Research article

Physicochemical and biological characterization of soils from the vicinity of the Arctowski Polish Antarctic Station

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Abstract: *Soil samples collected in 2006 from 4 different sites (Puchalski Hill and a moraine below this hill, an elephant seal colony and a penguin rookery) in the vicinity of Arctowski Polish Antarctic Station at King George Island were characterized in terms of physicochemical properties (pH, humidity, concentration of selected inorganic and organic substances), microbial colonization and the overall activity of selected enzymes (differentglycosidases and esterases). Activity of the latter enzymes was assayed by p-nitrophenyl and 4-methylumbelliferone derivatives of fatty acids (by spectrophotometric and fluorimetric method, respectively). The highest lipolytic activity was found in soils from the elephant seal colony and penguin rookery.*

Keywords: *Antarctica; soil organic matter; soil biodiversity; lipolytic activity; cold-adapted enzymes.*

Introduction

Arctowski Station is situated in the maritime Antarctic on the western shore of Admiralty Bay (King George Island, South Shetland Island)and terrestrial habitats which are not permanently covered by ice in contrast to more than 90% surface area of this island. Research on soils in these habitats, which have been conducted for approximately thirty years, revealed appreciable diversity of soil-dwelling bacteria, yeast and filamentous fungi, being the main source of enzymes in Antarctic soils and driving processes of organic matter decomposition, cycling of elements and humus synthesis [1].

The soil parameters affect processes of colonization and establishment of soil microflora. It has been estimated that 0.1-1% of the total soil population can be cultured by applying standard cultivation techniques, but 90-99% of the genetic diversity present in this microbial niche was lost because of difficulties in enriching and isolating microorganisms [2]. They do not grow under laboratory conditions due to specific nutritional requirements and complicated trophic

relationships, intercellular signaling, which are characteristic of their natural habitats. These unrecovered microorganisms represent an unexplored reservoir of novel strains, which may produce novel enzymes and other biomolecules. It is possible to obtain these valuable compounds through searching for relevant genes in total DNA libraries directly extracted from various environments that are named the metagenomic or environmental DNA (eDNA), followed by expression of isolated genes in mesophilic hosts [3]. Metagenomic studies of antarctic biotops have still been scarce [4] and most of them focused on evaluation of functional potential of strains that were culturable using traditional microbiological methods [5,6,7]. These experimental works revealed that Antarctic microorganisms from marine and soil habitats produced cold-adapted enzymes that were potentially interesting for biotechnology [8] because of applicability to catalytic processes requiring low temperature. Moreover, Antarctic microorganisms are adapted not only to low temperature but also – in the case of soil microbes - to low water activity, so their enzymes can efficiently catalyze transformations in organic solvents.

Examples of such enzymes are lipases (EC 3.1.1.3) and carboxylesterases (EC 3.1.1.1). Preferred substrates of lipases (named *true lipases*) are water-insoluble triacylglycerols containing higher fatty acids ($\geq C_{10}$), whereas carboxylesterases hydrolyze water-soluble esters and triacylglycerols with lower fatty acids ($\leq C_{10}$). It is a characteristic of lipolytic enzymes that in micro-water systems they preferably catalyze reactions of ester synthesis instead of their hydrolysis [9].Many of them have been used in food processing, production of pure enantiomers of chiral compounds (for example lipases from yeast *Candida antarctica* [10]), modification of polyester fibers (to increase water-binding and improve "washability" of stains) etc. Microbial cold-adapted lipases and esterases produced by Antarctic, psychrophilic and psychrotrophic microorganisms can potentially find novel industrial applications, e.g. in biotransformation of these substrates, which are stable only in the cold.

Therefore, apart from determination of physicochemical properties of 4 different Antarctic soils, this study embraced estimation of activity of selected microbial enzymes in these soils with focus on cold-active esterases/lipases and preliminary characterization of the latter enzymes.

