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# Sulphate Reducing Activity Detected in Soil Samples from Antarctica, Ecology Glacier Forefield, King George Island

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### Abstract

We determined sulphate-reducing activities in media inoculated with soils and with kettle lake sediments in order to investigate their potential in geomicrobiological processes in low-temperature, terrestrial maritime Antarctic habitats. Soil and sediment samples were collected in a glacier valley abandoned by Ecology Glacier during the last 30 years: from a new formed kettle lake sediment and forefield soil derived from ground moraine. Inoculated with these samples, liquid Postgate C and minimal media supplemented with various carbon sources as electron donors were incubated for 8 weeks at 4°C. High rates of sulphate reduction were observed only in media inoculated with soil. No sulphate reduction was detected in media inoculated with kettle lake sediments. In soil samples culture media calcite and elemental sulphur deposits were observed, demonstrating that sulphate-reducing activity is associated with a potential to mineral formation in cold environments. Cells observed on scanning microscopy (SEM) micrographs of post-culture-soil deposits could be responsible for sulphate-reducing activity.

Key words: Antarctica, biogenic minerals, biogeochemical reactions, psychrophiles, sulphate-reducing bacteria

## Introduction

Deglaciation in polar areas provides opportunities to observe microbial processes in the forefields of retreating glaciers. In this respect, the King George Island ice cap lost 65 km<sup>2</sup> between 1956 and 2000 (Simoes et al., 2004), including land exposed in the last 30 years near Ecology Glacier which calves into Admiralty Bay (Birkenmajer, 2002; Pudełko, 2008). Bacteria may play a significant role in the transformation and modification of the abiotic environment in these dynamic and developing ecosystems (Hodson et al., 2008). Despite many studies on anaerobic bacteria being present in soils, marine and freshwater sediments, those in cold environments remain poorly studied (Steven et al., 2006; Zdanowski et al., 2013). However, permafrost conditions are favorable for the activities of anaerobic bacteria such as denitrifiers, acetoclastic methanogens, hydrogenotrophic methanogens, Fe(III) reducers, and sulphate reducers (Gilichinsky et al., 2008).

Life in cold environments must overcome environmental stressors, but microbial activity and growth do occur at temperatures below 0°C (Grzesiak *et al.*, 2009). Morita (1975) termed such microbes "psychrophiles". The lowest temperature at which bacterial growth has been recorded so far is -12°C, for Psychromonas ingrahamii isolated from sea ice of Alaska (Breezee et al., 2004). Microbial activity has also been detected in permafrost at -20°C through uptake of radiolabeled substrates (Margesin et al., 2008). Snow and glacial ice hosts various microorganisms, although their abundance in englacial ice is low, at  $\sim 10^2 - 10^4$ /ml, increasing to as much as 10<sup>9</sup>/ml in the lower parts of a glacier where basement ice is enriched by rock and organic particles. Interestingly, analyses thus far appear to show that microbial communities in glaciers in different parts of the world are similar. Gram positive bacteria (particularly those with high CG content), dominate such communities, followed by Proteobacteria and those in the CFB group. This may reflect the fact that representatives of these groups are easily cultivated in the laboratory under aerobic conditions (Margesin et al., 2008).

In ecosystems low in or lacking diatomic oxygen, microbes obtain energy for metabolic processes through anaerobic respiration using oxidized compounds such as nitrates, nitrites, sulphates, and/or iron

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ions (Fe<sup>3+</sup>) as electron acceptors. Such environments contain products of these transformations in the form of biogenic gases, including nitrogen and hydrogen sulphide, as well as solid and amorphous mineral phases, *e.g.*, elemental sulphur, carbonates (dolomite, calcite), phosphates (*e.g.*, vivianite), or metal sulphides.

Studies of anaerobic microorganisms date back to the late 19<sup>th</sup> century, and they have been detected *inter alia* in marine sediments, microbial mats, rocks and permafrost (Steven *et al.*, 2006). However, little is known about the activity of psychrophilic bacteria and *Archaea* in anaerobic conditions. There are also little data on sulphate reducing bacteria and their role in biogeochemical processes in the terrestrial postglacial zones in Antarctica (Mikucki and Priscu, 2007).

