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Microbial community at the front of Ecology Glacier (King George Island, Antarctica): Initial observations

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Abstract: Since 1978 the retreat of Ecology Glacier in the vicinity of *Henryk Arctowski* Station has opened new ice-free areas for colonization by terrestrial organisms initiated by pioneer microbes. Samples were collected from the soil surface, at 0, 5 and 20 cm below surface close to glacier front, then stored at below -20°C. Total bacterial count (TC), estimated by epifluorescence microscopy, reached high values, of 10^{10} g^{-1} dry wt. Healthy looking bacterial cells of mean volume 0.0209 μm^3 at 0 cm to 0.0292 μm^3 at 20 cm made up from 7% at 0 cm, to 30% at 20 cm of total bacterial population. The number of colony forming units (CFU) accounted for only 0.02% of TC. Taxonomically they belonged to the α, β, γ subdivisions of the proteobacteria and to the *Cytophaga-Flavobacterium-Bacteroides* (CFB) group. Morphophysiologically CFU bacteria were diverse, from Gram variable short coccal forms to very long rods or filaments. Randomly selected CFU colonies were characterized by low sugar assimilation and high esterase/lipase activity. Spore forming bacteria – absent from 0 and 5 cm, formed a small fraction of 175 cells g^{-1} dry wt at the 20 cm depth. Filamentous fungi were relatively abundant and represented mainly by oligotrophs.

Key words: Antarctica, glacial retreat, ice-free areas, microbial communities.

Introduction

Increasing temperature in Western Antarctic including the Antarctic Peninsula and South Shetland Islands archipelago (Kejna *et al*. 1998; Vaughan *et al*. 2001; Turner *et al*. 2005) leads to a rapid retreat of ice fronts and increased water production (Rignot 1998; Cook *et al*. 2005). It is expected that changes in terrestrial and

marine ecosystems will drastically influence the activities of organisms in these areas (Ohtonen *et al*. 1999). On the other hand, recently deglaciated areas, present in different glacial zones in the world, are available for colonization and primary succession, especially by microorganisms, plants (Massalski *et al*. 2001) and animals (Kaufmann *et al*. 2002). During deglaciation, bacterial abundance and distribution, and high biodiversity in "new" marine and terrestrial environments has been observed at the edges of glaciers globally (Bolter and Kanda 1997; Kastovska *et al*. 2005; Nemergut *et al*. 2007), although the process of new environments colonization by microbes is still unknown.

The aim of this report is to describe the structure of the microbial community in terms of abundance and morphophysiology in barren soil at the front of Ecology Glacier in the vicinity of *Henryk Arctowski* Station (King George Is.). To understand better the specificity of such assemblages they were compared with those from other microbial habitats in the maritime Antarctic.

Materials and methods

Samples were collected from the following sites (Fig. 1):
– at the front of the glacier from 0, 5 and 20 cm depth; fine silty sand; (62°10'183"S, 58°28'209"W) – site 1;
– at the farther outskirts of glacier; surface; fine silty sand mixed with macroalgae detritus; (62°10'159"S, 58°27'989"W) – site 3; and in the vicinity of that, but without detritus (62°10'162"S, 58°27'854"W) – site 2.

For comparison we used microbial data obtained from soil moraine containing no identifiable plant debris, soil below a penguin rookery (Zdanowski and Węgleński 2001), fresh penguin guano and guano after 42 days of experimental exposure in situ (Zdanowski *et al.* 2005) and mixed fresh macroalgae and macroalgae decomposed to detritus.

All these sites were highly variable and included soils from newly ice-free areas open for the colonization after retreat of the glacier and from other bacteria habitats.

Analyses

Bacterial counts (total bacterial counts by epifluorescence microscopy, TC and colony forming units: CFU) were determined in 1 g wet weight of soil sample from all sites pooled together in conical Pyrex (100 ml) flasks. Each sample, suspended in 20 ml of sterile saline in 100 ml sterile flasks with glass beads, was shaken gently on a shaker at 120 r.p.m. for 20 min. at 5–10°C. After 10–20 min of particle sedimentation a decimal dilution series to 10^{-5} of the supernatant in 1% saline was prepared.