Materials and methods

Soil samples

Soil samples were collected at the end of February and at the beginning of March 2006 in the neighborhood of Henryk Arctowski Polish Antarctic Station at King George Island and kindly donated to the authors by Dr. Marek Zdanowski from the Department of Antarctic Biology, Polish Academy of Sciences, Warsaw (Poland). Sampling depth was 0-5 cm. The samples were kept without disturbance at 4°C until analyses, which were completed in April2008.

Two samples, which were virtually not contaminated with guano, were collected from the hill harboring the grave of the renowned Polish naturalist and photographer Włodzimierz Puchalski – designated as a historical site HSH No.51 (S 62° 09' 809, W 58° 28' 156, the sample designated as **HPu**) and from the moraine located below Puchalski Hill (S 62°09'730, W 58°28'163, the sample designated as **M**). The two other soil samples were derived from elephant seal colony (S 62°09'44,2",W58° 27'49,4, the sample designated as **SE**) and *Adelie* penguin rookery (S 62°09'46,6",W 58°27'42,7", the sample designated as **P**).

Physicochemical analyses

Soil was sieved through 2 mm-mesh sieve to discard rock rubble and analyzed for water and organic matter contents and pH. Dry weight (dwt) was determined after 24 h incubation at 65°C in a dry-box with circulating air, according to Zdanowski [11]. Measurements of pH of the air-dried soil samples suspended in distilled water (soil/water ratio of 1:2.5) were conducted using a glass combination electrode [12].

Total organic carbon (TOC) was quantified according to Tiurin [13].Total nitrogen was assayed by Kjeldahl method and quantified as nitrate in a flow injection analyzer [14]. Total phosphorus was determined by the colorimetric method of Rouser *et al*. [15] after mineralization of soil samples. Concentration of selected macro- and microelements was estimated after18 h extraction of soil samples with 2M HNO_3. It was conducted at 20°C and 110 rpm (INFORS shaker). Concentration of selected ions was determined using an atomic absorption spectrometer (GBC 932 pUrs).

Isolation of culturable soil microorganisms

A sample of antarctic soil (2 g dwt) was dispersed in a sterile liquid culture medium containing (% w/v): 1% bacto peptone; 0.5% NaCl; 0.36% Na_2HPO_4 and 0.15% K_2HPO_4 in order to isolate culturable microorganisms (colony forming units, cfu).This suspension was incubated at 10°C for 72 h to activate and increasethe number of microorganisms. Then a series of decimal dilutions (from 10^{-1} to 10^{-5}) was prepared (in physiological saline solution) and spread on standard agar media used for enumeration of bacteria (medium B, pH 7.0, containing (% w/v): 0.3% bacto beef extract, 0.25% yeast extract, 5% bacto-peptone, 0.1% glucose and 0.5% NaCl), yeast (YPG medium with 0.25 mg/ml chloramphenicol and 0.1 mg/ml ampicilin) and filamentous fungi (Čapek medium). Agar plates were incubated for 3 weeks at 6°C for isolation of bacteria and yeast, and for 4 weeks for isolation of filamentous fungi.

Total count (TC) of bacteria and yeast after the multiplication was determined by direct microscopic enumeration of their cells in Thom's chamber (1500-fold magnification). Suspension of cells was obtained by incubation for 72 h at 10°C in a sterile liquid culture medium containing (% w/v): 1% bacto peptone; 0.5% NaCl; 0.36% Na_2HPO_4 and 0.15% K_2HPO_4.