Sulphate reducing bacteria (SRB) are an important physiological group of microorganisms. The first described psychrophilic SRB capable of growth at low temperature was *Desulforhopalus vacuolatus* (Isaksen and Teske, 1996). SRB play a significant role in the biodegradation of organic matter in all anaerobic environments rich in sulphates and accessible organic compounds. Sources of sulphate for SRB in terrestrial environments may include the easily soluble mirabilite (Na<sub>2</sub>SO<sub>4</sub> · 10H<sub>2</sub>O), as well as poorly soluble gypsum (CaSO<sub>4</sub> · 2H<sub>2</sub>O) (Karnachuk *et al.*, 2002; Wolicka and Kowalski, 2006). On King George Island, however, sulphates are mostly of marine origin, being transported to land in aerosols and by direct coastal saturation (Barczuk and Tatur, 2003).

King George Island, located ~100 km north of the Antarctic Peninsula, is the largest of the South Shetland Islands. The island measures ~95 by ~25 km, and covers 1310 km<sup>2</sup> (Angiel and Dąbski, 2012; Birkenmajer, 2002). The geology of the study area is complex, and according to radiometric sphere integrating source (SIS) and micropaleontological data, rocks here formed in the Late Cretaceous to Miocene (Nawrocki et al., 2011); those rock formations include volcanic rocks (continental lavas and pyroclastic deposits), that are genetically linked by a calc-alkaline intrusive and extrusive island of arc magmatic processes (Birkenmajer, 2001). Plant remains have been found in some of the volcanoclastic sediments (Birkenmajer, 2001). Some of Eocene, Oligocene and Lower Miocene strata are represented by marine and glacial-marine deposits. Hypabyssal and plutonic magmatic intrusions have also been seen on the island. The former includes laccolites, dykes and sills. Erosion has exposed medium-sized magmatic plutons in the Barton block, and they co-occur with low-grade metamorphic rocks (Birkenmajer, 2001; Yeo et al., 2004).

The work described here focused on detecting psychrophilic SRB, and assessing their contribution to geomicrobial processes in glacial deposits of Ecology Glacier, King George Island, Antarctica.

# Experimental

# Materials and Methods

**Field site and samples.** Samples were collected from two sites: 1) Land-water interface of a recently formed (5 years ago) kettle lake 150 m from the front of the Ecology Glacier forefield, termed here 'W0' (GPS 62° 10' 7.5" S; 58° 27' 54.4" W) (Fig. 1); 2) At 50 cm intervals along a 1.5 m transect from the lake shore towards the dry ground moraine, termed 'W1,' 'W2,' 'W3' (GPS 62°10'2.5" S; 58°27'59.6" W) (Fig. 1).

These sites may be exposed to air or be periodically covered by lake water; the lake is located close to the glacial lagoon, which is periodically inundated by the sea during high tides, and also by water from the melting Ecology Glacier. Lake water pH was 6.8. Sampling sites were thus influenced (due to infiltration) by both the glacier and the nearby sea.

Up to 500 g of soil (0–20 cm deep), was collected with sterile plastic spatulas into sterile plastic bags during the 2008/2009 austral summer.

Samples were placed in a freezer  $(-20^{\circ}C)$  and stored until returned to a laboratory in Poland where geomicrobiological research was conducted.

**Media.** Sterile 250 ml glass bottles containing 200 ml Postgate C medium (Na<sub>2</sub>SO<sub>4</sub> – 4.5 g/l, NH<sub>4</sub>Cl – 1 g/l, yeast



Fig. 1. Location of sample sites. W0 – nearshore deposits of a kettle lake near the forefield of Ecology Glacier; W1, W2, W3
– from a ~1.5 m transect from the kettle lake shore towards the ground moraine (map modified from Pudełko, 2003)

extract – 1 g/l,  $KH_2PO_4$  – 0,5 g/l, sodium citrate  $\cdot$  2H<sub>2</sub>O  $- 0.3 \text{ g/l}, \text{ CaCl}_{2} \cdot 6\text{H}_{2}\text{O} - 0.06 \text{ g/l}, \text{ MgSO}_{4} \cdot 7\text{H}_{2}\text{O}$  $- 0.06 \text{ g/l}, \text{ FeSO}_{4} \cdot 7\text{H}_{2}\text{O} - 0.004 \text{ g/l})(\text{Postgate}, 1984)$ or minimal medium (Na<sub>2</sub>SO<sub>4</sub> – 4.5 g/l, NH<sub>4</sub>Cl – 1 g/l) were inoculated with 10 g of each soil sample and hermetically sealed to promote selection of sulphidogenic anaerobic bacteria. Different sole carbon or carbon/ nitrogen sources were provided: butanol (3 g/l), casein (6 g/l), glucose (6 g/l), lactose (6 g/l), acetate (6 g/l), phenol (0.5 g/l), ethanol (4 g/l), lactate (6 g/l), citrate (3 g/l), starch (4 g/l), urea (4 g/l), oxalate (4 g/l), benzene (0.5 g/l), toluene (0.5 g/l) ethylbenzene (0.5 g/l), and xylene (0.5 g/l). A total of 64 cultures were established, 32 of which contained material from sampling site W0, and 32 contained pooled material from sites W1-W3. The cultures were incubated at 4°C for eight weeks in a stationary manner in a Bioblock Scientific Incubator type 200031. After the incubation period, the cultures were transferred to 50 ml falcon-type tubes and cetrifuged at 10000×g for 10 min (type 5810 centrifuge, Eppendorf) to separate the post-culture liquid from the non-soluble deposit.

