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E. Buffan-Dubau, O. Pringault, R. de Wit. Artificial cold-adapted microbial mats cultured from Antarctic lake samples. 1. Formation and structure. *Aquatic Microbial Ecology*, 2001, 26, pp.115-125. 10.3354/ame026115 . hal-02080148

HAL Id: hal-02080148

<https://hal.science/hal-02080148>

Submitted on 7 May 2021

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Artificial cold-adapted microbial mats cultured from Antarctic lake samples. 1. Formation and structure

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ABSTRACT: Artificial microbial mats were cultured at 4 to 7°C in benthic gradient chambers using inocula of Antarctic lake mats (Lake Fryxell, Dry Valleys). Their formation and structure were studied based on both HPLC pigment quantification and microsensor measurements of oxygen profiles. Accretion rates (2 to 5 mm within the year) were consistent with previous estimations for artificial mats cultured at 17 to 25°C, suggesting that the microbial community was cold-adapted. Mats were vertically structured comprising an upper green layer dominated by cyanobacteria and an underlying pink layer including purple non-sulphur phototrophic bacteria. Pigment contents indicated that both groups of cyanobacteria (Oscillatoriaceae and possibly *Nostoc* sp.) adopted different patterns of vertical distributions within the mats. Heterogeneity of oxygen vertical distributions was determined, and net primary productivity rates were in the range of those previously reported for natural Antarctic microbial mats. Collective considerations of pigment contents and primary productivity strongly suggest that artificial mats presented some characteristics of natural polar mats, although some differences were established.

KEY WORDS: Antarctica · Artificial mats · Psychrotrophs · Pigments · HPLC · Net productivity

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INTRODUCTION

Microbial communities involving cyanobacteria dominate terrestrial and freshwater ecosystems in Antarctica (e.g. Vincent et al. 1993a, Vincent & Quesada 1994, Takacs & Priscu 1998). Microbial mats composed primarily of cyanobacteria, diatoms and bacteria occur throughout much of the benthic regions of Antarctic lakes of the Dry Valleys and contribute to a substantial fraction of the net primary production (Wharton et al. 1983, Simmons et al. 1993, Moorhead et al. 1997). Their morphology and species composition have been studied from field expeditions (reviewed in Simmons et al. 1993). Because microbial mat commu-

nities often inhabit shallow waters where they are exposed to the intensive solar radiation of the Antarctic summer, many previous works have focused on the strategies of adaptation by cyanobacteria to UV radiation (e.g. Vincent et al. 1993a, Vincent & Quesada 1994, Quesada & Vincent 1997, Quesada et al. 1998, Nadeau et al. 1999).

Cold water temperatures are obviously a characteristic feature of the polar aquatic environments. Thus, effects of temperature, and especially mechanisms of adaptation to cold by mat-forming cyanobacteria, have been a major research focus (Tang et al. 1997a, Roos & Vincent 1998, Tang & Vincent 1999). Experimental studies from polar cyanobacterial isolates have shown that many of these microorganisms can adjust their pigment content and photosynthetic capacity to optimise their growth over a broad temperature range (Tang et al. 1997, Tang & Vincent 1999). Recently, psychrophilic mat-forming cyanobacteria isolated

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from Antarctic meltwater ponds have been characterised; however, little is known about cold-adaptation processes in Antarctic mats (Nadeau & Castenholz 2000). Experimental works on Antarctic microbial mats may be partly complicated by difficulties encountered in preserving Antarctic mats without disturbing them over the long periods of time that are usually required for bringing samples from the field to the laboratory. Fenchel (1998a,b,c) has shown that artificial microbial mats from temperate environments can be obtained by experimental culturing using inocula of defaunated sediments. Further, the structure of these artificial mats was comparable to those of natural microbial mats, demonstrating the potential of this approach (Fenchel & Kühl 2000, Kühl & Fenchel 2000). Accordingly, the purpose of this study was to culture microbial mats initiated with Antarctic lake samples, at low temperature and under conditions mimicking field conditions. For that purpose, we used benthic gradient chambers (BGC). These devices have been previously designed to culture phototrophic microorganisms under physicochemical conditions occurring at the sediment-water interface (Pringault et al. 1996, 1998, Pringault & Garcia-Pichel 2000). The present paper describes the formation, the growth and the structure of artificial 'cold-mats' based on both pigment quantification and oxygen distribution. Part 2 (Pringault et al. 2001, this issue) reports the short-term temperature effects on oxygen turnover in these artificial cold-adapted mats.

MATERIALS AND METHODS

Site and sampling. Lake Fryxell (75° 36' S, 163° 35' E) is located in the Taylor Valley of the Dry Valley region, western Antarctica. The Dry Valleys are surrounded by 2500 to 3000 m high mountains (Transantarctic mountain range) and this results in an annual snowfall of only 2 to 4 cm (Pearl et al. 2000). Lake Fryxell has a surface area of 7 km² and a maximum depth of 19 m (Lawrence & Hendy 1985). The lake is permanently capped by thick (3.5 to 4.5 m) ice, although each summer, a peripheral moat area opens up around the ice cover (Spigel & Priscu 1998). Lake waters are mainly derived from glacial melt and are essentially freshwater at the top, whereas salinity increases with depth, reaching 10‰ at the bottom. The permanent ice-cap prevents direct wind-induced turbulence, which results in extreme stability of salinity and temperature gradients, as well as the nutrient regime in the water column (Lawrence & Hendy 1985, Priscu et al. 1989, Miller & Aiken 1996). Temperature ranges from 0°C immediately below the ice cover to 2°C at the bottom, and 4°C between 8 and 10 m

depth. The water column is anoxic below 9.5 to 10.5 m depth and life is dominated by microorganisms. Eukaryotic phototrophs, heterotrophic flagellates and bacteria dominate the planktonic community (Takacs & Priscu 1998, Simmons et al. 1993). Benthic microbial mats occur abundantly in shallow waters, i.e. where the benthic area is within the euphotic zone (Wharton et al. 1983, Simmons et al. 1993). Microbial mat samples were collected by Cathy Welch in January 1999 from the benthic area of Lake Fryxell. Mat samples were frozen (–20°C) and sent to France under frozen conditions. Delivery of mat samples took approximately 3 wk. In the laboratory, mat samples were stored either at –20°C for subsequent culturing in benthic chambers or in the dark at –80°C for HPLC pigment analyses.

