POLISH POLAR RESEARCH
vol. 32, no. 2, pp. 181–195, 2011 doi: 10.2478/v10183-011-0012-x

Analysis of cultivable aerobic bacteria isolated from bottom sediments in the Wijdefjorden region, Spitsbergen

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Abstract: The paper presents the first physicochemical and microbiological studies conducted in the northern area of Svalbard (Spitsbergen). Ten sediment samples were collected from the bottom of the longest fjord in the region, Wijdefjorden. Bottom sediments from ten lakes located along the shores of Wijdefjorden and Woodfjorden were also sampled. Organic matter content (LOI), water content, temperature, pH, and salinity of the sediments were determined. The quantity of aerobic bacteria cultured on various growth media at 4°C, 14°C, and 37°C ranged from 10^2 to 10^6 cfu/g of wet sediment mass, depending on the type of sampling station (fjord or lake). The number of bacteria did not correlate with organic matter content. Out of the 37 bacterial strains isolated from Wijdefjorden, 48% and 70% revealed ureolytic and proteolytic activity, respectively. The proportion of freshwater strains with ureolytic and proteolytic activity was 32% and 55%, respectively. Antibiotic resistance testing indicated that bacterial strains from the bottom sediments of the lakes were resistant to 8 antibiotics (out of the 18 investigated). Possible sources of this resistance are discussed. Using 16S DNA analysis, bacterial isolates from the lakes were identified as *Pseudomonas* sp., whereas frequently occurring strains in bottom sediment of the fjord were *Pseudoalteromonas* sp.

Key words: Arctic, Svalbard, psychrophilic bacteria, bottom sediments.

Introduction

The southern area of Spitsbergen (the Svalbard Archipelago in the Arctic Ocean) has been intensively studied in terms of biotic and abiotic environmental

factors for many years. In contrast, its northern coast remains less researched, both in terms of microbiology as well as physical and chemical factors. Hydrological conditions of Svalbard are shaped mainly by the circulation of two sea currents: the warm West Spitsbergen Current, carrying Atlantic waters, and the cold East Spitsbergen Current, originating in Arctic waters (Midttun 1990; Loeng 1991; Haugan 1999; Proshutinsky *et al*. 1999; Jones 2001; Løyning 2001).

Polar environments are successfully colonized by cold-adapted organisms (psychrophiles and psychrotrophes) thriving at temperatures close to the freezing point of water (Morita 1975; Hoyoux 2004). Psychrophilic microorganisms are mostly Gram-negative bacteria classified as α-, β-, and γ-proteobacteria, or belong to the *Cytophaga–Flavobacterium–Bacteroides* group, but some are also Gram-positive bacteria of the genera *Arthrobacter*, *Micrococcus*, and *Corynebacterium* (D'Amico *et al*. 2006; Männistö and Häggblom 2006).

The microflora of bottom sediments often contains bacteria with diverse and wide-ranging biochemical properties. Modern investigations of total microflora from polar regions include metagenomic analysis (Yergeau *et al.* 2010); however, to determine the level of metabolic activity of microorganisms it is still necessary to cultivate and isolate bacterial strains (Groudieva *et al.* 2004; Srinivas *et al.* 2009; Vardhan *et al.* 2009). Several publications have demonstrated that strains isolated from the bottom sediments of polar lakes and fjords are proteolytic and lipolytic at 5°C (Groudieva *et al.* 2004; Männistö and Häggblom 2006; Srinivas *et al.* 2009). Extremozymes, active at the temperature range from 0°C to 20°C, may also be a potential tool for biodegradation of organic waste and have some applications in the food and chemical industries (Dube *et al.* 2001; Alam *et al.* 2005).

Microorganisms with different patterns of biochemical activity are involved in biogeochemical processes. One of the more important ones is nitrogen circulation, where bacteria with proteolytic and/or ureolytic activity are essential for the mineralization of organic matter (Sutyła *et al.* 2009). Microorganisms from the polar regions (bacteria and archaea) could also be applied as indicators of biogeochemical changes (Bahr *et al*. 1996; Panzenböck *et al*. 2000). Human activity may cause alterations to environmental microflora. One of the symptoms of such alterations is the introduction of antibiotic resistance determinants, which were previously selected in human and animal populations as the result of the widespread use of antibiotic therapy or prevention (Davies J. and Davies D. 2010).

The main aims of the presented study were as follows:

- to quantify the physicochemical properties of the bottom sediments of Wijdefjorden and nearby freshwater lakes;
- to identify cultivable aerobic microorganisms using the molecular method;
- to measure the quantities of cultivable aerobic microorganisms (growth at 4°C, 14°C, and 37°C) isolated from two different classes of bottom sediments from northern Spitsbergen (Wijdefjorden and the freshwater lakes in the Wijdefjorden region);

– to test the proteolytic and ureolytic activity as well as antibiotic resistance of bacterial strains isolated from bottom sediments.