Analyses. Chemistry. Sulphate concentrations were determined at the end of the 8 week incubation period in a clear post-culture supernatant by a turbidimetric method using barium chloride and a Thermo spectrophotometer at  $\lambda = 400$  nm (Ristow *et al.*, 2005) Sulphate reduction activity was indicated as iron sulphide turbidity that was determined in uncetrifugated liquid medium at the end of the 8 week incubation period in a Shimadzu UV-1800 spectrophotometer at  $\lambda = 605$  nm (Hart *et al.*, 1992). Iron sulphide turbidity of  $OD_{605} < 0.3$  was considered as no  $SO_4^{2-}$  reduction (-), of  $OD_{605} = 0.3 - 0.7$  as poor  $SO_4^{2-}$  reduction (+), of  $OD_{605} = 0.7 - 2.0$  as good  $SO_4^{2-}$  reduction (++), and of  $OD_{605} > 2.0$  as excellent  $SO_4^{2-}$  reduction (+++). Sulphate reduction was calculated as a differential between the amount of sulphates introduced into the medium and the amount found at the end of the incubation period.

**Mineral composition.** Soil and post-culture deposits were analysed by powder X-ray diffraction (Lei *et al.*, 2009). Soil samples were pre-dried at 40°C, and only silt/clay was analysed. It was separated by sieve (0.063 mm mesh) and ground in an agate mortar. Post-culture deposits were centrifuged at  $10000 \times g$  before drying at 40°C in an oxygen-free atmosphere. Samples were analyzed in a diffractometer (X-Pert PRO MPD). Diffraction was measured in a capillary (DSH) or by a classical Bragg-Brentano method, depending on the amount of precipitate extracted (Edwards *et al.*, 2007).

**Microscopy.** Minerals and microorganisms in the soil and post-culture deposits, (along with their size, shape, form and spatial relationships were observed through scanning electron microscopy (SEM). Samples for SEM were prepared on a basic glass slide. One

drop of the post-culture fluid was transferred from the medium with a sterile pipette to the slide and air dried while covered. Dried preparations were coated with gold and observed in a JSM-6380LA Jeo Microscope in the Laboratory of Electron Microscopy and Microanalysis, Faculty of Geology, University of Warsaw.

### **Results and Discussion**

Sediment sample characteristics. Pedogenic processes in the postglacial King George Island area are at an early stage due to the low input of energy and short period of deglaciation. According to Lee et al. (2004), soil composition in such climatic conditions depends on the type of host rock, and the dominance of physical over chemical weathering processes. Multiple cycles of freezing and thawing are recorded in many soils in the area in the form of polygonal structures on the surface, as well as an increase in the degree of rounding and decrease in grain size (Ugolini and Bockheim, 2008). Mineral composition fundamentally influences the physico-chemical properties of rocks; that of the studied samples was extremely variable, with the presence of plagioclases, smectites and zeolites, and lesser amounts also of quartz and chlorites in the silt/clay fraction of all soil samples (Fig. 2). Samples were collected from the surface of a clay/silt bottom moraine of Ecology Glacier, composed of weathering products of basaltic andesite lavas, their pyroclastics and with minor participation of sedimentary rock derived from these rocks.

Selection of sulphidogenic microorganism communities. The aim of this paper was to determine the sulphate-reducing activity of enriched anaerobic bacteria communities and to estimate their influence on biogeochemical processes. Sulphidogenic microorganisms were cultivated on both modified Postgate C and a minimal medium. Modification of these media comprised use of different organic carbon sources, with a ratio of organic carbon to sulphates of 1:1, which is favourable for SRB development (Hao *et al.*, 1996). A total of 64 microcosm cultures were established for the samples collected, with 32 on Postgate C medium, and 32 on the minimal medium (Table I).

