytic zones (Fig. 2). The vegetavive cells were rigid cylindrical

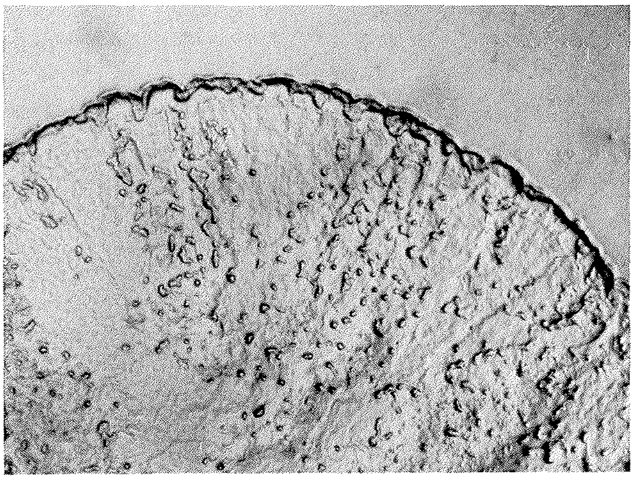

Fig. 1: P-type myxobacteria: swarm on a spot of Antarctic soil bacteria. Incubation 3 months at 4° C. Magnification 16x.

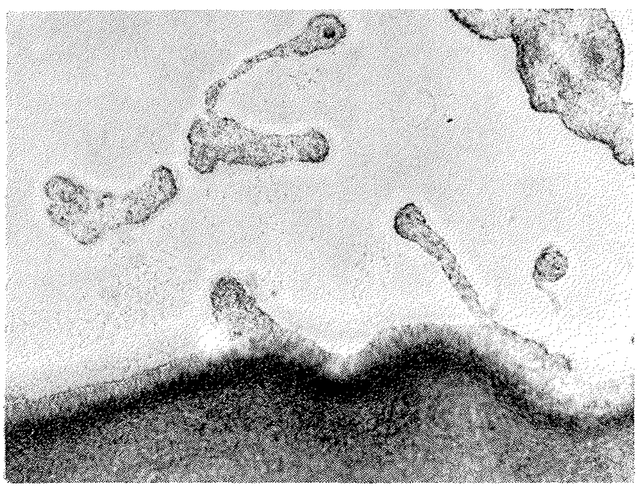

Fig. 2: P-type myxobacteria: cell aggregates in the bacteriolytic zone. Agar plate with food bacteria from Antarctic soils. Incubation 3 months at 4° C. Magnification 250x.

rods with broadly rounded ends (Fig. 3). In the center of these aggregations the cells were shorter and almost spherical, like stages of myxospores. Sporadically bright refractile cells, probably myxospores occurred, whereas sporangioles or fruiting bodies were never found. It was possible to subculture these myxobacteria on *E. coli* K12 or Antarctic bacterial spot plates at 4° C but not at 30° C. Cells of this type wee bacteriolytic and slightly agarolytic. The P-type occurred only in sample 856/149 from Battleship Promontory, a dolerite-rich soil that was collected under a rock.

T h e R - t y p e (rounded type). Myxobacteria of this type formed smooth-rimmed colonies which were sunken dish-like into the agar (Fig. 4). Vegetative cells were plump rods with rounded ends (Fig. 5), sporangioles and fruiting bodies were lacking. Subcultures grew on both kinds of bacterial spots, but only at 4 or 9° C. These myxobacteria were bacteriolytic and strongly agarolytic; they grew markedly slower than the P-type. The R-type cells were also found only in sample 856/149.