Materials and methods

Research area. — This study is focused on bottom sediments from two different environments: Wijdefjorden and freshwater lakes around Wijdefjorden (Fig. 1).

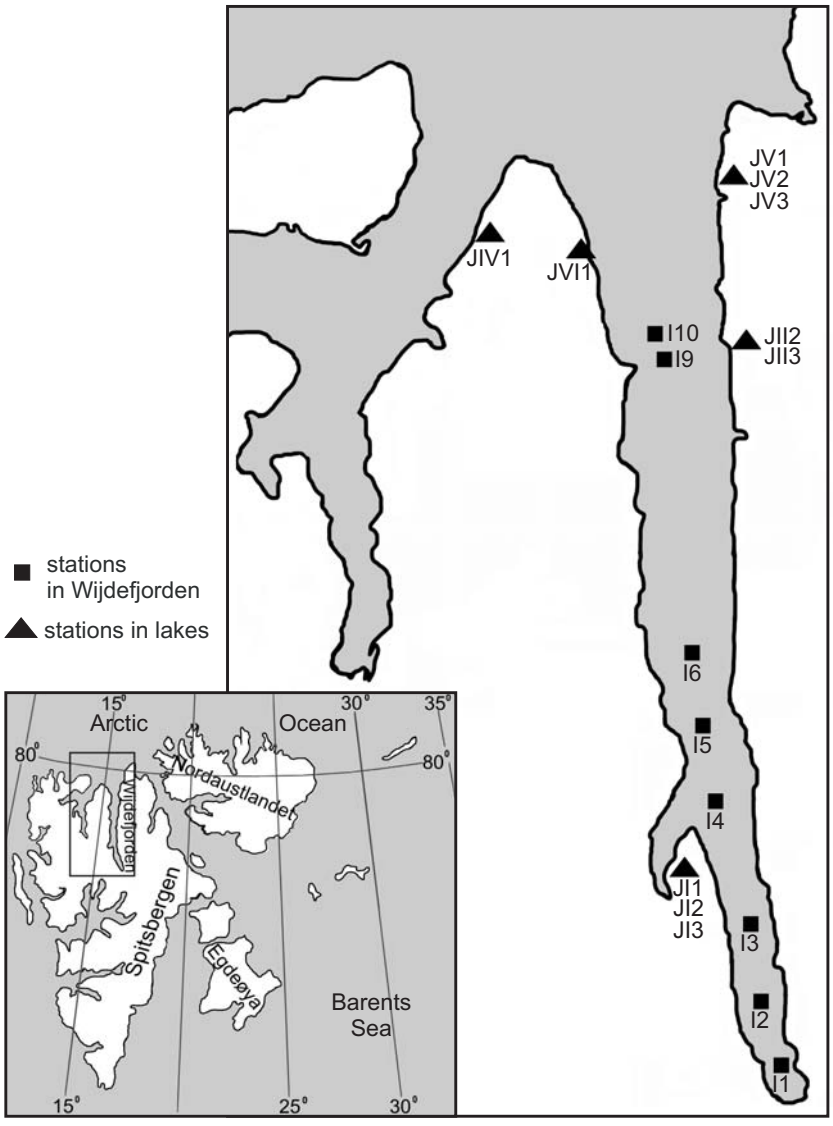

Fig. 1. Study area: location of sediment sampling stations.

The Wijdefjorden stations (I1–I6, I9 and I10) were located along the fjord. Stations JI1, JI2, JI3 were located in three small and shallow lakes near the mountain Skirshorg, close to the eastern coast of Vestfjorden (Wijdefjorden). The lakes have different genetic and morphometric properties. The hydrological conditions change from strong discharging in the rain and snowmelt season to (almost/complete) dried during the dry summer season. The area around the lakes in the terminal moraine of the Yggbreen Glacier is devoid of vascular plants such as ferns, gymnosperms, or angiosperms. Two stations (JII2 and JII3) are located in the northern part of the lateral moraine of the Nordbreen Glacier, which drains to Wijdefjorden. Plants around this basin were sparse. The lake with station JII2 was located in the slack ice at the border between moraine and glacier. The lake with station JII3 was located close to the draining place of the lateral moraine into the sea. Sampling station JIV1 was located in a big lake close to the east coast of Woodfjorden (Selungane region). The lake waters, with significant suspended matter content, originated from snowmelt, rains and glacier ablation. Station JV1 was located in a very big lake on the east coast of Woodfjorden, with its origins and properties similar to JIV1. Stations JV2 and JV3 were located close to the area of Woodfjorden. Station JV2 was in a big lake (about 0.1 ha) supplied by small rivers, with cold desert plants on the coast and outflow to the sea. Station JV3 was located in a smaller lake, seasonally without outflow. Sampling station JVI1 was located in a typical big fjord lake on the western side of Wijdefjorden (close to the Vogtdalen). The water in this lake originated from rivers and the glacier.