Mat cultures in BGC. BGC previously allowed culturing of microbial biofilms of phototrophic bacteria under conditions mimicking light, oxygen, sulfide, and pH gradients occurring in natural benthic habitats (Pringault et al. 1996, 1998, Pringault & Garcia-Pichel 2000). Briefly, the BGC is composed of an artificial sterile sediment core (45 to 55 mm inner diameter [i.d.]) of fine quartz sand (125 to 250 µm, Merck) sandwiched between an upper oxic chamber and a lower anoxic, sulphide-containing chamber to generate opposing oxygen and sulphide gradients. Both the upper and lower chambers were thermostated by a 15% glycerol solution at 3°C. In this study we present results concerning cultures in 2 BGC: BGC1 and BGC2. During the culturing phase, temperature was kept at 6 ± 1°C in BGC1 and at 5 ± 1°C in BGC2. The BGC were steam sterilised, and the uppermost 1 cm of each sterile sand core was cooled at 4°C. Two inocula of mat samples from Lake Fryxell were stored at 4°C for defrosting and were then aseptically mixed with the cold sand (10% of the total volume). The mixture was homogenised and added to the surface of the sterile sand core in each BGC. The BGC were exposed to an 18 h light:6 h dark cycle using 60 W incandescent lamps. The 2 sterile culture media in the upper and lower chambers had a similar composition comprising filtered (0.22 µm) diluted sea water (10‰ salinity), KH₂PO₄ (0.5 mM), SL12B without EDTA (1 ml l⁻¹) and the V7 vitamin solution (1 ml l⁻¹) (Pfennig & Trüper 1992). We anticipated that the availability of bound nitrogen would have a major impact on community structure. BGC1 was run at a high concentration of bound nitrogen, and NH₄Cl was therefore added at a final concentration of 5 mM in both the upper and lower medium. In contrast, in BGC2 NH₄Cl was added to the lower medium only (final concentration of 10 mM) but not to the upper medium. This way, the availability of bound nitrogen for growth of the microbial mat in BGC2 depended on diffusive delivery from

below; the estimated diffusive delivery was 40 to 50 nmol N cm⁻² h⁻¹. In addition, a third experiment was started with both media devoid of any bound nitrogen source in order to study formation of diazotrophic mats. However, the growth in this experiment was so slow that only a faint biofilm was obtained after 12 mo of incubation, when the experiment was aborted (unpubl.). Media were not replenished with nutrients in the course of the experiments. For both BGC, the upper medium was oxic and permanently saturated by a soft stream of sterile air while the lower medium was anoxic (addition of Na₂S to 30 mM final concentration). The medium of the upper chamber was amended with NaHCO₃ (4 mM final concentration). NaHCO₃ was also added to the lower chamber medium (80 mM final concentration) as a CO₂ source. Mat cultures were started in March 1999 (Mat 1 in BGC1) and in June 1999 (Mat 2 in BGC2). Microsensor measurements and sampling for HPLC pigment analyses were then carried out on both a 9.5 mo old mat (Mat 1) and an 8.5 mo old mat (Mat 2).

Microsensor measurements. Oxygen was measured with a Clark-type O₂ microelectrode (Revsbech 1989) under light and temperature conditions used for culturing, i.e. 70 μmol photons m⁻² s⁻¹ and 5°C, respectively. From steady-state profiles, the oxygen fluxes leaving upward and downward and the respiration of the mat were calculated using the procedure described by Kühl et al. (1996). This calculation procedure is detailed by Pringault et al. (2001). Gross photosynthesis rates of the artificial mats were determined by using the light-dark shift technique described by Revsbech & Jørgensen (1983).

HPLC analyses of pigments. Immediately after microsensor measurements, cores were collected from the artificial mats in the BGC using 1.3 cm i.d. plastic tubes. Either 4 replicate cores (Mat 1) or 6 replicate cores (Mat 2) were collected and immediately stored in the dark at -80°C. Since artificial mats comprised distinct layers, each replicate core was vertically partitioned into 3 layers: the green upper layer, the pink underlying layer and the colourless layer of sand underlying the mat. Layers were carefully separated on frozen cores using a cutter. Field mat samples from Lake Fryxell used for inoculating the BGC cultures were also analysed. There were no distinct layers in these samples and, thus, they were not vertically partitioned. Six subsamples (0.6 g wet weight) were randomly collected from frozen field mat samples. Freeze-drying of samples prior to extraction has been recommended because it improves extraction of benthic microalgal pigments (Buffan-Dubau & Carman 2000a). Thus, first analysed samples (Mat 1) were freeze-dried. However, since we observed that freeze-drying probably reduced the recovery of bacterial

carotenoids (unpubl.), subsequently we used wet samples for Mat 2 and field mat study. Pigments were extracted by both grinding and sonicating each sample for 30 s in 10 ml of 100% cold acetone (HPLC quality). Acetonic extracts were then incubated for 3 h in the dark at -20°C and separated from particles by centrifugation. The extraction procedure was applied 4 times to each sample and extracts were then combined. The combined extracts were filtered (0.22 μm) after methylation with diazomethane, totally evaporated using a speed-vacuum and stored in the dark at -80°C until analysis. Immediately before HPLC analysis, the 'dried' extracts were dissolved in Solvent A (100% methanol + 0.05 M pH 7.2 ammonium acetate + 100% acetonitrile, 50/45/5, v/v/v) and filtered (0.22 μm), and 50 to 60 μl of each extract was analysed by HPLC within 3 d following extraction.