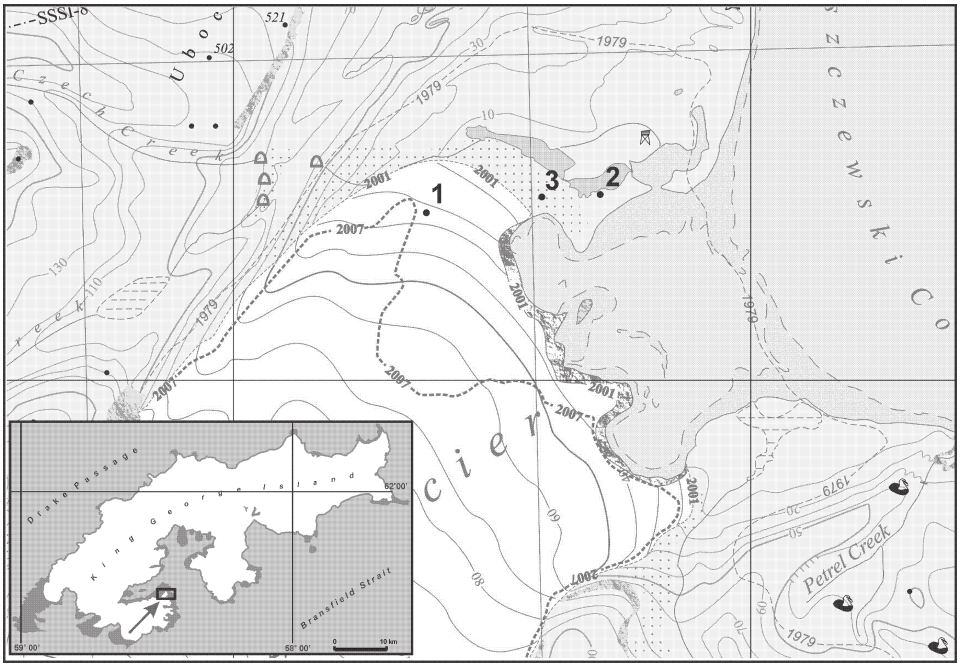

Fig. 1. Location of the sampling sites 1, 2 and 3 on the Ecology Glacier forefield. Part of the Admiralty Bay map (Pudełko 2003, 2008).

For direct counts homogenates were fixed with buffered formalin to a final concentration of 1%. Direct counts by epifluorescence microscopy were performed using 4'6-diamidino-2-phenylindole (DAPI) on black Nuclepore polycarbonate 0.2 mm pore size filters (Porter and Feig, 1980) under a Nikon E-200 microscope equipped with a 100 W Hg lamp and a 100× CFI 60 oil immersion objective, with digital DS Cooled Camera Head DS-5Mc-U1, using a filter block of wavelengths EX 330-380, DM 400, BA 420. Images of fields were analyzed in LUCIA 4.82 image processing and analysis software (Laboratory Imaging, Prague, CZ). A minimum of 400 cells in 20 fields per sample were counted automatically in the image analysis system. Average values from three measurements using three independently prepared filters were estimated.

Culturable bacteria and filamentous fungi (colony forming units, CFU) were enumerated by the spread plate method (0.1 ml) from the decimal series of the sample suspension on Soil Extract Agar (SEA) (for isolation of copiotrophic bacteria) and 100x diluted NA for oligotrophic bacteria (Ogram and Feng 1997). Spore-forming aerobic bacteria were described in terms of Colony Forming Units (CFUs): 10 ml of water was incubated at 80°C for 10 min. then mixed with 10 ml of double-strength NA and poured into Petri dishes (Fenchel and Hemmingsen 1974). Nutrient SEA medium was prepared as previously described (Fenchel and Hemmingsen 1974; Klement *et al*. 1990; Zdanowski *et al*. 2005). Mean CFU

counts were calculated from three replicates on the basis of the number of colonies present at the end (25–30 days) of the incubation at 4 °C.

A diversity analysis based on cell morphotypes (cocci, rods, curved) evaluated in five volume classes (< 0.1; 0.1–0.2; 0.2–0.5; 0.5–1.0 and > 1 μm^3) was conducted with the modified Shannon index (Świętecki 1997; Nübel *et al*. 1999).