Sample collection. — Surface sediment samples (0–5 cm) were collected in the period 23rd–30th July 2005. The samples were taken (Table 1, Fig. 1) using an Ekman grab with a surface of 23 × 23 cm. Water temperature was measured at each sampling station.

Physicochemical analysis. —Water content (W%) was determined by drying sediment samples at 105°C to the point of reaching constant mass (about 12 hours). Organic matter content was determined by loss on ignition (LOI %), which was measured by heating sediment samples to 550°C for 8 hours. Sediment type was determined on the basis of granulometric analysis (Myslinska 1998). Chemical analyses of the total amounts of phosphorus (P_{TOT}), nitrogen (N_{TOT}), and ammonium ions (NH_4^+) in the bottom sediment samples were performed by standard oceanographic methods (Grasshoff *et al*. 1983; Kirkwood 1994). For each sample, analyses were performed twice and mean values were calculated. Relative standard deviation (RSD) for ammonium ion concentration was less than 5%, and for phosphorus and nitrogen less than 9%.

Isolation and biochemical characteristics of bacterial strains. — Sampling of bottom sediments was done aseptically and the collected material was transported and stored at 4°C in aerobic conditions for up to one week prior to analysis. Microorganisms were isolated by intensive shaking of the bottom sediments in

Table 1

Characteristics of the research area and surface sediments. Note: ms – muddy-sandy (mud>sand), sm – sandy-muddy (sand>mud), smg – sandy-muddy with gravel (sand>mud).

Station	Geographical position		Depth [m]	Sediment type	Temperature [°C]	Salinity [psu]	Water content [%]	LOI [%]
	φ	λ						
Fjord								
I1	78°54.40' N	16°24.60' E	16	ms	2.7	32.0	32.8	4.77
I2	79°00.40' N	16°19.10' E	60	ms	2.1	29.3	40.5	4.97
I3	79°05.00' N	16°05.00' E	60	ms	3.6	29.9	41.2	4.35
I4	79°10.40' N	15°49.30' E	56	ms	3.3	31.4	37.9	4.25
I5	79°15.00' N	15°44.00' E	76	ms	1.3	29.9	38.5	3.89
I6	79°20.10' N	15°42.90' E	101	ms	2.2	34.9	48.6	4.75
I9	79°38.40' N	15°45.30' E	16	sm	4.6	34.6	32.8	1.65
I10	79°39.90' N	15°38.00' E	119	sm	3.2	31.7	43.0	3.21
Lakes								
JI1	79°06.20' N	15°37.91' E	0.3	ms	5.0	8.7	39.2	4.25
JI2	79°06.25' N	15°37.93' E	0.3	ms	4.3	11.4	24.5	2.79
JI3	79°06.34' N	15°37.91' E	0.3	ms	4.2	0.2	25.1	2.24
JII2	79°38.53' N	15°38.33' E	0.3	ms	0.8	0.0	16.7	0.43
JII3	79°38.45' N	15°38.93' E	0.3	ms	9.9	0.2	24.6	0.95
JIV1	79°43.58' N	14°24.12' E	0.3	ms	6.2	0.0	17.7	3.96
JV1	79°48.18' N	15°40.01' E	0.3	smg	3.2	0.0	11.1	0.48
JV2	79°48.20' N	15°37.87' E	0.3	sm	7.1	0.0	71.9	26.40
JV3	79°48.14' N	15°37.56' E	0.3	ms	7.1	0.0	39.1	6.24
JVI1	79°43.11' N	14°53.12' E	0.3	smg	6.8	0.4	12.7	2.78