The results indicate very different sulphidogenic potential of the two soils. Samples from the kettle lake shore towards the moraine were characterized by high sulphate reducing activity (iron sulphide precipitation) in almost all cultures, with the exception of those to which BTEX compounds were added. Iron sulphide precipitation was also noted, yet lower, in cultures grown on the minimal medium, as indicated by the incomplete blackening of the medium. In this respect, the Postgate medium was more favourable for sulphate reducing activity connected probably with the



Fig. 2. Field sample diffractograms. Soil samples were collected at 50 cm intervals along a 1.5 m transect from the lake shore towards the ground moraine.

W1 – 50 cm from the lake shore; W2 – 100 cm from the lake shore; W3 – 150 cm from the lake shore. Abbreviations: Pl – plagioclase, S – smectite, Cl – chlorite, Z – zeolite, Q – quartz

Table I Detection of iron sulphide precipitate in post-culture liquids of Postgate C and minimal medium with different single carbon and carbon/nitrogen sources as an indicator of sulphate reduction.

Carbon source	Sediment from land-water interface of a kettle lake		Soil from ground moraine	
	Medium			
	Postgate	Minimal	Postgate	Minimal
Oxalate	+	-	-	+
Butanol	+	-	+++	+
Casein	+	-	+++	+
Glucose	-	-	++	+
Lactose	-	-	++	+
Acetate	-	-	+++	+
Phenol	+	-	+	+
Ethanol	+	-	+++	+
Lactate	+	-	+	+
Citrate	+	-	+++	+
Starch	-	-	+	+
Urea	-	-	+	+
Benzene	-	_	-	-
Toluene	-	-	-	-
Ethylbenzene	-	-	-	-
Xylene	-	-	-	-

Iron sulphide turbidity of OD<sub>605</sub> < 0.3 was considered as no SO<sub>4</sub><sup>2-</sup> reduction (-), of OD<sub>605</sub> = 0.3–0.7 as poor SO<sub>4</sub><sup>2-</sup> reduction (+), of OD<sub>605</sub> = 0.7–2.0 as good SO<sub>4</sub><sup>2-</sup> reduction (++), and of OD<sub>605</sub> > 2.0 as excellent SO<sub>4</sub><sup>2-</sup> reduction (++)

growth and development of SRB. In cultures grown on this medium the highest sulphate reducing activity of communities was noted in those containing casein, citrate, butanol, ethanol and acetate as the sole carbon sources. In these microcosms, complete blackening of the medium was observed, so these substrates were the most preferable for SRB. This also reflects the inability of SRB to synthesize hydrolytic enzymes for anaerobic digestion of organic matter, and their use rather of low molecular weight organic compounds such as: organic acids (acetates, oxalates, lactates, citrates), or alcohols, e.g., ethanol, butanol (Fauque et al., 1991). It is likely that in both natural and anthropogenic conditions, synergistic sulphur cycling develops, i.e., 'sulfuretum', wherein bacteria are spatially and metabolically linked, such that the product of the activity of one group of is the substrate for another (Baas-Becking, 1925; Trüper, 1984).

Among the 64 microcosms, only those prepared from soil samples collected along the 1.5 m transect on the ground moraine were selected for further chemical and microscopic analysis. Sulphidogenic activity was not detected in the kettle lake deposits, *i.e.*, negligible or no blackening of the media was noted.

The amount of reduced sulphates in cultures on Postgate medium with butanol, casein, acetate, ethanol, or citrate exceeded  $2,5 \text{ g SO}_4/1$  after the 8 week incubation period (Fig. 3). Higher amounts of reduced sulphates were observed in Postgate medium than in the minimal medium. High percentage of reduced sul-



Fig. 3. Amounts of sulphates reduced (calculated as a differential between the amount of sulphates introduced into the medium and the amount found at the end of the incubation period in g/l) in post-culture media supernatant inoculated with soil from ground moraine on Postgate C and minimal media with different carbon sources after 8 weeks of incubation.