For each site an average of 15–20 colonies were randomly selected, and subcultured for purification through repeated transfers on the same medium and physiological investigations. Isolates represent the population of the most abundant bacteria forming similar colonies (based on colony growth characteristics and morphology). In all, 53 colonies were selected and examined for cell morphology and physiology analysis. Gram stain, ability to grow at 4, 22 and 32°C on a nutrient rich medium, ability to grow in the presence of 4% (w/v) NaCl in the medium, and responses in API 20NE, API ZYM and API 20 C AUX (API bioMérieux) were determined. Potential identification through API systems was based on comparing the observed profile to taxa in the API database (% i.d.) (ApiWeb®) and determining proximity to the most typical profile in each of the taxa (T index). A cluster analysis was used to compare isolates identified through the API system and 16S rRNA gene sequencing. A dendrogram showing the hierarchical classification of isolates based on the tests described was constructed by the simple matching coefficient of Sokal and Michener (1958) in association with the weighted pair-group-average algorithm (Sneath and Sokal 1974). Bacterial DNA isolation, rDNA amplification and sequence analysis were performed as described previously (Zdanowski *et al*. 2004).

Results and discussion

The sampling sites were situated in a *ca* 0.2 km^2 area at the front of Ecology Glacier. Relatively high TC abundance was observed (Table 1). This arise through enrichment of the soil surface bacterial population by microbes, eg. flushed from the glacier during melting (Christner *et al.* 2003; Stibal 2006). This is supported by oligotrophic and psychrotrophic character of the microorganisms detected in the soil in front of glacier. Interestingly, TC in this region were higher at the surface than at 5 cm depth, and the highest at 20 cm. This may result from rather unstable and/or diverged conditions (temperature, water flow, nutrition abundance and pH) on a soil surface. Observed TC value correlated with rather low biodiversity described by Shannon Index was the highest at 5 cm depth. The CFU/TC value correlated with the soil richness, was the highest (34.5% TC) in soil samples collected below penguin rookery (Table 2). On the other hand, samples from the front of the glacier contained only few CFU (0.03 of TC) and were dominated by non-sporulating oligotrophs which were more common in samples obtained from deeper layers (5 and 20 cm).

Table 1

Characteristics of microbial community in soil at the front of Ecology Glacier. Abbreviations: C_{bact} – bacterial carbon, CFUoligo., CFUcopio. – colony forming units oligotrophic and copiotrophic

Compound	At the forefield of the glacier			Further outskirts of glacier	
	0 cm	5 cm	20 cm	surface with macroalgae detritus	surface without detritus
Total count (g^{-1} dry wt)	1×10^{10}	4.01×10^{9}	7.67×10^{10}	1.45×10^{10}	5.96×10^{9}
C_{bact} (g^{-1} dry wt)	88.50	67.46	974.12	378.1	167.8
Biovolume ($\mu m^3 cell^{-1}$) av.	0.027	0.028	0.029	0.146	0.125
Length ($\mu m\ cell^{-1}$) av.	0.43	0.48	0.47	0.76	0.66
Width ($\mu m\ cell^{-1}$) av.	0.24	0.23	0.25	0.38	0.56
Shannon Index	0.34	0.51	0.34	0.78	0.79
CFUoligo. g^{-1} dry wt	5.58×10^{5}	1.80×10^{6}	1.63×10^{6}	3.44×10^{7}	3.52×10^{5}
CFUcopio. g^{-1} dry wt	2.18×10^{6}	2.41×10^{6}	7.95×10^{5}	2.30×10^{7}	1.08×10^{5}
Spore forming units (g^{-1} dry wt)	0	2	175	51	206
Fungi oligo. (g^{-1} dry wt)	5.20×10^{5}	2.46×10^{6}	6.07×10^{5}		
Fungi copio. (g^{-1} dry wt)	7.26×10^{3}	1.72×10^{6}	9.80×10^{4}		

Table 2

Colony forming units (CFU) to total count (TC) ratio in soil at the head of glacier and from area surrounding Ecology Glacier