sterile 0.85% water solution of NaCl. After the sedimentation of insoluble particles, the supernatant (undiluted and diluted up to 10^6 times) was spread on three Petri's plates with the following media: enriched (peptone 4.0 g/l, beef extract 0.4 g/l, enzymatic casein hydrolysate 5.4 g/l, yeast extract 1.7 g/l, sodium chloride 3.5 g/l, agar 10.0 g/l; Biomed), M9 (Na_2HPO_4 6.0 g/l, KH_2PO_4 3.0 g/l, NH_4Cl 0.5 g/l, agar 15.0 g/l, 1M $MgSO_4$ 1.0 ml, 1M $CaCl_2$ 0.1ml, 50% glucose 4.0 ml), King B (peptone 20.0 g/l, K_2HPO_4 1.5 g/l, $MgSO_4 \times 7\ H_2O$ 1.5 g/l, glycerol 10.0 ml, agar 15.0 g/l; Graso), Pochon (soluble starch 2.0 g/l, asparagine 0.05 g/l, Winogradsky standard salts 50.0 ml/l, nystatin 0.1 g/l, agar 15.0 g/l) and Sabouraud (tryptone 5.0 g/l, peptone 5.0 g/l, glucose 40.0 g/l, chloramphenicol 0.05 g/l, agar 15.0 g/l) agars. Plates were incubated in the dark under aerobic conditions at 4°C, 14°C, and 37°C for 14, 7 and 1 day, respectively. The amount of bacteria per 1 g of wet sediment was determined basing on plates which contained from 30 to 300 colonies. Isolated bacterial cells were stained using Gram's method. Proteolytic activity was determined on plates with agar supplemented with skimmed milk (Wassif *et al.* 1995; Männistö and Häggblom 2006), while ureolytic properties were tested on Christensen medium with urea (Christensen 1964; MacFaddin 1985). The determination of this activity was performed at 4°C, 14°C, and 37°C, depending on the

temperature used for the isolation of particular bacterial strain. The antibiotic resistance of isolated strains was determined at 4°C, 14°C, and 37°C, respectively. It was done by the diffusion-disc method and interpreted according to NCCLS and EUCAST standards. The collected bacterial strain suspensions, mixed with 50% glycerol (1:1 vol), were stored at -70°C.

Multidimensional scaling (MDS) analysis was done with the Primer software (Clarke and Gorley 2001). The analyzed factor was the antibiotic resistance pattern of bacteria isolated from the investigated sediment samples.

Analysis of 16S rDNA sequences. — DNA was isolated using Genomic Mini columns (DNA-GDANSK) or by boiling bacterial cells suspended in deionized water. The primers Com1: 5'-CAGCMGCCGCGGTAATWC and Com2: 5'-CCGTCAATTCMTTTRAGTTT (Lane 1985) were used for PCR. The concentration of the primers was 0.2 µM (each) and the concentration of magnesium was 1.5 mM. Approximately 0.1 ng of total bacterial DNA for each reaction was used as a template in PCR. The total reaction volume was 50 µl. For each reaction, 0.4 u of DyNAzymeII DNA polymerase (Finnzyme) was used. Reaction mixtures were placed at 95°C for 3 min and then subjected to 35 cycles of amplification by incubation for 1 min at 95°C, 30 s at 55°C and 1 min at 72°C. Finally, incubation for 5 min at 72°C was applied. The quality of the obtained PCR products was tested with electrophoresis on 2% agarose gel, followed by staining with ethidium bromide. Then, the PCR products were sequenced using the Beckman-Coulter CEQ™ 8000 Genetic Analysis System and 16S rDNA sequences were identified using the Ribosomal Database Project database, release 10 (http://rdp.cme.msu.edu/).

Results and discussion

The presented work concerns cultivable aerobic microflora from the bottom sediments of the Spitsbergen region, which was not investigated previously. The stations were located along Wijdefjorden (stations: I1 to I6 and I9, I10) and in the vicinity of Wijdefjorden and Woodfjorden (stations: JI1 to JVI1). The studied fjord, Wijdefjorden, is the longest fjord of Svalbard (120 km) with a varying depth of up to 246 m. The fjord opens into the Arctic Ocean and is influenced by Arctic and Atlantic waters during cold and warm years (NOAA, Walczowski and Piechura 2006, 2007). The depth, temperature and chemical parameters of the sediments varied (Table 1, Fig. 1). The content of phosphorus, nitrogen and ammonium ions was analyzed in Wijdefjorden sediments. Total nitrogen content increased along the fjord, except station I3 (Fig. 2). The examined parameters were much more diverse in lake sediments. Salinity in JI1 and JI2 was significantly higher than in the others lakes (8.7 and 11.4 psu, respectively) (Table 1). This might suggest incidental sea flows, infiltration and aerosols.