Pigment extracts were analysed using a liquid chromatograph consisting of a 100 μl loop refrigerated autosampler (TSP AS3000) and a Perkin Elmer binary pump coupled to a TSP UV6000 diode-array spectrophotometer programmed to obtain the on-line absorption spectra from 320 to 800 nm. The spectrophotometer was set at 440 nm for detection of carotenoid pigments, at 664 nm for detection of chlorophylls + pheopigments, and at 770 nm for detection of bacteriochlorophyll + bacteriopheopigments. Pigments were separated by reverse-phase liquid chromatography using a column Lichrospher 100RP18 (250 × 4 mm, 5 μm). The mobile phase was modified from the method described by Wright et al. (1991) for use with a binary solvent system: Solvent A (see above) and Solvent B (100% ethyl acetate + 100% methanol + 100% acetonitrile, 80/19/1, v/v/v). All organic solvents were HPLC grade (Prolabo). Pigments were identified by comparing their retention time and absorption spectra with those of authentic standards (International Agency for ¹⁴C Determination), with those of pure cultures of *Thiocapsa roseopersicina* (bacteriochlorophyll *a* [bchl *a*], bacteriopheophytin *a*) or with data from the literature (scytonemin [scyto], antheraxanthin, echinenone, hydroxyspheroidene). Literature references used for pigment identification and determination of algal or bacterial sources were Foppen (1971), Garcia-Pichel & Castenholz (1991) and Jeffrey et al. (1997). Standard solutions were quantified by spectrophotometry using the extinction coefficient listed by Jeffrey et al. (1997). Pigment quantification was based on the response factors calculated for each standard from the linear relationship between the concentration and the corresponding peak area obtained by HPLC. Pigments that were spectrally similar to, but did not have the same retention time as, standards were designated 'like-pigments' and were quantified using the response factor obtained from standards.

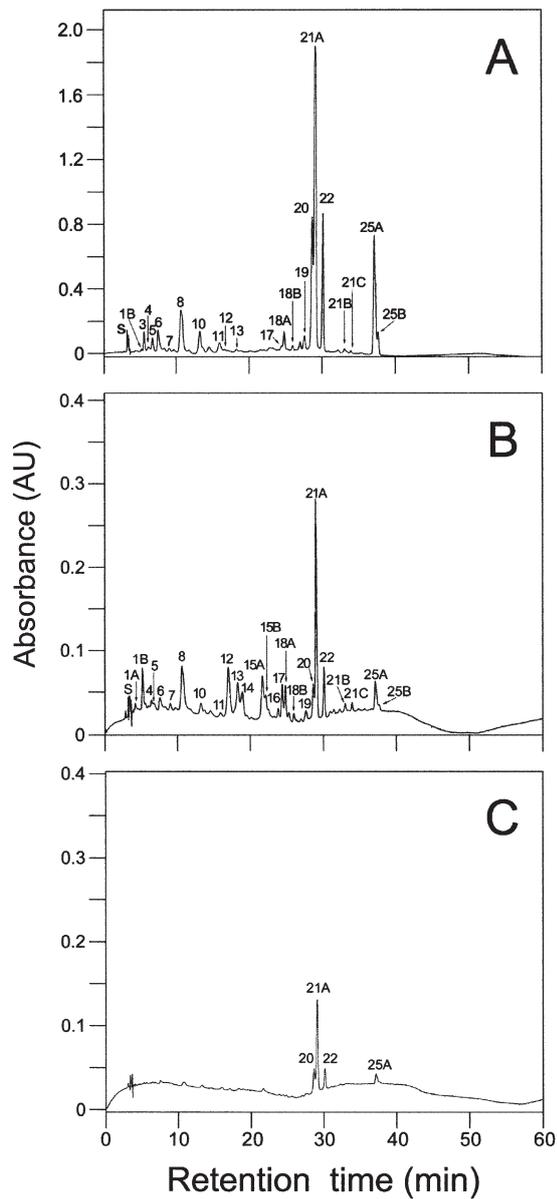


Fig. 1. Absorbance chromatograms at 440 nm of extracts of Artificial Mat 2 samples from (A) the upper green layer, (B) the underlying pink layer, and (C) the colourless sand underlying the mat. For peak identification see Table 1. AU: absorbance units; S: solvent peak

When authentic standards were not available, pigment contents were quantified from peak area assuming an extinction coefficients of $45 \text{ l g}^{-1} \text{ cm}^{-1}$ at 440 nm for scyto (Vincent et al. 1993b), $2350 (100 \text{ ml g}^{-1} \text{ cm}^{-1})$ at 440 nm for antheraxanthin (Jeffrey et al. 1997), $2158 (100 \text{ ml g}^{-1} \text{ cm}^{-1})$ at 440 nm for echinenone (Villanueva et al. 1994), $2785 (100 \text{ ml g}^{-1} \text{ cm}^{-1})$ at 440 nm for hydroxyspheroidene (Foppen 1971), and $84.1 \text{ l g}^{-1} \text{ cm}^{-1}$ at 770 nm for bchl *a* and bacteriopheophytins (Fenchel 1998a).