Assays of enzymatic activities by a microplate fluorimetric method

The assay of selected enzymes in soils was carried out according to Marx*et al* [16]. The substrates were 4-methylumbelliferone (4-MUB) derivatives of phosphate, β-D-glucopyranose, β-D-galactopyranose, β-D-xylopyranose, β-D-cellobiose, β-D-glucuronide and β-D-glucosamine (Sigma-Aldrich). The substrates used to determine the lipolytic activity of Antarctic soil samples were MUB-butyrate, MUB-heptanoate, MUB-palmitate and MUB-oleate. The substrates were pre-dissolved in 1 ml of ethylene glycol monomethylether, except 4-MUB-β-D-glucuronide and 4-MUB-phosphate, which were dissolved in sterile deionised water. To obtain soil suspension, 1 g of soil was weighed into an autoclaved 250 ml jar with a lid, mixed with 25 ml of 0.1 M MES buffer pH 6.1, sonicated for 4 min at 4°C (Ultrasonic Disintegrator 470 series Cole Parmer Instrument) and centrifuged (20 min, 12 000xg, 4°C). The solid debris was discarded and the supernatant was used for determination of enzymatic activities.

The fluorescence intensity was measured by a microplate fluorimeter (Fluoroscan *Ascent FL*, Labsystem), which shook each microplate for 5 s in order to homogenize reaction mixtures (180 μl containing 100 μM substrate and 120 μl enzyme), and incubated it for 60 min at 30°C.

Other assays of lipolytic activity

Activity of lipolytic enzymes was determined by the spectrophotometric method.Concentration of *p*-nitrophenol released from fatty acid *p*-nitrophenyl esters by these enzymes was measured at 420 nm. Reaction mixtures containing substrate solution (30 μl) in acetonitrile (1:4 vol/vol), soil extract (100 μl) and 50 mM sodium phosphate buffer (pH 8.0, 1.45 ml) were incubated at 30°C for 15 min. The extract from soil was prepared by 12 h extraction of 2.5 g soil with 10 ml of sodium phosphate buffer (pH 8.0, supplemented with 1% Triton X-100), followed by sonification (for 4 min with 6 s pauses every 25 s, at 4ºC and at the 30% amplitude) and centrifuging (20 min, 12 000xg, 4°C). *p*-Nitrophenyl butyrate (pNPB) (Sigma-Aldrich) was used as esterase substrate. Because long-chain fatty acid esters of *p*-nitrophenol (e.g. *p*-nitrophenyl palmitate and *p*-nitrophenyl stearate) are insoluble in acetonitrile, these substrates were dissolved in *n*-hexane.

Statistical analysis

Experiments and assays were conducted in triplicates and their results were analyzed with Microsoft Excel version 97. Quantitative results are given as the mean “±” standard deviation.

Results

Physicochemical soil characteristics

The tested Antarctic soils, in particular these originating from areas of penguin and elephant seal rookeries, were characterized by alkaline pH (8.4 and 8.9) while the other 2 soils were slightly acidic (pH of 4.7 and 6.3) (Table 1).The total water content was the highest in soil taken from the penguin rookery (almost 35%). The other soil samples were less humid (humidity below 10%, Table 1).

Table 1. Concentration of organic and inorganic components in soils from the examined Antarctic sampling sites and their pH; organic C, N, P and S), moisture. Concentration of selected micro-and macroelements in soils

Site	C ($g\ kg^{-1}$ dwt)	N ($g\ kg^{-1}$ dwt)	C/N	P ($g\ kg^{-1}$ dwt)	S ($g\ kg^{-1}$ dwt)	H_2O (% wt)	pH
HPu	3.7 ± 1.1	0.34 ± 0.01	10.8	7.4 ± 1.2	0.9 ± 0.1	9.57	4.7
M	1.6 ± 0.7	0.24 ± 0.05	6.6	1.7 ± 0.5	0.4 ± 0.1	5.60	6.3
SE	2.1 ± 0.8	0.32 ± 0.05	6.7	3.2 ± 1.2	1.2 ± 0.2	8.53	8.4
P	15.2 ± 4.2	8.1 ± 2.4	1.9	41.3 ± 15.	4.2 ± 0.8	34.86	8.9