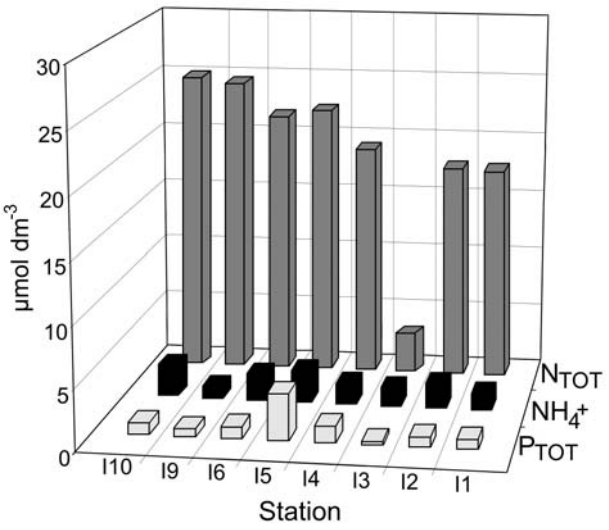

Fig. 2. Amount of phosphorus, nitrogen and ammonium ions in Wijdefjorden sediments.

Physicochemical conditions of bottom sediments, such as temperature, salinity, hydrostatic pressure, oxygen availability, pH, the quantity of organic and inorganic substances, water content, the occurrence of other organisms and, above all, the synergistic effect of all of these factors, determine the quantity and metabolic activity of microorganisms found in these habitats. Higher water and organic matter content in the sediment, and consequently its larger porosity and permeability, also increases the abundance of microflora (Bott and Kaplan 1985; Papageorgiou *et al*. 2007).

The quantities of microorganisms isolated from the samples collected in Wijdefjorden and in the neighboring freshwater reservoirs ranged from 10^2 to 10^6 cfu/g in wet sediments (Table 2). Similar discrepancies concerning the number of cells per gram of dry sediment mass in the topmost layer were also revealed in the studies of other authors: the numbers ranged from 10^3 (Norkrans and Stehn 1978) to 10^7 (Bowman *et al*. 2003).

Among the bacteria isolated from fjord sediments, the amount of psychrophilic strains varied depending on cultivation temperature. At 4°C, the quantity of colonies varied from 10^4 to 10^6 and was generally higher than the amount of bacteria grown at 14°C (from 10^2 to 10^5 at different stations). In three stations (I2, I6, I10), bacterial growth was also observed at 37°C, the number of colonies being about 10^2. This may result from the contact with Atlantic water masses, which flow into Wijdefjorden during the high activity of the West Spitsbergen Current. Along the length of the fjord, rock barriers may hamper water circulation (Kowalewski *et al*. 1990).

No particular tendency in the amount of bacteria cultivated from different lake sediments was observed. In the lake samples, the variation in the number of colonies was higher than in the fjord samples: at 4°C from 10^2 to 10^5, at 14°C from 10^3 to 10^6

Table 2

Amount of psychrophilic bacteria isolated from Spitsbergen sediments. Note: – no growth.

Sample	Amount of microorganisms cultivated on definite medium						
	Nutrient agar at temperature			King B	M9	Sabouraud	Pochon
	4°C	12-16°C	37°C				
Fjord							
I1	2×10^5	1×10^3	–	3.3×10^2	–	–	3.3×10^2
I2	2×10^5	9.9×10^3	1×10^2	6.5×10^3	–	–	4.4×10^3
I3	1×10^5	4.3×10^2	–	–	–	–	2.06×10^3
I4	2×10^5	8.3×10^3	–	4.16×10^3	–	–	1.5×10^3
I5	1×10^6	8.9×10^4	–	8×10^4	–	–	2.05×10^4
I6	2×10^4	2.6×10^4	1.9×10^2	9.1×10^3	9.7×10^2	–	1.2×10^4
I9	1×10^5	1.1×10^5	–	9.5×10^4	–	–	9.5×10^4
I10	2×10^5	3.1×10^3	5.5×10^2	3.8×10^3	–	–	8.2×10^3
Lakes							
JI1	1×10^5	4.2×10^6	1.5×10^3	9.2×10^5	–	–	8.1×10^4
JI2	1×10^4	3.4×10^4	4.7×10^3	3.1×10^2	–	–	1.5×10^3
JI3	8×10^4	4×10^3	–	9.8×10^3	4×10^2	3.2×10^2	6.5×10^4
JII2	9×10^4	1.6×10^4	–	2.2×10^3	–	3.7×10^3	9.9×10^3
JII3	1×10^5	8.3×10^4	–	8.3×10^4	–	–	8.3×10^4
JIV1	4×10^3	–	1.2×10^4	–	–	–	–
JV1	1×10^5	3.2×10^3	–	4.5×10^3	–	–	2.8×10^3
JV2	6×10^4	2.4×10^4	1.2×10^3	4.2×10^3	–	6×10^2	1.1×10^4
JV3	1×10^3	4.5×10^6	–	1.5×10^4	1.3×10^4	5.8×10^3	1.8×10^6
JVI1	9×10^2	1×10^3	–	1.07×10^3	–	–	8.4×10^2

(but in one sample (JIV1) there was no growth at all at this temperature), and at 37°C from 10^3 to 10^4 (but growth was observed only in JI1, JI2, JIV1 and JV2). The significant majority of the number of colonies cultivated in 4°C or 14°C in samples from lakes located in the northern part of Wijdefjorden region was not observed.