Bacterial habitat	TC	CFU	CFU/TC (%)
(1) at the head of glacier (average)	1.03E+10	1.79E+06	0.017
(2) soil without detritus	5.96E+09	1.08E+05	0.002
(3) soil with detritus	1.45E+10	2.30E+07	0.159
(4) streamlet near glacier	6.43E+08	4.00E+05	0.060
(5) moraine with plant debris	6.65E+08	5.05E+07	7.59
(6) soil below penguin rookery	9.76E+08	3.37E+08	34.5
(7) fresh penguin guano - PG	1.02E+10	2.82E+07	0.276
(8) PG remnants after 42 d	2.19E+11	2.00E+11	91.3

Additionally, besides bacteria, surprisingly high counts of oligotrophic filamentous fungi were observed in barren soil samples at the head of glacier. This may suggest a contribution of water runoff from glaciers to the enrichment of barren soils at the front of glaciers in microbial populations from cryoconite holes (Christner *et al.* 2003; Mueller and Pollard 2004). Such high fungi counts were found at the highs of the Scotia Arc moss-peat communities (Vishniak 1993). The frequency of filamentous fungi at the head of Ecology Glacier was correlated with the sampling depth. In contrary to bacteria, fungi were most abundant in soil collected from 5 cm depth. This observation may suggest some negative interactions between bacteria and filamentous fungi.

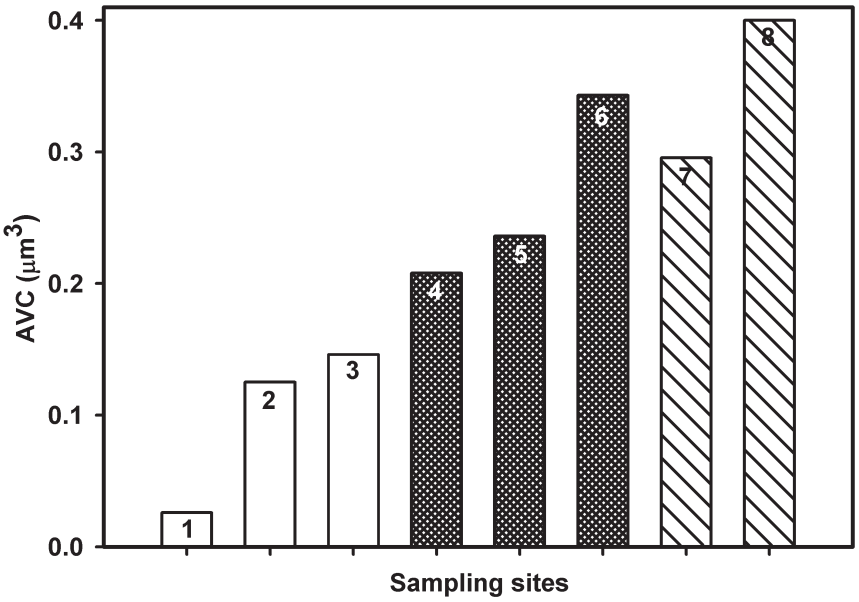

Fig. 2. Comparison of average cell biovolume (AVC) in samples from the sites at the forefield of the glacier (site 1) and at outskirts of glacier (site 2 – silty sand without macroalgae detritus and site 3 – silty sand mixed with detritus) with those from different sites and materials located outside the Ecology Glacier: 4 – soil below penguin rookery, 5 – fresh penguin guano, 6 – guano after 42 days of experimental exposure *in situ* (penguin rookery), 7 – fresh macroalgae (mixed species), 8 – macroalgae decomposed to detritus.

Biotic and abiotic conditions affect not only bacterial abundance (TC) and diversity (Shannon Index) but also their average cell biovolume (AVC), and correlate especially with mineral and organic nutrition level (Fig. 2). The smallest bacteria were observed near the front of the glacier and the largest (almost 40 times larger) in macroalgae decomposed to detritus. Significantly larger bacteria were observed in a sample of fine silty sand without macroalgae detritus. It may be an effect of the enrichment of silty sand by nitrogen compounds as a consequence of soil exposure on ammonia originating from penguin rookery (Zdanowski *et al.* 2005) and the activities of microorganisms converting some minerals to soluble and bioavailable forms. The high TC and biovolume of bacteria detected in the soil enriched by macroalgae or marine derived detritus suggested their important role in bacterial succession in areas exposed through the consequence of glacial melting.