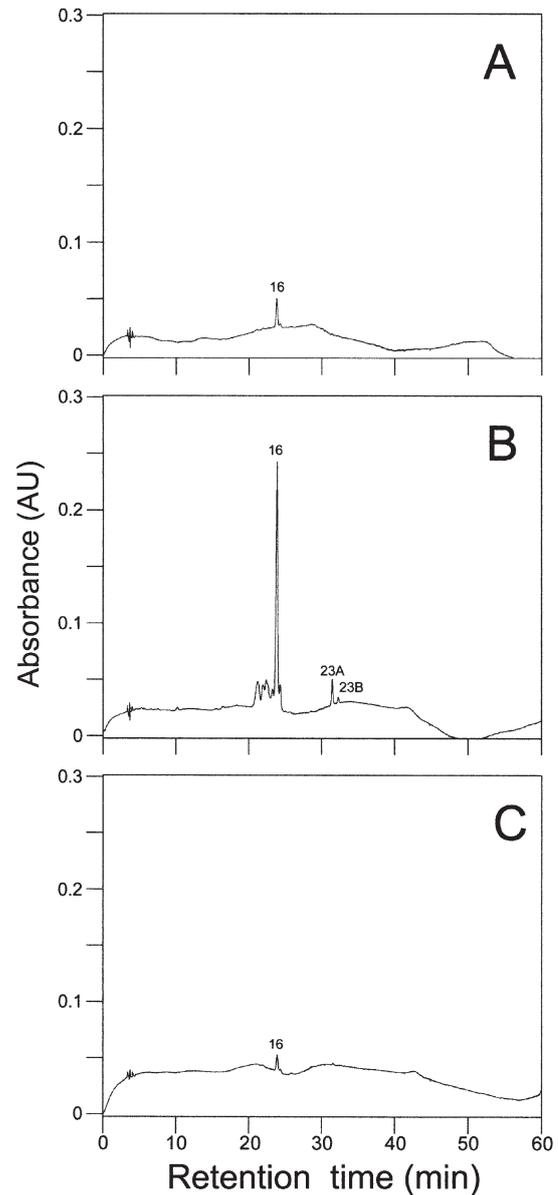


Fig. 2. Absorbance chromatograms at 770 nm of extracts of Artificial Mat 2 samples from (A) the upper green layer, (B) the underlying pink layer, and (C) the colourless sand underlying the mat. For peak identification see Table 1

RESULTS

Formation of artificial mats

Development of mats was macroscopically observed throughout the culturing periods. After 3 (Mat 1) or 5 wk (Mat 2), first signs of microbial development were observed in BGC with the appearance of green spots widespread at the sand surface (approximately 1 to 5 mm diameter). A month later, a light

Table 1. Averaged (mean \pm SD) concentrations ($\mu\text{g g}^{-1}$ sediment dry wt) of pigments detected in the green upper layer and in the underlying pink layer (see text for depths) of both Artificial Mat 1 (n = 4) and Mat 2 (n = 6) after 9 mo in benthic gradient chambers (BGC) at 4 to 7°C and in field mat samples (n = 6). For unidentified pigments, the wavelengths of maximum absorption are given in brackets. +: detected but not quantified; -: not detected; Chl: chlorophyll; TR: retention time

| TR (min) | Peak no. | Pigment | Artificial Mat 1 | | | | Artificial Mat 2 | | | | Field mat | |
|----------|----------|-------------------------------------|------------------|------|------------|-------|------------------|------|------------|------|-----------|------|
| | | | Green layer | | Pink layer | | Green layer | | Pink layer | | Mean | SD |
| | | | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| 4.3 | 1A | Scytonemin1 (red form) ^a | - | | 1.1 | 0.9 | - | | 0.78 | 0.76 | 163 | 84 |
| 5.2 | 1B | Scytonemin2 (green form) | 3.5 | 2.0 | 4.9 | 0.9 | 2.3 | 1.5 | 3.1 | 1.4 | 216 | 25 |
| 5.6 | 2A | Violaxanthin | 5.4 | 5.3 | 0.05 | 0.04 | - | | - | | - | |
| 5.6 | 3 | Chlorophyllide a | - | | - | | 3.2 | 2.4 | 0.33 | 0.33 | - | |
| 6.2 | 4 | Neoxanthin | 3.1 | 3.4 | 0.06 | 0.04 | 0.92 | 0.57 | - | - | - | |
| 6.7 | 2B | Violaxanthin-like | 3.5 | 4.0 | 0.02 | 0.01 | - | | - | | - | |
| 6.8 | 5 | Antheraxanthin | - | | - | | 2.5 | 1.1 | 0.17 | 0.08 | - | |
| 7.5 | 6 | Myxoxanthophyll | 3.4 | 1.4 | 0.06 | 0.03 | 9.6 | 4.0 | 0.75 | 0.29 | 14.5 | 3.6 |
| 9.0 | 7 | (372-436-463 nm) | + | | - | | + | | + | | - | |
| 10.7 | 8 | Lutein | 17.4 | 15.2 | 0.24 | 0.19 | 10.7 | 2.6 | 1.26 | 0.41 | - | |
| 10.9 | 9 | Zeaxanthin | - | | - | | - | | - | | 7.0 | 1.5 |
| 13.2 | 10 | (467) | - | | - | | + | | - | | + | |
| 15.8 | 11 | Canthaxanthin | 1.5 | 0.8 | 0.03 | 0.01 | 3.9 | 0.7 | 0.18 | 0.07 | 2.8 | 0.6 |
| 17.0 | 12 | (483) | - | | + | | + | | + | | - | |
| 18.3 | 13 | (480) | + | | + | | + | | + | | - | |
| 18.9 | 14 | (372/472) | - | | - | | - | | + | | - | |
| 21.6 | 15A | Hydroxyspheroidene1 | - | | 0.03 | 0.02 | + | | 0.49 | 0.28 | - | |
| 22.1 | 15B | Hydroxyspheroidene2 | - | | - | | - | | 0.23 | 0.13 | - | |
| 23.8 | 16 | Bacteriochlorophyll a | - | | - | | 1.6 | 0.7 | 6.0 | 1.2 | 0.33 | 0.19 |
| 24.4 | 17 | (443-450/684-688) | - | | - | | + | | + | | + | |
| 24.7 | 18A | Chl b | 69 | 63 | 0.91 | 0.66 | 17.6 | 7.7 | 3.7 | 1.5 | - | |
| 25.9 | 18B | Chl b-like | 1.8 | 1.8 | - | | 3.4 | 1.3 | 0.7 | 0.2 | - | |
| 27.6 | 19 | Chl a allomere | - | | - | | 20.9 | 9.2 | 1.7 | 0.7 | - | |
| 28.6 | 20 | Echinenone | 13.4 | 6.8 | 0.18 | 0.07 | 40.3 | 10.8 | 1.1 | 0.5 | 16.8 | 4.6 |
| 28.9 | 21A | Chl a | 347 | 194 | 5.5 | 2.7 | 652 | 162 | 30.9 | 9.5 | 239 | 71 |
| 30.0 | 22 | Chl a epimer | 7.1 | 4.9 | 0.09 | 0.07 | 155 | 42.9 | 5.6 | 2.4 | 25.2 | 9.8 |
| 31.5 | 23A | Bacteriopheophytin a1 | - | | 0.005 | 0.006 | - | | 0.52 | 0.31 | - | |
| 32.3 | 23B | Bacteriopheophytin a2 | - | | - | | - | | 0.10 | 0.09 | - | |
| 32.9 | 21B | Chl a-like1 | 5.6 | 4.7 | 0.05 | 0.06 | 2.0 | 0.2 | 0.9 | 0.3 | - | |
| 33.8 | 21C | Chl a-like2 | 4.4 | 3.5 | 0.02 | 0.05 | 3.0 | 0.9 | 0.7 | 0.3 | - | |
| 34.9 | 24A | Pheophytin a | 8.2 | 5.8 | 0.16 | 0.07 | 3.4 | 1.9 | 0.3 | 0.3 | 82.5 | 37.4 |
| 35.8 | 24B | Pheophytin a-like1 | - | | - | | - | | - | | 14.3 | 7.3 |
| 37.0 | 25A | β -carotene | 7.4 | 4.4 | 0.09 | 0.05 | 27.6 | 8.1 | 0.9 | 0.4 | 11.6 | 3.3 |
| 37.4 | 25B | cis- β -carotene | 0.85 | 0.89 | 0.022 | 0.018 | 3.5 | 0.85 | 0.12 | 0.05 | 1.66 | 0.49 |
| 37.7 | 25C | Pheophytin a-like2 | - | | - | | - | | - | | 2.14 | 0.76 |