Site	Chemical composition ($g\ kg^{-1}$dwt)									
	Mg^{2+}	Ca^{2+}	K^{+}	Na^{+}	Cu^{2+}	Zn^{2+}	Ni^{2+}	Co^{2+}	Mn^{2+}	Fe^{2+}
HPu	14.4	0.6	9.7	26.2	0.7	0.2	0.00	0.05	0.5	167.5
M	76.8	6.6	8.4	28.8	0.6	0.1	0.04	0.1	1.3	112.8
SE	28.6	6.7	9.1	28.8	0.3	0.2	0.00	0.1	0.8	85.3
P	80.5	12.8	13	85.9	0.6	0.2	0.05	0.1	2	147.9

± - standard deviation

The soil from the penguin rookery was the richest in nitrogen (*circa*8.0 g kg^{-1} dwt), organic carbon (*circa* 15 g kg^{-1} dwt) and phosphorus (*circa* 40g kg^{-1} dwt) (Table 1). High contents of carbon and nitrogen at site P coincided with a relatively high sulfur level (*circa* 5g kg^{-1} dwt).The lowest concentrations of nutrients (organic carbon, nitrogen, phosphorus and sulfur of approximately 2, 0.2, 2 and 0.4 g kg^{-1} dwt respectively) were found in the soil sample taken from the moraine adjacent to Puchalski Hill (Table 1). Apart from N, C and P the soil samples contained numerous macro- and microelements. The most abundant of them were Mg^{2+} (14.4 – 80.5g kg^{-1} dwt), Na^{+} (26.2-8.6 g kg^{-1} dwt) and K^{+} (8.4-13 g kg^{-1} dwt) ions. Mn^{2+} (0.5-1.3 g kg^{-1} dwt) and Fe^{+2} (85.3-168 g kg^{-1} dwt) were the dominating heavy metal ions while concentrations of Ni^{2+} and Co^{2+} ions varied between 0 and 0.05 and between 0.05 and 0.1 g kg^{-1} dwt, respectively. The highest concentrations of Na^{+}, K^{+}, Ca^{2+} and Mg^{2+}ions were found in the soil from penguin rookery (Table 1).

Isolation of culturable soil microorganisms

Single colonies showing different morphological attributes were picked from agar plates, which were used to isolate culturable microorganisms from the examined soils. They were streaked for purification, which yielded pure bacterial (17), yeast (6) and filamentous fungi (8) cultures. The cultures were subsequently used to screen for lipolytic enzyme activity (results not included).

Enumeration of the total number of activated microbial cells (TC) in Thom's chamber gave 2 orders of magnitude higher results for bacteria (10^9 versus 10^7CFU g^{-1} soil dwt) and yeast (10^7 versus 10^5CFU g^{-1} soil dwt) than the agar plate method (Table 2).The colony forming bacteria (CFU) accounted for 1.6-3.5% total microbial count (TC). Although small rods tended to dominate each population, microscopic observations revealed differences between microbial populations in soil from each site. The number of activated microbial cells was the lowest in soil from penguin rookery. Even the number of bacteria in this sample was relatively low (TC close to 10^6 g^{-1} soil dwt) and yeast cells were undetectable (Table 2). In the other activated soil samples, the number of yeast CFU was by 2 orders of magnitude lower than bacterial CFU and accounted for 0-5.0% TC. The number of filamentous fungi capable of growing in Čapek medium was relatively low and ranged between 1.0 x 10^2 and 48.0±10.2 x 10^2 g^{-1} soil dwt (Table 2).