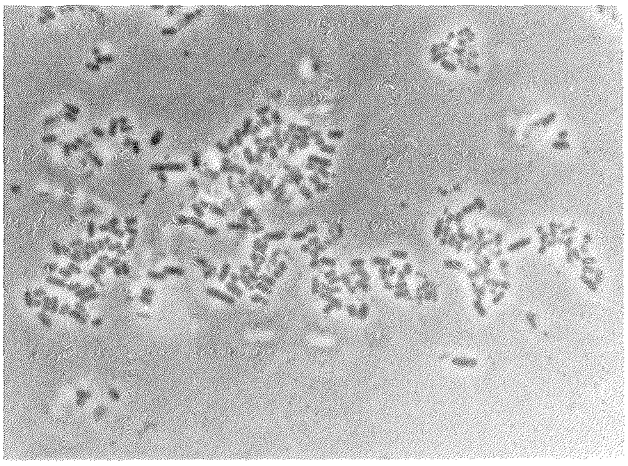

Fig. 3: P-type myxobacteria: vegetative rod-shaped cells, grown on a spot of Antarctic bacteria. Incubation 4 months at 4° C. Phase contrast, magnification 1000x.

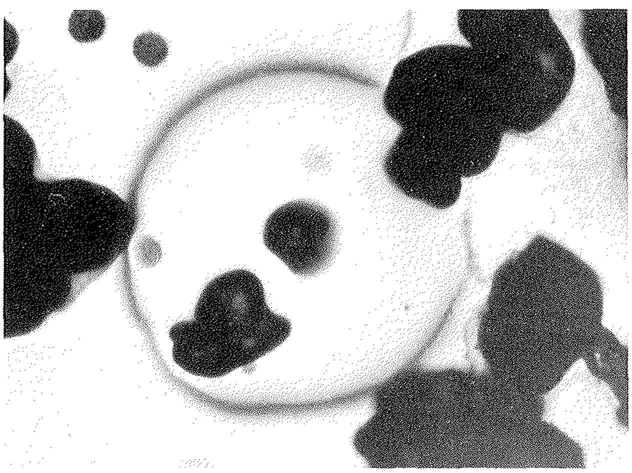

Fig. 4: R-type myxobacteria: Swarm on an Antarctic bacteria spot. Incubation 5 months at 4° C. Magnification 16x.

The frequency of both types was studied. 72% of the *E. coli* spots were myxobacteria positive; the P-type occurred in 6%, the R-type in 66% of the spots. 54% of the Antarctic bacterial spots were myxobacteria positive; the P-type occurred in these to 30% and the R-type to 24% of the spots.

T h e N - t y p e (*Nannocystis*-like type). This type formed swarms reminescent of *Nannocystis exedens*; the

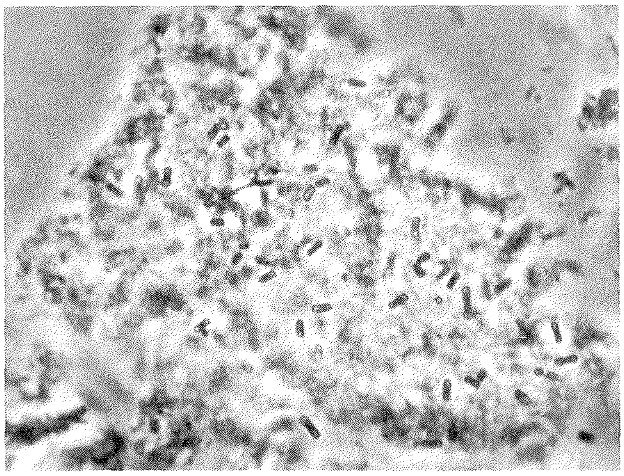

Fig. 5: R-type myxobacteria: vegetative rod-shaped cells in agar. Grown on *E. coli* spots. Incubation 4 months at 4° C. Phase contrast: magnification 1000x.

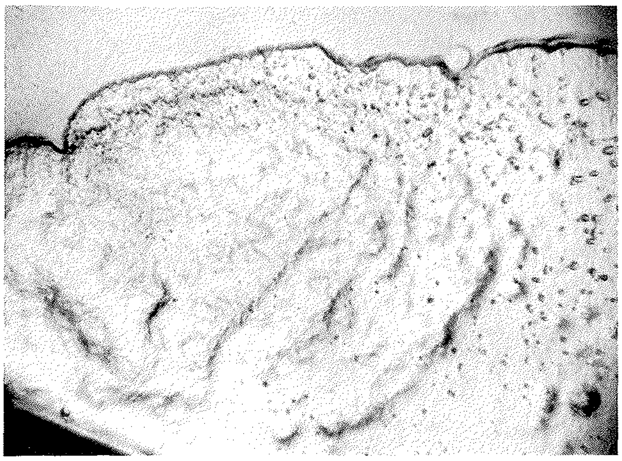

Fig. 6: N-type myxobacteria: swarm on an Antarctic bacteria spot. Incubation 4 months at 4° C. Magnification 6.4x.

