INTERNATIONAL JOURNAL OF ENVIRONMENTAL SCIENCES Volume 4, No 5, 2014

Research article ISSN 0976 – 4402

Isolation, identification and hydrolytic enzymes production of aerobic heterotrophic bacteria from two Antarctic islands

Tomova I.[1], Gladka G.[2], Tashyrev A.[2], Vasileva-Tonkova E.[1]
1- Departments of General and Applied Microbiology, The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Bl. 26, 1113 Sofia, Bulgaria
2 - Department of Biology of Extremophilic Microorganisms, Zabolotny Institute of Microbiology and Virology, National Academy of Sciences of Ukraine, 154 Zabolotny Str., D 03680 Kiev, Ukraine
evaston@yahoo.com
doi: 10.6088/ijes.2014040404501

ABSTRACT

The microbial communities in some Antarctic regions still have not extensively investigated. In the present study, we isolated 24 bacterial strains under aerobic conditions from terrestrial samples at two locations in maritime Antarctica: Deception Island and Galindez Island. Phylogenetic analysis based on the 16S rRNA gene sequencing revealed affiliation of the Antarctic isolates to *Gammaproteobacteria* (54.2%), *Betaproteobacteria* (8.3%), *Firmicutes* (20.8%), and *Actinobacteria* (16.7%). The majority of isolates (92%) are psychrotolerant, and 75% are halotolerant. About 63% of isolated Antarctic bacteria were able to produce hydrolytic enzymes suggesting important role of the strains in carbon and nitrogen cycling in their habitats. Among isolates, producers of proteases (58.3%), ureases (45.8%), polygalacturonases (41.6%), β-glucosidases (33.3%), phytases (20.8%) and ribonucleases (16.6%) were detected. The results revealed higher potential of isolates from Deception Island to produce hydrolytic enzymes than those from Galindez Island. To the best of our knowledge, this is the first report for polygalacturonase production by Antarctic bacteria and β-glucosidase production by culturable Antarctic *Burkholderia* strain. The results obtained contribute to better understanding of the diversity of culturable heterotrophic bacteria in maritime Antarctica and their potential for production of hydrolytic enzymes allowing detection of promising psychrotolerant producers of industrially important enzymes.

Keywords: Antarctica, Culturable aerobic bacteria, Deception Island, Galindez Island, Hydrolytic enzymes

1. Introduction

Environmental conditions are generally unfavorable in terrestrial Antarctic environments, with low thermal capacity of the substratum, frequent freeze-thaw and wet-dry cycles, low and transient precipitation, reduced humidity, rapid drainage and limited organic nutrients. Despite the extreme conditions, microorganisms including bacteria, archaea, microfungi and microalgae, are the dominant life form in the Antarctic ecosystems representing relatively simplified system sensitive to perturbations (Niederberger et al., 2008; Gesheva, 2009; Gesheva and Negoita, 2012). Bacteria play a major role in food chains, biogeochemical cycles and primary biomass production in Antarctic ecosystems (Aislabie et al., 2006; Margesin and Miteva, 2011; Peeters et al., 2011).

Received on February 2014 Published on March 2014

Psychrophilic and psychrotolerant microorganisms have developed various structural and functional adaptations allowing them to survive in harsh environments. Enzymes are essential target for adaptation of microorganisms to cold environment (Feller and Gerday, 2003; Ferrer et al., 2007). In the past decades, production of enzymes by psychrophilic/psychrotolerant microorganisms has been widely studied for developing diverse industrial applications (Cavicchioli et al., 2011; Kumar et al., 2011). Although more than 3000 different enzymes have been identified (Van den Burg, 2003), a lot of them with industrial application, they are not sufficient to meet all requirements of industry. Due to the high catalytic activity at low temperatures, low thermostability and high specific activity, enzymes from cold-adapted microorganisms might offer novel opportunities for biotechnological exploitation (Russell, 2000). In this regard, isolation and characterization of novel psychrophilic/psychrotolerant microorganisms is one of the most active fields of applied microbiology research as they could be inexhaustible source of novel enzymes (Cavicchioli et al., 2011; Kumar et al., 2011; Gesheva and Vasileva-Tonkova, 2012; Loperena et al., 2012).