There was no clear correlation between the quantity of cultured bacteria and the content of the organic component in the sediments. This was also reported by Mauro and Danovaro (1998), who observed high bacterial biomass and density at stations with poorer trophic conditions. However in the present work, a slight relationship between the quantity of cultured bacteria (in particular for strains cultivated at 14°C) and total phosphorus concentration was noted for bacteria isolated from the Wijdefjorden sediments. Phosphorus is crucial for bacterial cells, as it is a component of numerous molecules (e.g., DNA, RNA, phospholipids or ATP). Moreover, Miettinen *et al.* (1997) reported that phosphorus is a factor limiting bacterial growth in fresh waters.

Bacteria isolated from the lakes were more influenced by the environmental factor like higher and lower temperatures than those from Wijdefjorden stations. The variety of bacteria grown from the samples may be explained by the fact that

some bacteria (psychrotrophs) are able to grow in a temperature-unstable environment (Swiecicka *et al*. 1997).

Fungal cells were observed only in freshwater reservoirs. Bacterial colonies were also found on Sabouraud agar. In all samples, numerous strains grew on Pochon medium; the majority of them stained as Gram-negative rods. *Actinomycetales* were not observed on this medium, which was confirmed microscopically. It cannot be excluded that Pochon medium, which is specific for *Actinomycetales* isolation according to Polish Standard PN-Z-04111-02:1989, in fact may not be optimal for the cultivation of microorganisms from Arctic sediments. Hamaki *et al*. (2005) show that the best medium for soil *Actinomycetales* is soil-extract agar, while other rich or mineral media give poorer results.

From the investigated sediment samples, 123 bacterial strains were isolated, including frequently presented as well as unique colonies. For all the isolated strains, Gram staining and the determination of proteolytic and ureolytic activity were performed. Most of the isolated bacteria stained Gram-negative with the exception of three strains isolated from Wijdefjorden and 11 strains isolated from the lakes. The majority of the bacterial strains isolated from the sediments of Wijdefjorden had the ability to decompose milk proteins (26 out of 37 strains, which is 70%) and urea (18 out of 37 strains, which is about 48%).

Proteolytic and ureolytic activity was also observed for strains isolated from the freshwater reservoirs of the Wijdefjorden and Woodfjorden coast. Over 55% (48 out of 86 strains) were able to hydrolyze milk. Most microorganisms found in

Table 3

Amount of bacterial strains isolated from Spitsbergen sediments with ureolytic and/or proteolytic activity. Numbers in the columns indicate the quantity of strains with a specific activity; +++ strong activity; ++ moderate activity; + weak activity; – no activity.

Station	Proteolytic activity				Ureolytic activity			
	+++	++	+	–	+++	++	+	–
Fjord								
I1	1	3	0	0	0	0	0	4
I2	1	2	0	1	1	0	0	3
I3	0	2	1	0	0	1	0	2
I4	0	1	0	4	1	1	0	3
I5	0	2	2	0	2	0	0	2
I6	0	5	0	2	4	2	0	1
I9	0	3	0	0	2	0	0	1
I10	2	1	0	4	2	2	0	3
Lakes								
JI1	2	0	0	5	0	1	0	6
JI2	1	2	0	5	0	0	0	8
JI3	12	0	0	4	1	3	1	11
JII2	5	0	0	3	0	0	1	7
JII3	6	0	0	0	2	0	0	4
JIV1	3	0	0	0	0	1	0	2
JV1	4	2	0	1	1	3	2	1
JV2	1	2	1	14	3	2	1	12
JV3	1	2	0	2	1	3	0	1
JVI1	2	2	0	4	0	1	1	6

the lakes revealed a strong reaction (zone of total milk hydrolysis of over 13 mm, which was marked in Table 3 as +++). In the case of ureolytic activity, only 32% (28 out of 86 strains) of the isolated bacteria were characterized by the ability to decompose urea (Table 3). Few works have been published about the activity of hydrolytic enzymes from bacteria isolated in polar regions, especially Spitsbergen. Groudieva *et al.* (2004) showed that about 56% of bacteria isolated from sea ice and sea water from four fjords in Spitsbergen revealed proteolytic activity in contrast to strains from sediment samples from Kongsfjorden or sediment and soil samples from Ny-Ålesund (Svalbard) (Srinivas *et al.* 2009). Analyses of the ureolytic activity of psychrophilic bacteria are even fewer. Shivaji *et al.* (2011) studied sediments from a freshwater lake in Antarctica and found that only about 20% of the strains revealed ureolytic activity. The above data are completely contradictory. As our results are inconsistent with them, it seems possible that the Wijdefjorden area has an unique microflora.

