3.8 Psychrophilic Myxobacteria from Antarctic Soils #

By Wolfgang Dawid®, Claudia A. Gallikowski® and Peter Hirsch®

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 carotenoids formed. They were bacteriolytic, agarsoluble, and psychrophilic myxobacteria that did not grow at 18°C or 30°C. Specifically, myxosporous occurred, while myxococci or fleshy bodies were not found so far. On the basis of cell shape and extracellular bodies the three types appeared to belong to the suborder Streptomycetes. All isolates came from one sample: the other 23 samples were negative for these myxobacterial types. When rabbit feces pellets were used for bait, or when agar plates with food bacteria were inoculated 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 other three types. This type was agarsoluble but not bacteriolytic and its colonies were similar to the agar in a single-filament phase. The myxobacteria consisted, besides the myxobacteria, also represented bacteria and Myxobolus spp. as one sample ameba.


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 Alaskan and Canadian artic. Myxobacteria were not found in 13 samples, but of the remaining four samples, two yielded Myxococcus xanthus and three contained Polysporangium soredianum. None of the strains developed at low temperatures. RÜCKERT (1985) investigated five crust 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 viridans 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, 1980–84).

1Dedicated to Prof. Dr. Karl Ernst Wohlfahrt-Borsboom (Hons) on the occasion of his 65th birthday.
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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 -1.5°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 Linnacus 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.

<table>
<thead>
<tr>
<th>Strain*</th>
<th>Isolation from sample</th>
<th>Morphology</th>
<th>Gram-reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA-604</td>
<td>845/224</td>
<td>thin orange rods</td>
<td>negative</td>
</tr>
<tr>
<td>AA-609</td>
<td>845/224</td>
<td>thick orange short rods</td>
<td>negative</td>
</tr>
<tr>
<td>AA-723</td>
<td>845/225</td>
<td>pink pigmented cocci</td>
<td>positive</td>
</tr>
<tr>
<td>AA-730</td>
<td>845/225</td>
<td>pinkish small cocci</td>
<td>positive</td>
</tr>
<tr>
<td>AA-742</td>
<td>845/226</td>
<td>yellow thin rods</td>
<td>positive</td>
</tr>
<tr>
<td>AA-819</td>
<td>845/247</td>
<td>grayish small and thin rods</td>
<td>positive</td>
</tr>
</tbody>
</table>

Tab. 2: List of Antarctic soil bacterial cultures used as food bacteria for the enrichment of myxobacteria. *Abbreviations: *E. coli K 12: Experimental design see Materials and Methods: **experimental strains.

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 CaCl2·2H2O, pH 7.2 (REICHENBACH & DWORNIK 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 KRZEMIEŃIEWSKA & KRZEMIEŃIEWSKI (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 at 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.

<table>
<thead>
<tr>
<th>Number of</th>
<th>Food bacteria or substrate offered*</th>
<th>Incubation (°C)</th>
<th>Number of</th>
</tr>
</thead>
<tbody>
<tr>
<td>samples tested</td>
<td></td>
<td></td>
<td>aliquots</td>
</tr>
<tr>
<td>24</td>
<td><em>E. coli</em> K 12</td>
<td>30</td>
<td>1,300</td>
</tr>
<tr>
<td>24</td>
<td>Antartic soil bacteria**</td>
<td>4</td>
<td>1,200</td>
</tr>
<tr>
<td>24</td>
<td><em>E. coli</em> K 12</td>
<td>4</td>
<td>1,200</td>
</tr>
<tr>
<td>24</td>
<td>Antartic soil bacteria**</td>
<td>30</td>
<td>400</td>
</tr>
<tr>
<td>24</td>
<td>rabbit dung pellets***</td>
<td>4</td>
<td>400</td>
</tr>
</tbody>
</table>

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 elevated rim that was reminiscent of Polyangium spp. (Fig. 1). Cell aggregates were found in bacteriolytic zones (Fig. 2). The vegetative cells were rigid cylindrical...
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 were 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.

The R-type (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.

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.

The N-type (*Nannocystis*-like type). This type formed swarms reminiscent of *Nannocystis excedens*; the
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. extensus (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 sporangioid-like structures on an Antarctic soil bacteria spot. Incubation 8 months at 4 °C. Magnification 40x.

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

Fig. 9: T-type bacteria: torus-shaped colony with rock particles in the center. Grown on an Antarctic soil bacteria spot. Incubation 9 months at 4 °C; magnification 16x.
Fig. 10: N-type myxobacterial vegetative rod-shaped cells grown on the agar surface. Incubation 9 months at 4°C. Phase contrast; magnification 1000x.

<table>
<thead>
<tr>
<th>Origin (sample number)</th>
<th>Type</th>
<th>Appearance of swarm or colony</th>
<th>Vegetative cells size (μm)</th>
<th>Mysospores size (μm)</th>
<th>Fruiting bodies or sporangi--cles</th>
<th>Lytic properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>856/049</td>
<td>P-type</td>
<td>Polyallgill-like</td>
<td>1.4 x 4.6 - 5.8</td>
<td>1.2 x 3.0</td>
<td>-</td>
<td>+++ ++</td>
</tr>
<tr>
<td>856/049</td>
<td>R-type</td>
<td>round, smooth, convex, disk-like</td>
<td>1.0 x 3.3 - 4.8</td>
<td>0.3 x 1.8</td>
<td>-</td>
<td>+ +</td>
</tr>
<tr>
<td>856/049</td>
<td>N-type</td>
<td>Namysospore-like</td>
<td>1.4 x 3.2 - 3.8</td>
<td>1.2 x 1.8</td>
<td>embedded in agar, oral</td>
<td>+++ ++</td>
</tr>
<tr>
<td>856/072, 856/077, 856/086, 856/097, 856/019, 856/026, 856/029, 856/034, 856/132, 856/143, 856/149</td>
<td>T-type</td>
<td>terrace-shaped</td>
<td>1.2 x 3.8</td>
<td>n.d.</td>
<td>-</td>
<td>++</td>
</tr>
</tbody>
</table>

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 sporangiules 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 *Mycobacterium* 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 Linnaeus 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, cannot 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.).

5. ACKNOWLEDGEMENTS

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References