Microbial studies of Antarctic terrestrial habitats have mostly been restricted to local regions and relatively little is known about patterns of terrestrial microbial diversity across Antarctica (Yergeau et al., 2007). Isolation of microorganisms from more Antarctic habitats and investigation of their biosynthetic potential would allow more reliable conclusions on microbial distribution and endemism. The aim of the present study was to isolate and characterize aerobic heterotrophic bacteria from terrestrial biotopes in two Antarctic islands, and to screen isolates for production of hydrolytic enzymes aiming to find promising psychrotolerant producers of industrially important enzymes.

2. Materials and methods

2.1 Sampling sites

Surface samples (0-5 cm) used for isolation of heterotrophic bacteria were collected from terrestrial biotopes in two Antarctic islands: stream sediment sample from Deception Island (South Shetland Islands), and ornithogenic soil sample from Galindez Island (Argentine Islands). Samples were taken during the Antarctic Research Expeditions in 2008 and 2010. They were shipped to the laboratory under ice and stored frozen at –20°C in sterile containers until analysis.

2.2 Isolation and cultivation of heterotrophic bacteria

Heterotrophic enrichment cultures were performed from each sample at 12 and 20°C. One gram of each sample was placed in Erlenmeyer flasks and suspended in 15 ml of each of the enrichment culture media (pH 7.0): R2A broth, and nutrient broth (NB, Oxoid, UK). After incubation for 14 days including two transfers, a loopful of each inoculum source was streaked on the surface of each of the following agar media: nutrient agar (NA), R2A agar, peptone-corn extract agar (PCA), and yeast-malt extract agar (YMA). Petri plates were incubated at the indicated temperatures and growth was monitored at 24 h intervals up to 10 days in order to isolate a variety of growing bacteria. After incubation, colonies with different morphology were picked out and isolates were obtained in pure cultures by two successive transfers on NA/R2A plates. The pure cultures were stored at +4°C by transferring every three months on NA to control purity and viability. The bacterial suspensions, mixed with 30% (v/v) glycerol, were stored at –80°C.

2.3 16S rDNA amplification and restriction analysis

Phylogenetic affiliation of the Antarctic isolates was determined by16S rDNA analysis. Bacterial genomic DNA was extracted from isolates using GenElute Bacterial Genomic DNA kit (Sigma-Aldrich) in accordance with the manufacturer's instructions. The 16S rRNA gene was amplified from the extracted DNA using universal bacterial primers 8F and 1492R (Weisburg et al., 1991). PCR was performed as described in our previous work (Tomova et al., 2013). The amplified 16S rRNA genes were subjected to RFLP analysis using four base pairs restriction enzymes (MspI and HaeIII, Fermentas) as described previously (Tomova et al., 2013). Isolates with identical RFLP profiles were considered to be phylogenetically identical. One or more representatives of each RFLP pattern group were chosen for sequencing, which was done in Macrogen, South Korea. Sequence analyses were performed using BLASTn search analysis to determine the closest relatives and approximate phylogenetic affiliation of isolates. Phylogenetic tree was constructed using MEGA software version 4.1.

2.4 Growth temperature range and salt tolerance of bacterial isolates

Growth of the Antarctic bacteria was monitored in NB at a temperature range of 0-37°C. After inoculation (2% of each 24 h pre-culture), the strains were cultivated for 2 to 5 days at 4, 10, 15, 20, 25, 30 and 37°C. Salt tolerance of bacterial isolates was tested in NB containing 5% and 10% (w/v) NaCl. After inoculation, the tubes were incubated at 18 ± 2°C for 2-3 days. Growth of the strains was measured by monitoring the turbidity at 570 nm (OD_{570}).