To describe an impact of the organic matter on microbial populations development TC and CFU values were determined in soil from different areas near *H. Arctowski* Station. A correlation between TC/CFU ratio and organic matter availability was observed (Table 2). CFU counts were low in samples not containing, or containing only traces of organic matter (samples collected near glacier) and they increased in samples containing macroalgal detritus. Similar trends of higher CFU:TC ratio in terrestrial environments in maritime Antarctica were observed by

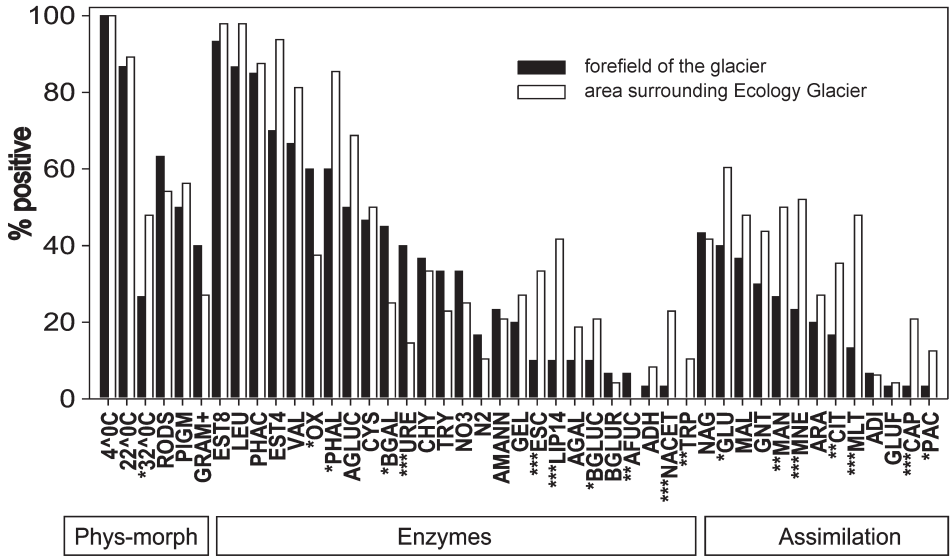

Fig. 3. Differences between morhophysiological properties of CFU bacterial populations isolated from the area at the head of the glacier (n = 30 strains) and appropriate data collected by Zdanowski and Weglenski (2001) from the area surrounding Ecology Glacier. Significance level given by Chi square tests ($\div^2$) comparing frequency of positive responses in each habitat are indicated as: $*p < 0.05$; $**p < 0.01$, $***p < 0.001$. Following tests were carried out: ability to grow at 4°C, 22°C, 32°C; morphological analyses which comprised counts of pigmented colonies (PIGM), two microscopic tests – Gram reaction (GRAM) and morphology (ROD); the API 20NE system which allows nine biochemical tests: reduction of nitrate to nitrite (NO_3), and to nitrogen (N2); indole production (TRP); glucose fermentation (GLU_F); arginine dihydrolase (ADH); urease ((URE); b-glucosidase (ESC); gelatin hydrolysis (GEL); cytochrome oxidase (OX); and twelve tests for assimilation of carbohydrates as sole carbon sources; glucose (GLU); arabinose (ARA), mannose (MNE); mannitol (MAN); N-acetyl-glucosamine (NAG); maltose (MAL); gluconate (GNT); caprate (CAP); adipate (ADI); malate (MLT); citrate (CIT); phenyl-acetate (PAC); the API ZYM system tests for the presence of 18 constitutive enzymes: alkaline phosphatase (PHAL); esterase – C_4 (EST4); esterase lipase – C_8 (EST8); lipase – C_{14} (LIP14); leucine arylamidase (LEU); valine arylamidase (VAL); cystine arylamidase (CYS); trypsin (TRY); chymotrypsin (CHY); acid phosphatase (PHAC); a-galactosidase (AGAL); b-galactosidase (BGAL); b-glucuronidase (BGLUR), a-glucosidase (AGLUC); b-glucosidase (BGLUC); N-acetyl-b-glucosaminidase (NACET); a-mannosidase (AMANN); a-fucosidase (AFUC).