Table 2. Distribution of microorganisms in the examined Antarctic soils

Soil sample	Bacteria		Yeasts		Filamentous fungi	Total culturable microorganisms
	CFU x $(10^7)^a$	TC x $(10^9)^b$	CFU x $(10^5)^a$	TC x $(10^7)^b$	CFU x $(10^2)^a$	CFU x (10^7)
HPu	4.32 ± 0.26	1.5 ±0.52	9.6 ± 4.8	35.6 ± 9.2	2.65 ± 0.41	4.42 ±0.79
M	3.13 ± 1.0	0.9 ±0.03	0.05 ± 0.01	0.01 ±0.001	48.0 ± 10.2	3.14 ±0.15
SE	2.19 ± 0.38	1.4 ±0.68	1.25 ± 0.34	8.8 ± 5.89	1.0 ± 0.21	2.20 ±0.24
P	0.01 ± 0.001	0.001 ±0.000076	ND	ND	1.0 ± 0.18	0.008 ±0.00064

[a] - colony forming units of bacteria, yeast and fungi (CFU x g^{-1}dwt)

[b] - total count of bacteria and yeasts (TC x g^{-1}dwt) after 72 h activation of microbial strains at 10°C.

± - standard deviation; ND-not detected

Biochemical analyses

Activity of selected enzymes

The highest activity of lipases for 4-MUB derivatives of fatty acids was found in extracts of soil samples from elephant seal and penguin rookeries (5.4 - 107.2 and 0.9 - 21.9 u g^{-1} soil dwt, respectively) and the lowest lipolytic activity was

displayed by soils from moraine (0-5.4 u g^{-1} soil dwt) and Puchalski Hill (0.3-14.4 u g^{-1} soil dwt) (Table 3). From 4 applied fluorescent substrates, the derivative of 7 carbon atom fatty acid (ester of 4-MUB heptanoate) was the preferred substrate of these lipolytic enzymes.

Table 3. Activity of selected enzymes in extracts from the examined soil samples

		Activity of soil samples [u g^{-1} dwt][a]			
		HPu	M	SE	P
	AF	ND	2.11 ±0.32	ND	ND
	S	0.35 ±0.07	0.07 ±0.012	0.56 ±0.1	0.22 ±0.013
	β-Gal	ND	ND	ND	2.44 ±0.0.34
	β-Glc	1.23 ±0.23	ND	2.03 ±0.4	0.51 ±0.102
	β-Xyl	ND	ND	ND	0.33 ±0.076
	β- GlcA	0.88 ±0.16	0.56 ±0.06	ND	0.78 ±0.056
	N-Ac	0.17 ±0.02	0.17 ±0.032	1.37 ±0.25	16.3 ±3.16
	β-Cel	ND	ND	ND	0.22 ±0.44
Lip	**4-MUB butyrate**	7.0 ±0.07	1.5 ±0.15	21 ±4.1	9.2 ±0.1
Lip	**4-MUB heptanoate**	14.4 ±2.6	5.4 ±1.05	107.2 ±17.44	21.9 ±2.38
Lip	**4-MUB palmitate**	1.2 ±0.2	ND	2.2 ±0.32	1.3 ±0.17
Lip	**4-MUB oleate**	0.3 ±0.04	ND	12.6 ±1.34	0.9 ±0.12

AF - alkaline phosphatase; S - sulphatase; β-Glc - β-glucosidase;
β-Gal - β-galactosidase; β-Xyl - xylobiase; β-GlcA - β-glucuronidase;
N-Ac - N-acetyl-glucosaminidase (chitobiase); β-Cel - cellobiase; Lip - lipase
± - standard deviation; ND-not detected
[a] - one unit of enzymatic activity (u) is equivalent to 1 nmol of methylumbelliferone released from the substrate in 60 min per 1 g soil dwt

The activity of other 8 enzymes, which were assayed in the soil extracts is shown in Table 3. The activity of alkaline phosphatase (*circa* 2 u g^{-1} soil dwt) was contained only in the moraine soil while activities of β-galactosidase, xylobiase and cellobiase were found only in the extract from penguin rookery soil (*circa* 2.4, 0.3 and 0.2 u g^{-1} soil dwt, respectively). Apart from the alkaline phosphatase the moraine soil displayed only the activities of sulphatase, β-glucuronidase, and N-acetyl-glucosaminidase (*circa* 0.07, 0.56 and 0.17 u g^{-1}dwt, respectively). Only activities of sulphatase (0.07-0.56 u g^{-1}dwt) and N-acetyl-glucosaminidase (chitobiase, *circa* 0.17-16.6 u g^{-1}dwt) were displayed by all the

examined extracts (Table 3). The latter activity was the highest in the penguin rookery soil.