^aGarcia-Pichel & Castenholz (1991)

green thin biofilm was formed, releasing oxygen bubbles under light exposure. During the following months, mats became thicker and mainly grew above the sediment surface. After 8.5 to 9.5 mo mats reached a thickness of 2.5 to 5 mm, comprising both a green upper layer (1.5 to 3 mm thick) and an underlying light pink layer (1 to 2 mm thick). The surface of mats was mainly dark green with a few light green and brown patches. Structures similar to the pinnales described for Antarctic microbial mats (Simmons et al. 1993) were also present at the surface of the mats.

Pigment composition and structure of artificial mats

In artificial mats, 97 to 98% of the total chlorophyll a (chl a) pool was concentrated in the green upper layer (Table 1, Fig. 1) while bchl a, when it was detected (Mat 2), mainly occurred in the underlying pink layer (74% of total concentration). Thus, the underlying pink layer included the bulk of phototrophic purple bacteria (Fig. 2). Further, the presence of hydroxyspheroidene in the pink layers indicated that these anoxyphototrophs comprised the rhodospirillacean purple non-sulphur bacteria (Imhoff & Trüper 1992).

HPLC analyses of biomarker pigments (Table 1, Fig. 1) indicated that the green upper layers comprised both cyanobacteria (echinenone, myxoxanthophyll [myxo] and scytonemin2) and green microalgae (chl *b*, lutein, violaxanthin, violaxanthin-like neoxanthin and antheraxanthin). Canthaxanthin (cantha) and β -carotene may be found both in cyanobacteria and in green microalgae (Jeffrey et al. 1997). Microscopic observations of aliquots from the green upper layers suggested that green microalgae were mainly *Chlorella* sp. that were previously found in Antarctic ice, freshwater and benthic mats (Malatoni & Tesolín 1997). Using the pigment ratio lutein/chl *a* of 0.22 from pure cultures of *Chlorella* sp. (Schlüter et al. 2000), it was estimated that green microalgae represented 23% in Mat 1 and 7.5% in Mat 2 of the total chl *a* pool in the green upper layers. This suggests that cyanobacteria were dominant in the green upper layers. It has been reported that scyto may yield a red form when it is exposed to a reduced environment and especially to H₂S (Garcia-Pichel & Castenholz 1991). This 'reduced' red form of scyto was detected in the pink layer (scytonemin1), indicating that cyanobacteria associated with purple phototrophic bacteria contributed to the pink coloration of the underlying layer (Table 1). The chl *a*/pheopigments ratio averaged 34 to 59 in Mat 1 and 95 to 227 in Mat 2. Although bchl *a* was not detected in Mat 1, in Mat 2 the bchl *a*/bacteriopheophytin *a* ratio reached 4 to 33. These values indicate that cyanobacteria and purple phototrophic (Mat 2) communities were both in a healthy state.

Comparison with pigment composition of natural field microbial mat

Most of the biomarker pigments detected in field samples were also present in artificial mats (Table 1). However, zeaxanthin was found in field samples but not in artificial mats. Although zeaxanthin may be found in chlorophytes, the absence of chl *b* in field mat samples indicated that zeaxanthin had originated from cyanobacteria (Fig. 3). This suggests that the artificial conditions of culturing did not allow the growth of zeaxanthin-producing cyanobacteria from field mat. No green microalgal pigments were detected in field samples, indicating that, in contrast, the artificial mat community was enriched in microalgae (Table 1). The non-cyanobacterial pigment detected in field samples was bchl *a*; however, the averaged concentration was 5 times lower than in Mat 2, indicating that Mat 2 was also enriched in purple phototrophic bacteria.