Zdanowski and Węgleński (2001) and Zdanowski *et al*. (2005). However, "high variability in CFU:TC ratio is one of the unanswered questions of interest for microbial ecologists" (Zdanowski and Węgleński 2001). We observed that the conversion to the reduced form provided always excellent conditions for growth of the CFU soil bacteria. In terms of biodiversity it should be noted that within the bacterial communities the heat resistant spore forming bacteria made up only small fraction, up to 200 cells/g dry weight of soil at 20 cm depth, whereas nearly none were observed at the surface.

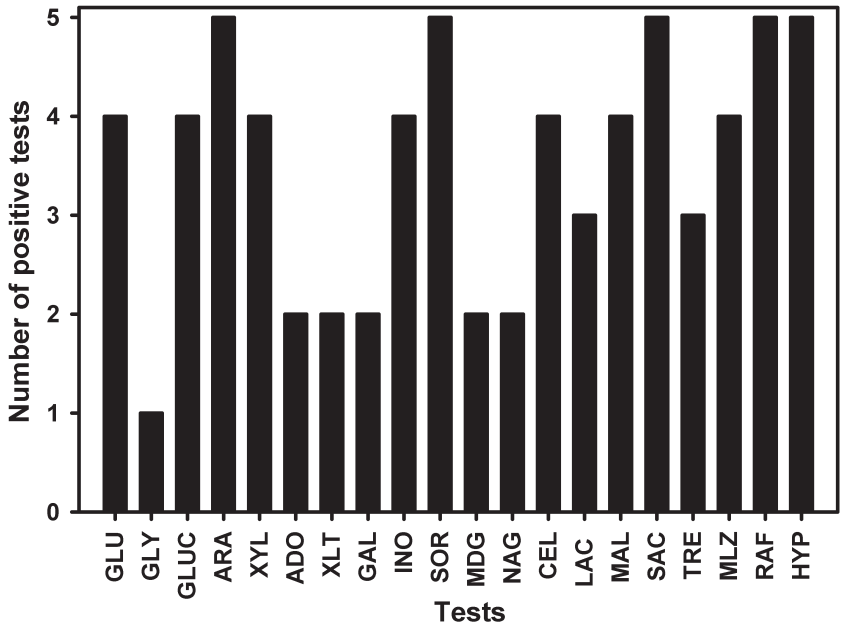

Fig. 4. Morpho-physiological characteristics of CFU fungal community. The following tests were carried out: D-Glucose (GLU); Glycerol (GLY); Gluconate (GLUC); L-Arabinose (ARA); D-Xylose(XYL); Adonitol (ADO); Xylitol (XLT); D-Galactose (GAL); Inositol (INO); D-Sorbitol (SOR); Methyl-aD-Glucopyranoside (MDG); N-Acetyl-Glucosamine (NAG); D-Cellobiose (CEL); D-Lactose (LAC); D-Maltose (MAL); D-Saccharose (SAC); D-Trehalose (TRE); D-Melezitose (MLZ); D-Rafinose (RAF); Hyphae (HYP).

More then 80% of CFU in all analyzed samples were psychrotolerant and only less than 20% were psychrophiles. Some biochemical differences between bacteria isolated from the soil near the glacier and from more distant sites were observed (Fig. 3). Generally, bacteria from the glacial site secreted less hydrophilic enzymes then bacteria from distant localization. The most dramatic physiological differences were related to lipolytic activities. In populations distant from the glacier, more than 40% of CFU hydrolyzed long chained (C14) lipids, while in samples collected near glacier only ~8% of bacteria expressed C14 lipase. Similar results were obtained for other catabolic enzymes (other lipids and sugars hydrolyzing enzymes). Based on morpho-physiological properties CFU belonged in the α, β and γ subdivisions of the proteobacteria; *Cytophaga–Flavobacterium–Bacteroides* (CFB) were also present, along with spore forming bacteria and filamentous fungi. Five isolated psyhrotolerant fungi were examined according to their physiological properties. All formed hyphea and utilized arabinose, sorbitol, saccharose and rafinose as a sole carbon source (Fig. 4). Only one utilised glycerol and all remaining 18 tested carbon sources, including xylitol, adonitol, D-galactose, inositol, D-trehalose, D-cellobiose and N-acetyl-glucosamine.The last source sugests that it may participate in chitin degradation.