For 26 bacterial strains with proteolytic and/or ureolytic activity, patterns of sensitivity to 18 different antibiotics were identified (Table 4). The majority of the strains isolated from Wijdefjorden sediments were sensitive to the antibiotics tested. Only one strain (out of the nine examined) was resistant to antibiotics belonging to three different classes (out of the eight examined). A frequent occurrence (five of the 9 strains) of resistance to sulfamethizole was found. In contrast to the fjord isolates, strains from the lakes were resistant to amoxicillin (100% of the tested strains), aztreonam (59%), cefotaxime, imipenem and nalidixic acid (29% each), doxycycline (18%), tetracycline (41%), sulfamethizole (88%), trimethoprim (94%), cinoxacin and chloramphenicol (76%). Multidimensional scaling analysis (MDS, Fig. 3), based on the Bray-Curtis faunistic similarity measure, revealed few clusters of antibiotic resistance patterns of isolated strains. This may in-

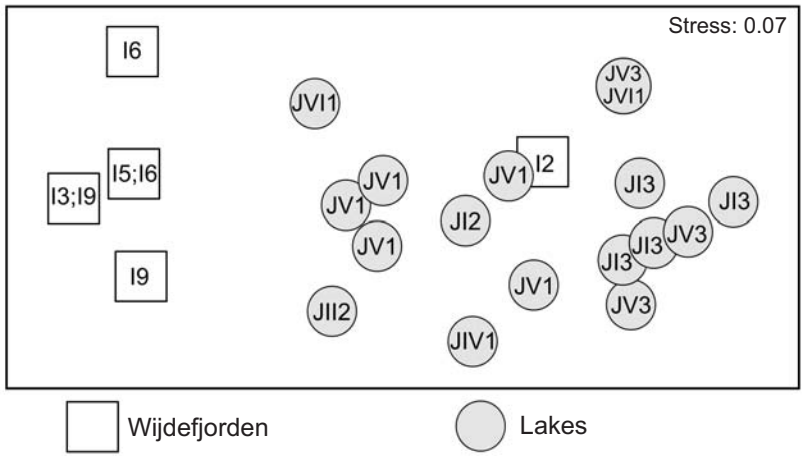

Fig. 3. MDS analysis (Primer-E v.5) of resistance to antibiotics of bacterial strains isolated from Spitsbergen sediments.

Table 4

Antibiotic resistance patterns of Gram-negative bacteria isolated from sediments in the investigated fjord and lakes. Note: AMX – amoxicillin, ATM – aztreonam, CFP – cefoperazone, CTX – cefotaxime, IPM – imipenem, PIP – piperacillin, TZP – piperacillin + tazobactam, D – doxycycline, TE – tetracycline, GM – gentamycin, NN – tobramycin, C – chloramphenicol, TH – sulfamethizole, TM – trimethoprim, CIN – cinoxacin, CIP – ciprofloxacin, NA – nalidixic acid, OFX – ofloxacin; R – resistant; I – intermediate; S – sensitive; * in the inhibited growth zone there were several colonies of resistant clones.

Station	No. of strains with particular pattern	Antibiotic																	
		AMX	ATM	CFP	CTX	IPM	PIP	TZP	D	TE	GM	NN	TH	TM	CIN	CIP	NA	OFX	C
Fjord																			
I2	1	R	R	I	I	S	S	S	S	S	S	S	R	R	I	S	I	S	R
I3	1	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
I5	2	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S
I6	2	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S
	1	S	I	S	S	I	S	S	S	S	S	S	S	S	S	S	S	S	S
I9	1	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S
	1	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Lakes																			
JI2	1	R	S	I*	S	S	S	S	S	S	S	S*	R	R	R	S	I	S	R
JI3	1	R	R	I	R	R	I	S*	R	R	S	S	R	R	R	S	R	S	R
	1	R	R	I	R	R	S	S	S	R	S	S	R	R	R	S	I	S	R
	1	R	R	S	I	R	S	S	I	R	S	S	R	R	R	S	R	S	R
	1	R	R	S	I*	I	S	S	I	R	S	S	R	R	R	S	R	S	R
JII2	1	R	S	S	S	S	S	S	S	S	S	S	I*	R	R	S	S	S	I*
JIV	1	R	R	S	R	S	S	S	S	S	S	S	S	R	R	S	S	S	R
JV1	1	R	R	S	R	S	S	S	S	R	S	S	R	R	R	S	S	S	R
	1	R	R	I	S	S	S	S	S	S	S	S	R	R	R	S	I	S	R
	1	R	I	S	S	S	S	S	S	I	S	S	R	R	I	S	S	S	S
	1	R	S	S	S	S	S	S	S	S	S	S	R	R	I	S	S	S	R
	1	R	I	S	S	S	S	S	S	S	S	S	R	R	I	S	S	S	S
JV3	1	R	I	I	I	R	I	I	S	S	S	S	R	R	R	S	I	S	R
	1	R	R	S	R	S	S	S	R	R	S	S	R	R	R	S	R	S	R
	1	R	R	I	I	I	S	S	R	R	S	S	R	R	R	S	R	S	R
JV1	1	R	I	I	I	R	I	I	S	S	S	S	R	R	R	S	I	S	R
	1	R	R	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	I