swarms were strongly bacteriolytic and agarolytic (Fig. 6). The vegetative cells were cylindrical rods with broadly rounded ends (Fig. 7). After lysis of the food bacteria, at the rim of the swarm, structures developed sunken into the agar which were very similar in shape and size to the sporangioles of *N. exedens* (Fig. 8). The interior of these structures contained shorter rods, probably stages of myxospores. Subculturing was easy on *E. coli* K12 and on Antarctic bacterial spots. The N-type was strongly bacteriolytic and agarolytic. It was only found in sample 856/149, with a frequency of 3%.

A fourth type was found; it was agarolytic but not bacteriolytic and had colonies that degraded the agar in a terrace-like fashion, beginning from the center in which there were always soil particles (Fig. 9). This type, called the T-type (terrace forming) grew extremely slowly. The cells were rod-shaped with rounded ends (Fig. 10). Colonies were detected only after 8—10 months of incubation. The T-type occurred in 10 of the 24 soil samples (Tab. 4). The average frequency was about 12%. At present it can not be decided whether this type is really a myxobacterium. Table 4 summarizes our current knowledge of these three (or four) myxobacterial types.

4. DISCUSSION

The three myxobacterial types developed on bacterial spots only in the temperature range between 4° C and 9° C. At room temperature (18—20° C) growth was inhibited. At 30° C the cultures were killed within 2—3 weeks.

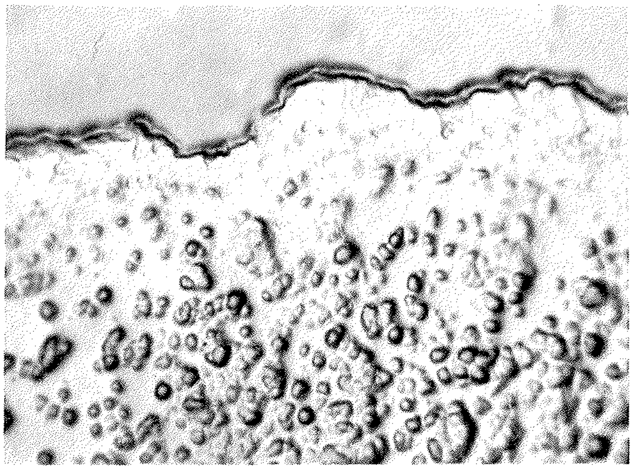

Fig. 7: N-type myxobacteria: swarm with sporangiole-like structures on an Antarctic soil bacteria spot. Incubation 8 months at 4° C. Magnification 16x.

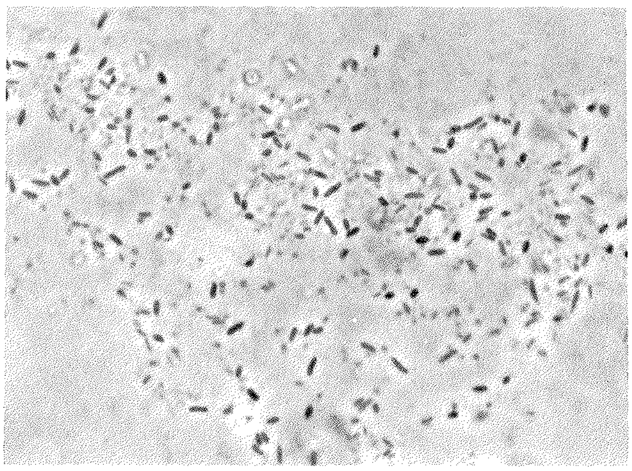

Fig. 8: N-type myxobacteria: vegetative rod-shaped cells, grown on *E. coli* spot. Incubation 3 months at 4° C. Phase contrast, magnification 1000x.