Activity for p-nitrophenyl derivatives of fatty acids

Conditions of extraction of extracellular (adsorbed on soil particles) and intracellular enzymes from the examined Antarctic soil samples were optimized prior to determination of the total activity of microbial carboxylesterases and lipases. It was found (results not presented) that the most efficient method of enzyme extraction from Antarctic soil was sonification of its suspension in sodium phosphate buffer, pH 8.0.

Table 4. Substrate specificity of lipolytic enzymes from soil extracts

Substrate	Specific lipolytic activity [u $mg_{protein}^{-1}$][a]	
	SE	P
p-nitrophenyl acetate	15±2.7	75±5.02
p-nitrofenyl butyrate	312±15.6	70±9.24
p-nitrofenyl caprylate	243±26.73	64±5.5
p-nitrophenol decanoate	201±16.24	65±7.15
p-nitrophenyl palmitate	175±24.8	7±0.42
p-nitrophenyl stearate	135±13.09	4±0.692

± - standard deviation

[a] - one unit of lipolytic activity (u) is equivalent to 1 nmol of *p*-nitrophenol released from the substrate in 1 min per 1mg protein

Substrate preferences of lipases contained in soils from penguin and elephant seal rookeries, which showed higher activity than lipases from the other two soil samples, were determined for six synthetic substrates (p-nitrophenyl esters of carboxylic acids). P-nitrophenyl butyrate was the preferred substrate of esterase extracted from elephant seal soil (312 u $mg_{protein}^{-1}$) (Table 4). Activity of these enzymes for derivatives of longer fatty acids like p-nitrophenyl palmitate was almost twice lower (175 u $mg_{protein}^{-1}$). Also enzymes from penguin rookery soil preferred p-nitrophenyl esters of shorter carboxylic acids and displayed lower activity for longer fatty acid derivatives (the lowest for p-nitrophenyl stearate (Table 4).

Discussion

The four described soil samples had different physicochemical characteristics. Contents of organic carbon and nitrogen in these soil samples were relatively similar to those of the other examined Antarctic soils [17,18]. With an exception of the soil from *Adélie* penguin rookery, levels of C and N in the other 3 soil samples were similar to those from some soils in the western Enderby Land Coast [19] but lower than in soils on Bailey Peninsula at Wilkes Land in Coastal East Antarctica [20].The highest C/N ratio was observed in soil collected from Puchalski Hill and it indicates the dominance of plant material in soil covered with mosses and dense plant of *Deschampsia antarctica* [17]. Total phosphorus levels in the 4 presented soils were higher than those reported by Aislabie *et al.*

[21] in soils of Marble Point and Wright Valley (Victoria Land) and in soils from Wilkes Land [18]. According to Beyer and Bőlter [22] the apparent differences in soil organic matter composition indicate that metabolism of organic compounds is strongly affected by their origin and structure, the microclimate and activity of soil microorganisms. Generally, the content of bio-available carbon in soils from cold regions is lower as compared to warmer ecosystems because of lower CO_2 assimilation by organisms such as lichens, mosses, photo- and chemoautotrophic bacteria [23]. Only in places populated by macrophytes the soil carbon concentration is slightly higher but due to very low rates of organic matter conversion also humus formation in these soils is minimal [18]. High contents of carbon and nitrogen in soil from penguin rookery coincided with relatively high sulfur level. This confirms the animal origin of organic matter at this site since much reduced sulphur occurs in feathers and other epidermal keratinous structures of birds [17].