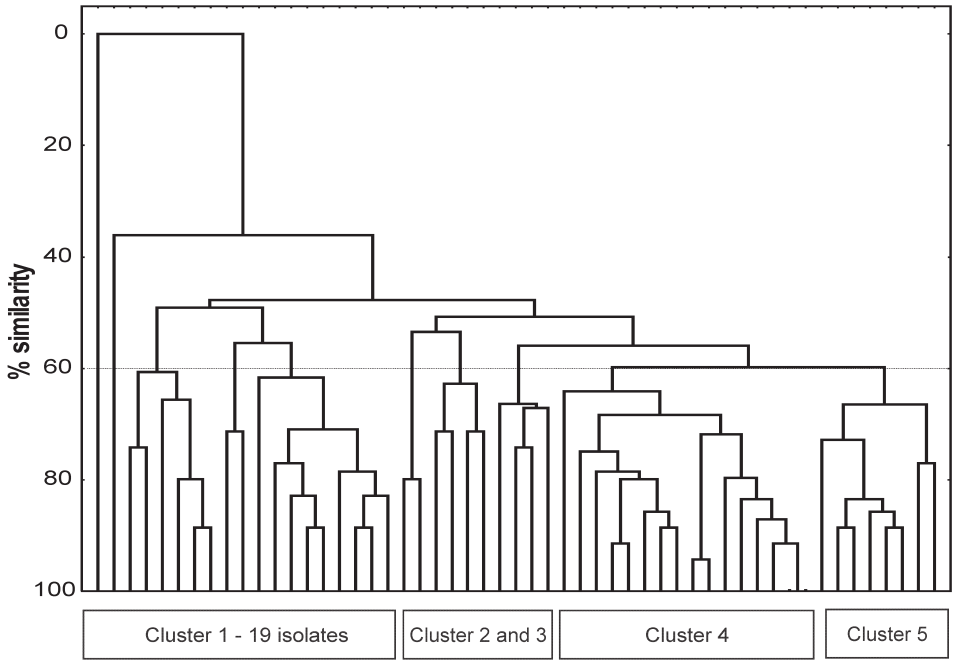

Fig. 5. Hierarchical classification of the 53 bacterial isolates based on their responses in 48 morpho-physiological tests (as described in Fig. 2).

In order to more precisely characterise the isolated bacteria strains fifty three isolates were classified on the basis of morphological and physiological features (Fig. 5). At the ≥ 60 % level of similarity four clusters (numbers 2, 3, 4 and 5) were distinguished. Two additional clusters of the 6 and 9 strains at the same level of similarity were grouped (group 1) together with 4 strains unclustered but situated in the same part of the tree. On the basis of their numerical profiles in the diagnostic system used (ApiWeb®, 2007) strains of this group, mainly Gram negative cocci, were mostly similar to *Sphingomonas* (alphaproteobacteria). Also strains (Gram negative cocci and rods) in clusters 2 and 3 were homologous with alpha subclass of Proteobacteria, while strains in cluster 4 were dominated by gamma-proteobacteria, mainly Moraxellaceae and Pasteurellaceae.

Generally, bacterial communities isolated from soil at the edge of the glacier were dominated by different species belonging to the alpha subclass of Proteobacteria (ApiWeb®. 2007). Strains of this group were distributed both, near the glacier and at a distance of the glacier front. Interestingly, dominance by alpha-proteobacteria was not observed in our earlier studies on soil microbial populations not directly adjacent to melting glacier (Zdanowski and Węgleński 2001) or in avian guano in the Antarctic (Zdanowski *et al.* 2005), nor in the Arctica (Zdanowski *et al.* in preparation), where bacteria communities were dominated by gammaproteobacteria.

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