Symbols indicate carbon source: O – oxalate, B – butanol, C – casein, G – glucose, L – lactose, A – acetate, P – phenol, E – ethanol, La – lactate, Ci – citrate, S – starch, U – urea

phates (in comparison to the same amount introduced to all the media) were noted in cultures with alcohols and organic acids as the sole carbon source (butanol, 65% reduction of  $SO_4^{2-}$ ; ethanol, 62.5% reduction), citrate (62.5% reduction) acetate (62.5% reduction), and also with casein (63.0%). Lower sulphate reducing activity was noted on the minimal medium with the same carbon sources, ranging from 2.5% with oxalate, to 44% with butanol.

Minerals such as sulphur, calcite, and apatite were detected in the post-culture deposits of only 4 of the selected cultures (Fig. 4). SRB communities take part in the formation of minerals, e.g., carbonates such as calcite, dolomite and siderite (Perry and Taylor 2006), phosphates, e.g., apatite (Wolicka and Kowalski, 2006), elemental sulphur (Wolicka and Kowalski, 2006) or sulphides (Labrenz et al., 2000). The formation of calcite or aragonite in environments in which SRB are typically found, and conditions favouring the reduction of sulphates, usually occur in anaerobic, high-salinity lagoons (Warthman et al., 2000), while gypsum and anhydrite (Peckmann et al., 1999) also form in high-salinity lakes that are rich in sulphates (Wright and Wacey, 2004). The calcite occurrence in SRB enrichment cultures results by the reaction of calcium cations contained in the medium with carbonate anions derived from bacterial respiration, forming the insoluble CaCO<sub>3</sub>. Elemental sulphur in such enrichment cultures is formed due to oxidation of sulphides by trace amounts of oxygen and other oxidizing agents (Wolicka and Kowalski, 2006).

In cold environments microorganisms may also participate in weathering processes. Skidmore *et al.* (2005) concluded that sulphide oxidation proceeds in postglacial waters, and that such environments contain bacteria responsible for these reactions. Maurice *et al.* (2002) suggested that decomposition of muscovite in the dry valleys of the Antarctic could result from the activity of bacteria, although there is no direct evidence supporting this process. However, it is possible that microbes producing organic acids, coordination complexes and exopolymers may indirectly influence



Fig. 4. Post-culture deposit diffractograms of media inoculated with soil from ground moraine on Postgate medium with ethanol (A), buthanol (B), lactate (C), acetate (D) as the sole carbon source after 8 weeks of incubation. Abbreviations: G – gypsum, S – sulphur, C – calcite, A – apatite, Q – quartz



Fig. 5. Cells observed in post-culture media inoculated with soil from ground moraine on Postgate medium with casein (A), citrate (B), buthanol (C), ethanol (D, F) and acetate (E) in SEM after 8 weeks of incubation

the decomposition of minerals present in the rocks, as in other environments. The only impediment may be the generally low availability of water to transport products of bacterial metabolism in the Antarctic. In the South Shetland Islands, however, water availability is relatively abundant, and the activities of microorganisms seem to be considerably high. For example, microbes here cause decomposition and leaching of minerals and rocks, particularly below the cells, and on their surfaces covered by exopolymeric substances (EPS) through accumulation of active substances and their impeded diffusion (Buss *et al.*, 2007). EPS are composed mainly of polysaccharides and amino acids, and are most likely responsible for the accumulation of free metal ions. Moreover, many SRB are capable of developing their own EPS, through which they control precipitation of calcium carbonate (Braissant *et al.*, 2007). SEM revealed the presence of different bacterial cell morphologies, including cocci, bacilli, spirilla and vibrio (Fig. 5). Different mineral forms and EPS were also visible around the microbes.

**Conclusions.** Our findings indicate that sulphatereducing activity was barely detectible in the soil water interface of the kettle lake (W0) through the microcosm method. This may reflect low abundance of culturable sulphate-reducing bacteria in such an environment or that the media composition and incubation period were inappropriate for this type of community. Sulphate-reducing activity was detected at sites exposed earlier by the receding glacier (W1-W3), sites which may have been enriched in nutrients and sulphates through marine aerosols and seabird activity. The mineral-transforming activity in samples with sulphate-reducing activity although efficient in vitro, seems to be of minor impact in the early soils of the Ecology Glacier foreland as none of the post-culture compounds were found in the native samples. However, high sulphate-reducing activity observed in the relatively nutrient-rich media hints that it could be an important mineral processing factor in environments where organic matter is abundant, e.g., tundra covered soil, or penguin rookeries. Further, interdisciplinary studies comprising microbiology, geochemistry and mineralogy are required to provide scales and rates of these activities in such environments.

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