The chl *a*/pheopigments ratio, averaging 2.7 ± 1.3 in the field mat, was 12 to 84 times lower than in artificial

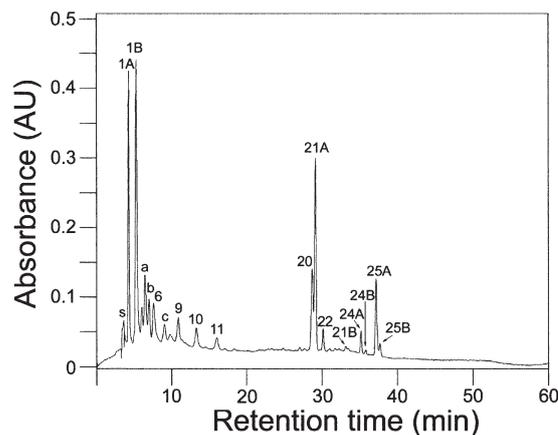


Fig. 3. Example of absorbance chromatogram at 440 nm obtained from extracts of field mat subsamples (Lake Fryxell, Antarctica). For peak identification see Table 1. Myxoxanthophyll derived pigments were probably the cause of peaks a, b and c

mats. This difference mainly resulted from both the higher concentration of pheophytin *a* and the presence of 2 additional forms of pheophytin *a* (pheophytin *a*-like) in field samples (Table 1, Fig. 3). These pheophytin *a*-like pigments have been previously found in pigment samples from natural microalgal communities (e.g. Villanueva et al. 1994, Buffan-Dubau et al. 1996, Le Bris et al. 1998, Buffan-Dubau & Carman 2000b). The senescence of microalgae results in accumulation of pheopigments in sediments. Thus, the low chl *a*/pheopigments ratio in natural samples reflected the difference in age between field and artificial mats. Chl *a* concentration in field sample was either similar to or lower than that in artificial mats. Collectively, these results suggest that the artificial conditions used for culturing artificial mats were appropriate. However, additional differences in pigment ratios were noticed. In natural field samples the chl *a*/scyto ratio varied between 0.3 and 1 only, against 40 to 854 in the upper green layer and 0.7 to 11.6 in the pink underlying layer of artificial mats. Further, the chl *a*/(myxo + cantha) ratio was higher in artificial mats (from 35 ± 6 to 68 ± 23) than in field mat samples (14 ± 4).

Steady-state profiles of oxygen and photosynthesis in artificial microbial mats

For each mat, steady-state oxygen profiles were measured at 6 different locations (spots) under the light exposure that we used for culturing. These measurements indicated that oxygen distribution through the mat was heterogeneous (Fig. 4). The maximum depth of oxygen penetration varied from 1.5 to 4 mm

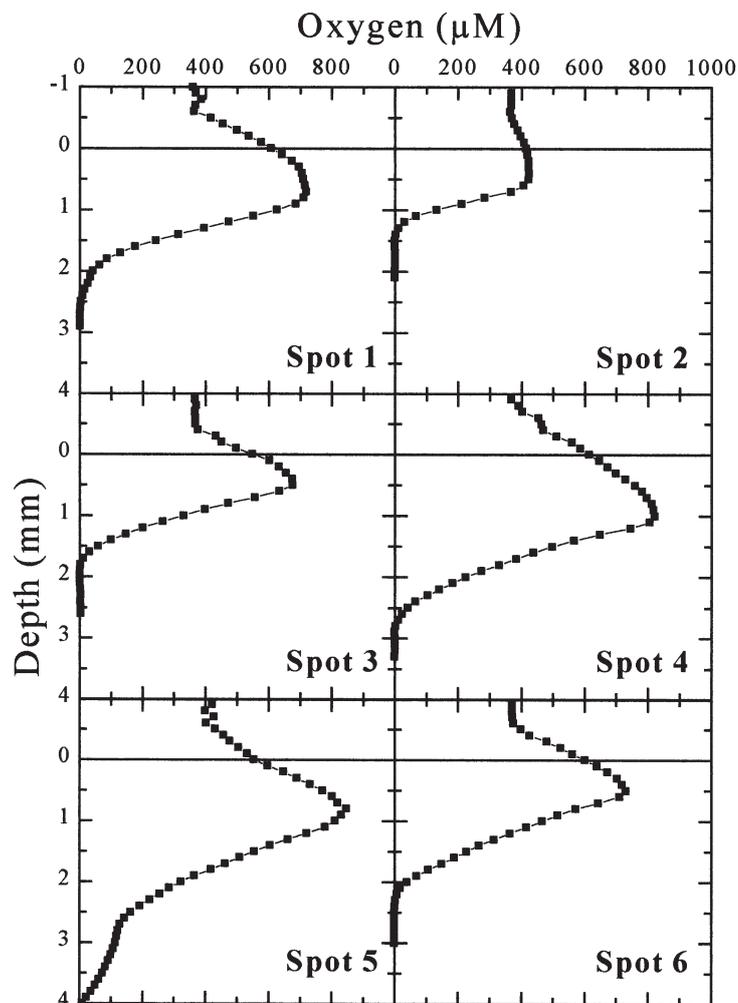


Fig. 4. Averaged light steady-state microprofiles ($n = 3$) of oxygen at 6°C and at 6 different spots of Artificial Mat 1. Incident irradiance was $70 \mu\text{mol photons m}^{-2} \text{s}^{-1}$

and averaged 2.5 mm. This range of oxic layer thickness matched the thickness of the green upper layers of mats. A peak of oxygen concentration appeared between 0.5 to 1 mm below the mat surface (Fig. 4) and the maximum concentration ranged from 420 ($1.12 \times$ air saturation) to 840 μM ($2.24 \times$ air saturation). An example of gross photosynthesis rate profile and its associated oxygen fluxes is shown for Mat 1 in Fig. 5. The profile of gross photosynthesis determined a photic surface layer corresponding to the upper part of the green layer depth horizon (Fig. 5). The maximum rate of gross photosynthesis ($4.2 \text{ nmol cm}^{-3} \text{ s}^{-1}$) was located at the depth horizon of the oxygen peak. The net productivity ($3.1 \text{ nmol cm}^{-2} \text{ min}^{-1}$) represented 40% of the gross oxygen production (primary production, Fig. 5) whereas 45% of this oxygen production (i.e. $3.5 \text{ nmol cm}^{-2} \text{ min}^{-1}$) was consumed by the mat itself for respiration in the light, and 15% was dedicated to respiration in the aphotic layer (Fig. 5).