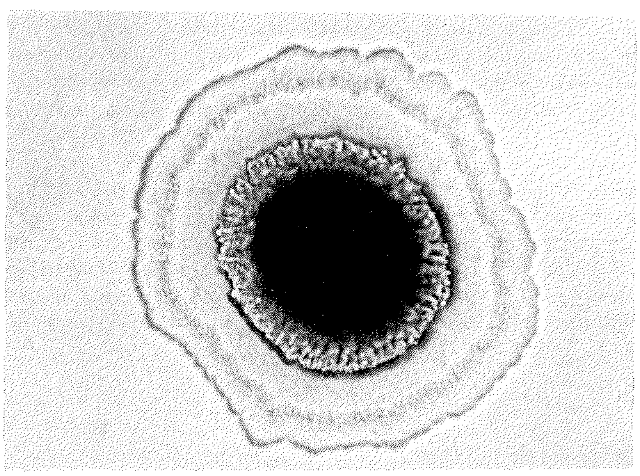

Fig. 9: T-type bacteria: terrace-shaped colony with rock particle in the center. Grown on an Antarctic soil bacteria spot. Incubation 9 months at 4° C; magnification 16x.

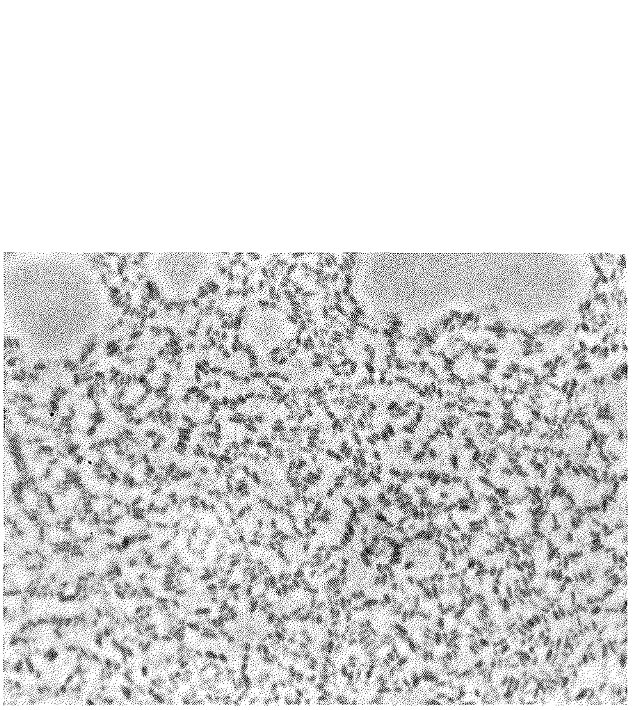

Fig. 10: N-type myxobacteria: vegetative rod-shaped cells grown on the agar surface. Incubation 9 months at 4° C. Phase contrast; magnification 1000x.

Origin (sample number)	Type	Appearance of swarm or colony	Vegetative cells size (um)	Myxospores size (um)	Fruiting bodies or sporangioles	Lytic properties bac	ag
856/149	P-type	*Polyangium*-like	1.4 x 4.6 – 5.8	1.2 x 3-0	–	+++	++
856/149	R-type	round, smooth-rimmed, dish-like	1.0 x 3.2 – 4.8	0.8 x 1.8	–	+	+
856/149	N-type	*Nannocystis*-like	1.4 x 3.2 – 3.8	1.2 x 1.8	embedded in agar, oval	+++	+++
856/102, 856/107, 856/108, 856/121, 856/126, 856/129, 856/134, 856/142, 856/143, 856/159	T-type	terrace-shaped	1.2 x 2.8	n.d.	–	–	++

Tab. 4: Types of myxobacteria found and some of their characteristics. bac = bacteriolytic, ag = agarolytic, n.d. = not detected.