dicate that bacterial strains from Wijdefjorden sediments have a more uniform pattern in comparison to bacteria from fresh water reservoirs. The only exception among the fjord -isolated bacteria is the strain 7 from station I2, which has an antibiotic resistance pattern similar to the strains from lake sediments.

The 16S rDNA sequencing method was applied to identify cultivable aerobic bacteria isolated from bottom sediments. Seven strains were selected for analysis, based on their frequencies of isolation and antibiotic resistance patterns. Strain marked as no. 7 is resistant to five of the tested antibiotics (Table 4), and as such it belongs to a minority of the strains isolated from I2 station. Strains 19 and 28 were frequently isolated from the bottom sediments of Wijdefjorden. Strains 19 and 18 were sensitive to most of the antibiotics used. Strains 64, 68, 83, and 115 were isolated from lake sediments and represented diverse antibiotic resistance patterns; two of them were widespread and two of them were rare, respectively. The results of PCR analysis of 16S rRNA genes of the seven strains and its sequencing results are presented in Table 5. The frequently occurring strains 19 and 28 isolated from Wijdefjorden sediments (sensitive to the antibiotics tested) belonged to the genus *Pseudoalteromonas*. Strain no. 7, which was highly resistant to the antibiotics tested, was classified as *Pseudomonas* sp. Four strains (64, 68 83 and 115) isolated from lake sediments belong to genus *Pseudomonas* (Table 5).

Table 5

Identification of isolated bacterial strains by 16S rDNA sequencing. Note: R – rare (less than 10 colonies per plate); F – frequent (more than 10 colonies per plate).

Station	Frequency of appearance	Strain no.	The nearest taxonomic neighbour/ accession number	Similarity (%)
Fjord				
I2	R	7	*Pseudomonas* sp. /FJ002582.1	100
I5	F	19	*Pseudoalteromonas* sp. /EU935098.1	99
I9	F	28	*Pseudoalteromonas* sp. /EU935098.1	100
Lakes				
JI3	F	64	*Pseudomonas* sp. /FJ424504.1	99
	R	68	*Pseudomonas* sp. /FJ424509.1	98
JIV1	R	83	*Pseudomonas* sp. /FJ386496.1	99
JV3	F	115	*Pseudomonas* sp. /FJ379535.1	97

It is worth stressing that bacterial strains isolated from lake sediments are resistant to 8 out of the 18 antibiotics tested. Based on EUCAST standards (www.eucast.org/eucast_disk_diffusion_test), the isolated strains may be considered non-wild isolates as a result of resistance to aztreonam and imipenem.

The sources of multi-antibiotic resistant strains isolated from the bottom sediments of lakes in the Wijdefjorden area remain to be elucidated. One possible explanation is horizontal gene transfer (Nwosu 2001; D'Costa *et al*. 2006; Baquero *et al*. 2008; Davies J. and Davies D. 2010). Another possibility is long-range transport of pollutants or bacterial cells (Pacyna *et al.* 1985; Barrie 1986; Beine *et al.*

1996; Burkow and Kallenborn 2000; Rose *et al*. 2004; Sapota *et al*. 2009; Yukimura K. *et al.* 2009). Analysis and isolation of bacterial strains from areas with limited human activity could be a good indicator of the occurrence of anthropogenic pressure on industry-free and clean natural areas, such as the studied Wijdefjorden region.

Acknowledgements. — The authors would like to thank J. Różański, the captain of s/y *Eltanin*, for his help in collecting samples, as well as students M. Suska, J. Banasik, and E. Małysza for their work with bacterial strain isolation. This study was supported by grant no. NN304044639 from the Ministry of Science and Education, Poland and grant no. BW-1411-5-0256-5 from Gdansk University. Some of the experiments were run on apparatus purchased with EU grant *2.2 Innovation Industry*.

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Received 21 December 2010
Accepted 14 April 2011