DISCUSSION

Artificial cyanobacterial mats from temperate environments have been previously cultured with success (Fenchel 1998a,b, Fenchel & Kühl 2000). Such artificial systems are of great value for the study of physiology and turn-over rates in microbial mats and can be used to study the adaptation of community metabolism to different environmental conditions, e.g. temperature (Pringault et al. 2001). However, to our knowledge, we report for the first time the formation of cold-adapted artificial microbial mats and their structure, during experimental cultures of microbial communities from Antarctica. We provided the opportunity to study living cold-adapted microbial mat communities as components of undisturbed structured artificial mats over long periods of time.

Mat thickness was consistent with measurements from Antarctic benthic mats (Vincent et al. 1993a). The increment of artificial mats was estimated as their thickness above the mineral sediment surface and assuming that growth was near to linear (Fenchel & Kühl 2000). The increment would have ranged from 2.3 to 5.3 mm within the year. This is consistent with the estimate of 2 mm yr^{-1} previously determined for artificial microbial mats grown between 17 and 25°C (Fenchel 1998a, Fenchel & Kühl 2000). More generally, accretion of the artificial mats at 4 to 7°C was in the range of the rates

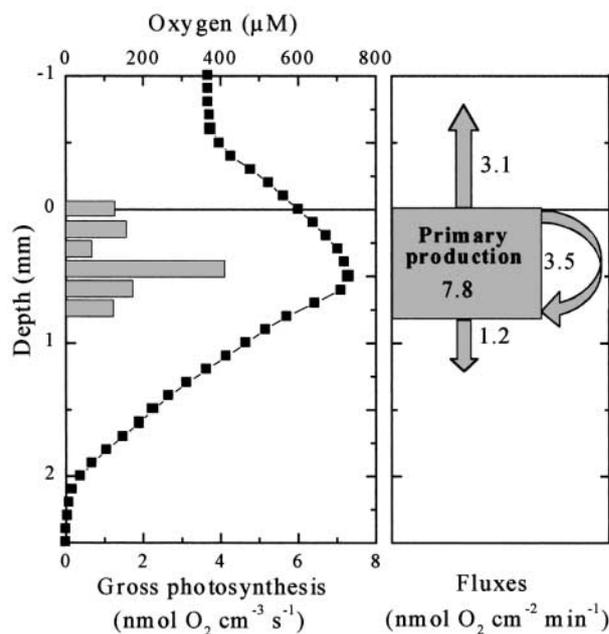


Fig. 5. Example of gross photosynthesis, oxygen distribution (left side), and primary production and its related oxygen fluxes (right side), for Spot 6 in Mat 1

found for natural cyanobacterial mats: from 0.5 to 5 mm yr⁻¹ in saline lakes (Bauld 1981) and from 1–2 to 10 mm yr⁻¹ in benthic marine environments (van Gernerden 1993). Thus, the artificial mat microbial communities grew in BGC as rapidly as many field mat communities in their environment. This strongly suggests that artificial mat microbial communities were adapted to cold conditions. Physiological cold adaptation and temperature effects on the metabolism of these microbial mat communities were studied by Pringault et al. (2001).

The basic vertical zonation of phototrophic microorganisms in artificial mats was similar to those reported for natural cyanobacterial mats, i. e. an oxic layer dominated by cyanobacteria overgrowing an anoxic pink layer of purple phototrophic bacteria (Wharton et al. 1983, van Gernerden 1993, Vincent et al. 1993a). A detailed comparison of the cyanobacterial species composition requires, however, the study of genetic information such as a fingerprinting technique for the 16S ribosomal RNA genes. The artificial mats formed pinnacles, which are also a feature of some naturally occurring benthic microbial mats in Antarctic lakes (Simmons et al. 1993). However, the artificial mats were unusual in respect to the presence of green microalgae. Generally, when microalgae are present in microbial mats, diatoms rather than green microalgae occur with cyanobacteria in the oxic layer (Simmons et al. 1993). These green microalgae (mainly *Chlorella* sp.) were not detected in field samples, suggesting that the experimental conditions of culture favoured their growth. Fast-growing green microalgae such as *Chlorella* sp. have high nutrient uptake capabilities (Tang et al. 1997b), and Antarctic lakes from Dry Valleys are characterised by low concentrations of nutrients (Priscu et al. 1989). Thus, growth of green microalgae in artificial mats was probably favoured by the high ammonium content of the artificial culture media (approximately 90 mg l⁻¹ NH₄⁺ at the beginning of the experiments) compared to field conditions (10 to 20 µg l⁻¹ NH₄⁺, Priscu et al. 1989). This hypothesis is consistent with the observations that we reported, indicating that green microalgae were relatively less abundant in Mat 2 than in Mat 1 (see 'Results'). For Mat 1, ammonium was directly added in the upper medium of BGC1 and, thus, was instantaneously available for the nutrient requirement of the fast-growing *Chlorella* sp. (see 'Materials and methods'). In contrast, for Mat 2, ammonium was only added in the lower medium underlying the core and, thus, became progressively available for the growth of *Chlorella* sp. by the diffusion process.