This proves that the three types are genuine psychrophiles. So far only mesophilic myxobacteria are known, which grow in the range of 18 to 35° C. It remains to be seen if similar psychrophilic myxobacteria occur in other cold stressed environments (mountains, glacier soils etc), or if the Antarctic isolates were indigenous, adapted forms. In this connection it is interesting to note that the myxobacterial isolates from Battleship Promontory (on the slope of Mt. Gran, north of Linnaeus Terrace) grew well on the spots of soil bacteria isolated from Linnaeus Terrace. Additional tests should be made with bacterial isolates from Battleship Promontory; perhaps under such conditions the number of positive samples would increase, as the local myxobacterial population might be better adapted to the local bacterial population.

A taxonomic identification of the isolates to the species level was not possible so far, as neither fully developed myxospores, nor sporangioles or fruiting bodies were formed. Most probably they belong to the suborder Sorangineae. This is supported by the shape and size of their vegetative cells, the gliding movement on agar surfaces, the swarm structure (except for the R-type) and the type of agarolysis.

Presently enrichments are still being incubated and may yield further myxobacterial growth. Our studies have raised a number of questions, such as the late appearance (2—5, or even 9 months) after inoculation of the food spots. Perhaps the myxobacterial population in these Antarctic soils is very small and it may be limited by slow growth and low numbers of the food bacteria present. Another problem is the extremely slow development of the myxobacterial growth in our experiments. At in situ temperatures below zero, myxobacterial growth may not be possible, and it could be assumed that these organisms survive naturally in an inactive form which would have to be "activated" for growth.

Members of the genus *Myxococcus* have not been found in our enrichments, although this genus is widely distributed in almost all soils. Why, on the other hand, were there three (or possibly four) myxobacterial types in this one sample (856/149)? What was so specific about this sample? The soil in this case had been taken from under a dolerite boulder in Battleship Promontory. This site is perhaps more protected and may have been more moist than Linneaeus Terrace sites. The nearly black dolerite boulder would collect more heat than the surrounding sandstone, so that underneath there may have been a much more favourable environment for bacterial (and hence myxobacterial) growth. This assumption is supported by the observation of amebae in this very sample. The opposite question, why did the other soils not contain myxobacteria, can not be answered as yet. More samples need to be studied with additional techniques, variable environmental parameters, and long incubation times. Preliminary experiments have shown that growth conditions, bacterial growth rates, and environmental parameters vary greatly on Linnaeus Terrace (P. HIRSCH unpubl.).

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References

Brockmann, E. R. & Boyd, W. L. (1963): Myxobacteria of the Alascan and Canadian Arctic. — J. Bacteriol. 86: 605—606.

Dawid, W. (1984): Myxobakterien in ungestörten Hochmooren des Hohen Venn (Hautes Fagnes, Belgien). — System. Appl. Microbiol. 5: 555—563.

Gallikowski, C. A. & Hirsch, P. (1988): Characterization and preliminary identification of 1984/85 Antarctic soil microorganisms of Linnaeus Terrace (McMurdo Dry Valleys). — Polarforschung 58: 93—101.

Hirsch, P., Gallikowski, C. A. & Friedmann, E. I. (1985): Microorganisms in soil samples from Linnaeus Terrace, Southern Victoria Land: preliminary observations. — Antarct. J. U.S. 29: 183—186.

Krzemieniewska, H. & Krzemieniewski, S. (1926): Die Myxobakterien von Polen. — Acta Soc. Botan. Polon. 4: 1—54.

Reichenbach, H. & Dworkin, M. (1981): The Order Myxobacterales. — In: Starr, M. P., Stolp, H., Trüper, H. G., Balows, A., Schlegel, H. G. (eds.) The Prokaryotes. A Handbook of Habitats, Isolation and Identification of Bacteria, 328—355. Berlin—Heidelberg—New York. Springer Verlag.

Rückert, G. (1985): Myxobacteria from Antarctic soils. — Biol. Fert. Soil 1: 215—216.

Singh, B. N. (1947): Myxobacteria in soils and composts; their distribution, number and lytic action on bacteria. — J. Gen. Microbiol. 1: 1—10.

Staley, J. T. (1968): *Prosthecomicrobium* and *Ancalomicrobium*: new prosthecate freshwater bacteria. — J. Bacteriol. 95: 1921—1942.