The K^+ and Na^+ levels in the examined non-ornithogenic soils were lower (about 9 and 27 g kg^{-1} dwt.; Table 1) than in the ornithogenic soils (13 and 86 g kg^{-1} dwt.; Table 1) contaminated with guano. This is consistent with the results of Beyer and Bőlter [22]. Generally, all Antarctic soils are rich in K^+, Na^+ and Mg^{2+} ions which are mainly brought by wind as sea water aerosols with lesser contribution of avian and animal faeces [20].

Two of the examined Antarctic soils, originating from penguin and elephant seal rookeries, were characterized by alkaline pH (between 8.4 and 8.9) while the other soils were slightly acidic (pH between 4.7 and 6.3; Table 1). pH is one of the most important properties of soil. This quantity changes with concentration of organic substances. Soil pH close to neutral is characteristic of soils in the early stage of development and soil acidification is often a result of microbial activity[37].

The examined soil samples from 4 different sites in the neighborhood of Henryk Arctowski Station were populated by bacteria, yeast and filamentous fungi. Bacteria were more abundant than the other two groups of activated microorganisms (Table 2). The number of viable microbes in soil, their growth and metabolic activity are strongly affected by water content [24]. Although the content of water was the highest in soil taken from the penguin rookery (almost 35%) and the other soil samples were less humid (moisture content below 10%), the soil from penguin rookery contained the lowest number of culturable bacteria (8×10^4 CFU g^{-1} dwt of soil after activation, Table 2) that is thought to result from the relatively high concentration of acrylic acid (known as a microbial antagonist) in the guano [18].

Zdanowski *et al*. [25,26] found that the properties of Adélie penguin guano resulted from the penguin's diet, which was extremely rich (95.4%) in krill (*Euphausia superba* Dana). Our results are consistent with those of Meyer *et al.* [27] who also found that guano had relatively low bacterial counts. In contrast, Beyer and Bőlter [22] and Zdanowski *et al*. [25] observed greater bacterial

colonization in ornithogenic soils in comparison to the non-ornithogenic ones. Besides, they suggest that colonization of the ornithogenic soils with lichens and /or mosses depresses bacterial growth. According to Zdanowski *et al*. [26] the guano can be populated by microbes of diverse origin, also by penguin's enteric microflora. Results related to soil-dwelling Antarctic microflora are also affected by long-term changes in soil, air temperature and seasonal changes in climatic conditions. Zdanowski *et al.* [26] postulated that also the considerable discrepancies in experimental set-ups complicated the comparison of results.

Population of activated yeast varied between 0 and 9.6×10^5 CFU g^{-1}dwt of soil, however they were not found in the soil from penguin rookery. Counts of filamentous fungi are harder to interpret as fragmentation of hyphae and dispersal of spores during soil processing prior to agar medium inoculation can lead to exaggerated counts [18]. Thus, the values of 265-4800 CFU g^{-1}dwt of soil after activation (Table 2) should be viewed as maximum values. Fungi (yeast, yeast-like, and filamentous species) were also found by other researchers in Antarctic soils [28].These eukaryotes play important roles in degradation of organic matter, in early site colonization and development of soil structure. Besides, they synthesize sterols that are essential for soil invertebrates [29].

It is to note that total counts of activated bacteria and yeasts that were determined by using Thom's chamber (10^9 and 10^7 TC g^{-1}dwt, respectively, Table 2) were on average 2 orders of magnitude higher than the respective results of plate tests (10^7 and 10^5 CFU g^{-1}dwt, respectively, Table 2). This finding is consistent with that of Zdanowski and Węgleński [17] and confirms the opinion that the majority of soil-dwelling microorganisms (above 90 %) rank among "unculturable" species [30]. Recently, there is a growing belief that the term "uncultutrable" is inappropriate and that we have to develop the correct culture conditions mimicking natural environments.