It could be expected that the cyanobacterial community of both artificial mats and field samples comprised the major mat-forming species previously identified in

Antarctic mats, such as the filamentous *Phormidium frigidum* Fritsch, ubiquitous in Dry Valley lakes, *Lyngbya martensiana*, often present in Lake Fryxell (Simmons et al. 1993), and *Nostoc commune*, found in Fryxell Stream (Vincent & Quesada 1994). We compared pigment analysis results with those reported by Vincent & Quesada (1994) for *Nostoc commune* mats originating from Fryxell Stream and for oscillatoriacean mats from Brack Pond (McMurdo Ice Shelf, Antarctica). It appeared that the HPLC absorbance chromatogram from *Nostoc commune* mats resembled those obtained in the present study for field samples (Fig. 3): i.e. scytonemin was its major peak and it reflected a high scyto/chl *a* ratio (Vincent & Quesada 1994). In contrast, oscillatoriacean mats contained no scyto but were rich in myxo, echinenone and cantha. All these pigments were well represented in the present study (Table 1). This supported our microscopic observations of samples indicating that Oscillatoriacea were present in both artificial and field mats. However, in artificial mats, 63% of total scyto concentration occurred in the underlying pink layer while 93 to 98% of total concentration of oscillatoriacean carotenoids was concentrated in the upper green layer. This indicates that both types of cyanobacteria were differently distributed within the mats: while the carotenoid-producing cyanobacteria (Oscillatoriacea) were restricted to the green upper layer, scyto-producing cyanobacteria (probably *Nostoc commune*, although further studies are required for identification) occurred deeper, from the surface throughout the green and the pink layers. Pringault et al. (2001) have indicated that sulfide was present in the artificial mats, at the depth horizon of the underlying pink layer. It is known that sulfide may be toxic for cyanobacteria. Cohen et al. (1986) have shown that the cyanobacterial resistance level to sulfide toxicity varies among species. Thus, it is possible that the observed differences in vertical distributions of cyanobacteria were partly based on their differential sensitivity to sulfide toxicity.

Scyto is a UV screening pigment from the extracellular sheath of some cyanobacteria playing an important role of photoprotection against the deleterious effects of the high UV radiation occurring in Antarctica (Garcia-Pichel & Castenholz 1991, Vincent et al. 1998, Dillon & Castenholz 1999). Myxo and cantha are also involved in the photoprotection of cyanobacterial cells, and all of these pigments are UV-inducible (Garcia-Pichel et al. 1992, Vincent & Quesada 1994, Cockell & Knowland 1999). This probably explains the drastic differences in chl *a*/photoprotectant-pigment ratios between artificial mats and field samples that pigment analyses have pointed out, i.e. ratios were drastically lower in field mat samples: on average 62 to 183 times lower for chl *a*/scyto ratio and 3.4 to 5 times lower for

Table 2. Net primary productivity inferred from oxygen microprofiles in different microbial mats. NP: net productivity in $\text{nmol O}_2 \text{ cm}^{-2} \text{ min}^{-1}$

| Origin | Type | NP | Irradiance ^a | Temp. (°C) | Source |
|---------------------------|------------|------------|-------------------------|------------|-----------------------|
| Wismar Bay (Germany) | Natural | 12 | 180 | 21 | Epping et al. (1999) |
| Solar Lake (Egypt) | Natural | 6 to 23 | 425 | 25 to 40 | Wieland & Kühl (2000) |
| Cap Evans (Antarctica) | Natural | 0.5 to 2.6 | 10 to 40 | -1.8 | McMinn et al. (2000) |
| Nivå Bay (Denmark) | Artificial | 7.2 | 413 | 20 | Kühl & Fenchel (2000) |
| Lake Fryxell (Antarctica) | Artificial | 0.5 to 3.1 | 70 | 6 | This study |

^aIrradiance value for photosynthetic active radiation (PAR) in $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$

chl *a*/(myxo + cantha) ratio. This probably resulted from the difference in quality and intensity between the artificial incandescent light used for the cultures of mats (very low UV-A radiation, Pringault et al. 1996) and the natural high UV radiation occurring in the field linked to the development of the Antarctic ozone hole. This suggests that the artificial light was less harmful than the field natural light to the mat cyanobacterial community from Lake Fryxell.

Chl *a* concentrations determined for artificial and field mats (Table 1) were lower than values reported for cyanobacterial mats from non-polar environments: e.g. 1500 to 2100 $\mu\text{g g}^{-1}$ dry weight at the surface of solar saltern microbial mats originating from the Ebre Delta in Spain (Villanueva et al. 1994), and between 900 and 2000 $\mu\text{g g}^{-1}$ dry weight in the top 1 mm of cyanobacterial mats from hypersaline ponds in Guerrero Negro, Mexico (Palmisano et al. 1989). To compare our results with previous reported values from Antarctica (Vincent et al. 1993a, Quesada et al. 1998), chl *a* concentrations were converted into $\mu\text{g cm}^{-2}$: $59.4 \pm 21 \mu\text{g cm}^{-2}$ for Mat 1 and $117 \pm 59 \mu\text{g cm}^{-2}$ for Mat 2 in the green upper layer. Subsequently, it appeared that chl *a* concentrations were in the range of those previously published for natural Antarctic cyanobacterial mats: around 45 to 50 $\mu\text{g cm}^{-2}$ for Ross Island Pond (Vincent et al. 1993a) and 17 to 28 $\mu\text{g cm}^{-2}$ (Quesada et al. 1998). As observed for chl *a* concentration, net primary productivity values inferred from oxygen microprofiles in artificial microbial mats were lower than data published for non-polar environments but were in the range of those reported for natural Antarctic microbial mats (Table 2). These observations are consistent with recent studies indicating that psychrotrophic cyanobacteria reduced their chl *a* content and their photosynthetic capacity at low temperatures (Tang et al. 1997a, Tang & Vincent 1999). Thus, collective considerations of pigment content and primary productivity indicated that the artificial microbial mats presented some characteristics of natural polar mats, although some differences between artificial mats and field mat have been determined.

Acknowledgements. We are grateful to Cathy Welch, who carried out sampling in Lake Fryxell (Antarctica), and the Long-Term Ecosystem Research Program (LTER). The US Antarctic program facilitated the sampling. Thanks to Astrid Massé, Emmanuelle Lemaire and H el ene Lecordier for assistance in the laboratory work. This work was supported by a grant from the European research program MICROMAT (Microbial Diversity of Microbial Mats in Antarctica, contract No. BIO4-CT98-0040).

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*Editorial responsibility: Karin Lochte,
Rostock, Germany*

*Submitted: February 26, 2001; Accepted: October 5, 2001
Proofs received from author(s): November 27, 2001*