Agar plate cultures revealed considerable differences in macroscopic appearance of bacterial colonies derived from different soil samples. For example, the majority of culturable bacteria from the elephant seal colony soil were yellow and beige while these from Puchalski Hill and the moraine adjacent to this hill were mainly dark orange. The prevalence of variously pigmented isolates derived in this step of investigations might result from the sampling depth of 0-5 cm. Natural pigments were found to protect against different types of UV irradiation. Some pigments synthesized by bacteria protect them against UV-B irradiation and thus promote the selection of UV resistant strains [31]. The presence of chromogenic bacteria at the soil surface was also observed by Cameron *et al*. [32] and Zdanowski and Węgleński [17].

Our intention was to find in maritime Antarctic soils some microbial producers of enzymes that may find application in biotechnology. The *in situ* assays of enzymatic activities in the examined soil samples revealed that all of them contained sulphatase, β-glucosidase, β-glucuronidase and chitobiase (Table 3). A very weak activity of alkaline phosphatase was found only in the extract of

the moraine soil, and activities of β-galactosidase, xylobiase and cellobiase were detected only in extracts of penguin rookery soil (Table 3). The latter soil displayed the highest activity of chitobiase. This can be explained by chitin deposition to soil with guano containing 32.1% chitin [33]. This in turn stimulates the development of chitin-digesting microflora. Chitin sources in guano are exoskeletons of krill, *Euphausia superba* Dana, and other crustaceans constituting the penguin's diet. Penguin rookery-dwelling microorganisms presumably synthesize also the enzyme chitinase (apart from N-acetyl-glucosaminidase) but this activity has not been assayed by us. The values of β-glucosidase activity reported here are smaller than those typically measured in temperate soils[34].Similarly, the smaller activity of this enzyme was observed by Hopkins *et al*. [38] in an Antarctic dry valley soil. The authors explain that cellulose and cellobiose – the principal substrates for β-glucosidase – are very scarce in the Antarctic dry valleys.

Synthesis of diverse hydrolases by bacterial isolates from Maritime Antarctica was reported by Zdanowski and Węgleński [17] who by means of API Zym tests found that of 134 soil bacterial strains more than 90% produced alkaline phosphatase, 30% – chitobiase (N-acetyl-β-Dglucosaminidase), more than 20% – β-glucosidase and β-galactosidase and only 5% – β-glucuronidase.

Our study focused on cold-active microbial lipolytic enzymes. Their recovery was most efficient when soil samples were subjected to sonification in sodium phosphate buffer pH 8.0 at 4°C. Lipolytic activity of cell-free extracts obtained from soils was assayed for fatty acid 4-methylumbelliferyl (Table 3) and p-nitrophenyl derivatives (Table 4) and olive and rapeseed oils (data not included). All these methods revealed the highest lipolytic activity in soils from elephant seal colony and penguin rookery. These soils contain remnants of lipids of animal origin and probably the availability of the latter stimulates development of lipolytic microorganisms. Estimation of substrate preferences of enzymes contained in these samples showed that microorganisms populating these habitats synthesize both carboxylesterases and true lipases, whose activity in hydrolysis of oils was higher than the activity in hydrolysis of synthetic substrates.

The overall lipolytic activity of soil depends on the number of cells producing these enzymes and the number of different lipolytic species seems to be less important. Our results showed that the lipolytic activity was the highest in cell-free extracts obtained from penguin and elephant soils but we can only speculate that the number of lipolytic microorganisms in these soils was the highest. It is to note that our metagenomic study of these two antarctic soils resulted in isolation of a gene of a unique lipase lip1 from the elephant soil sample [4]. We have been trying to achieve its expression in *Escherichia coli* host. Also these cold-active lipases and esterases, which are synthesized by psychrophilic and psychrotrophic, culturable microorganisms have been mainly derived through expression of lipase-encoding genes in recombinant *E. coli* strains because of slow growth of wild-type strains and low yield of enzyme synthesis. Examples of such enzymes

are bacterial lipases from *Pseudomonas fluorescens* B68 [35]and enzymes from yeasts *Mrakia psychrophila* [36].

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