2.5 Hydrolytic enzymes activity assay

The production of hydrolytic enzymes by the Antarctic isolates was determined using a diffusion method on solid media with specific substrates. Basal mineral agar medium (pH 7.0) used contained (%): KH_2PO_4 0.1, $(NH_4)_2SO_4$ 0.5, $MgSO_4$.$7H_2O$ 0.01, NaCl 0.01, and agar 2.0. Inoculated plates were incubated for 3 to 10 days at 18 ± 2°C. Two replicates were carried out for each sample. The growth of cultures, zones of clearing around the colonies or color-diffusion zones on respective specific media were used as an indication of the presence of enzyme activity. The activities of protease (PRO) on skim-milk (sm) and gelatine (gel), α-amylase (AMY), lipase (LIP), ribonuclease (RNAse)/desoxiribonuclease (DNAse), cellulase (CEL) on carboxymethyl cellulose (cmc), urease (Ure), phytase (PHY), β-glucosidase (β-GLU) and polygalacturonase (PGAse) were determined as described previously (Tomova et al., 2013). Endo-cellulase, xylanase (XYL) and chitosanase (CSN) activities were detected in peptone-yeast extract agar (PYA) supplemented with 0.05% (w/v) of each insoluble azurine cross-linked hydroxyethyl-cellulose (AZCL-HE-cell), AZCL-Xylan and AZCL-Chitosan (Megazyme, Bray, Ireland). Substrate degradation appeared as a blue zone around the colony as a result of substrate hydrolysis when the chromophore compounds are released in the agar medium (Pedersen et al., 2009). The β-galactosidase (β-GAL) activity was determined by the appearance and intensity of blue-colored colonies during growth on LB agar with X-Gal (5-Bromo-4-chloro-3-indolyl-β-D-galactopyranoside) and IPTG (isopropyl-β-D-thiogalactopyranoside) as an inducer (Karasová et al., 2002).

3. Results and discussion

3.1 Isolation and identification of the Antarctic bacteria

We isolated and cultured 24 aerobic heterotrophic bacteria from two terrestrial biotopes in maritime Antarctica (Table 1).

Table 1: Characteristics of bacterial isolates from two Antarctic islands

Isolate code	Isolation medium	Temperature (°C) for:			Closest related species / Accession number / Similarity (%)
		Isolation	Maximal growth rate	Maximal growth	
Sample type: Stream sediment. Location: Deception Island					
A1-1	R2A	10	25	30	*Pseudomonas* sp. CCM 7848 / HQ844526.1 / 98
A1-2*	NA	10	25	30	*Pseudomonas* sp. AC10 / FJ424489.1 / 99
A1-4	NA	10	25	30	*Janthinobacterium lividum* KOPRI 25595 / HQ824865.1 / 99
A1-7	NA	18	25	37	*Pseudomonas migulae* D67 / JN228320.1 / 99
A1-8	NA	10	25	30	*Pseudomonas* sp. AC10 / FJ424489.1 / 99
A1-10	NA	10	25	30	*Pseudomonas* sp. AC10 / FJ424489.1 / 99
A1-12	R2A	10	25	30	*Pseudomonas* sp. AC10 / FJ424489.1 / 99
A1-13	YA	18	30	30	*Pseudomonas migulae* KP-24CO / JX454785.1 / 100
A1-14	PCA	18	20-25	30	*Rhodococcus luteus* 7Y / AJ576249.1 / 99
A1-15	PCA	18	20-25	30	*Arthrobacter psychrochitiniphilus* JCM 13874 / HM165461.1 / 99
A1-16	PCA	18	25	30	*Arthrobacter psychrochitiniphilus* IARI-R-98 / JX429031.1 / 99
A1-17	PCA	18	20	37	*Arthrobacter livingstonensis* LI2 / GQ406811.1 / 99
Sample type: Soil. Location: Galindez Island					
A2-1	NA	10	25	30	*Pseudomonas* sp. HC6-6 / JF313016.1 / 99
A2-2	NA	10	15-20	25	*Pseudomonas* sp. C16w / GQ247788.1 / 99
A2-3	NA	18	25	30	*Sporosarcina* sp. SS16.26 / KC160946.1 / 99
A2-4	NA	18	25	30	*Bacillus* sp. SS16.1 / KC160930.1 / 100
A2-5	NA	18	20	30	*Sporosarcina globispora* OS-253 / AM237400.1 / 99
A2-6	NA	18	20	37	*Sporosarcina aquimarina* SF237 / GQ141979.1 / 99
A2-7	NA	10	15-20	30	*Pseudomonas* sp. AC10 / FJ424489.1 / 99
A2-8	NA	18	25	30	*Pseudomonas putida* NBRC 12653 / AB680304.1 / 99
A2-13	R2A	18	25	30	*Pseudomonas gessardii* NBRC 101045 / AB681347.1 / 99
A2-15	PCA	18	15-20	25	*Pseudomonas fluorescens* MLB-43 / JX205210.1 / 99
A2-16	PCA	18	20	30	*Burkholderia sordidicola* BLN14 / GQ181054.1 / 99
A2-17	YMA	18	30	37	*Sporosarcina aquimarina* 38 / JX475902.1 / 99

*strains A1-8, A1-10, A1-12 and A2-7 refer to A1-2 restriction group and were not sequenced

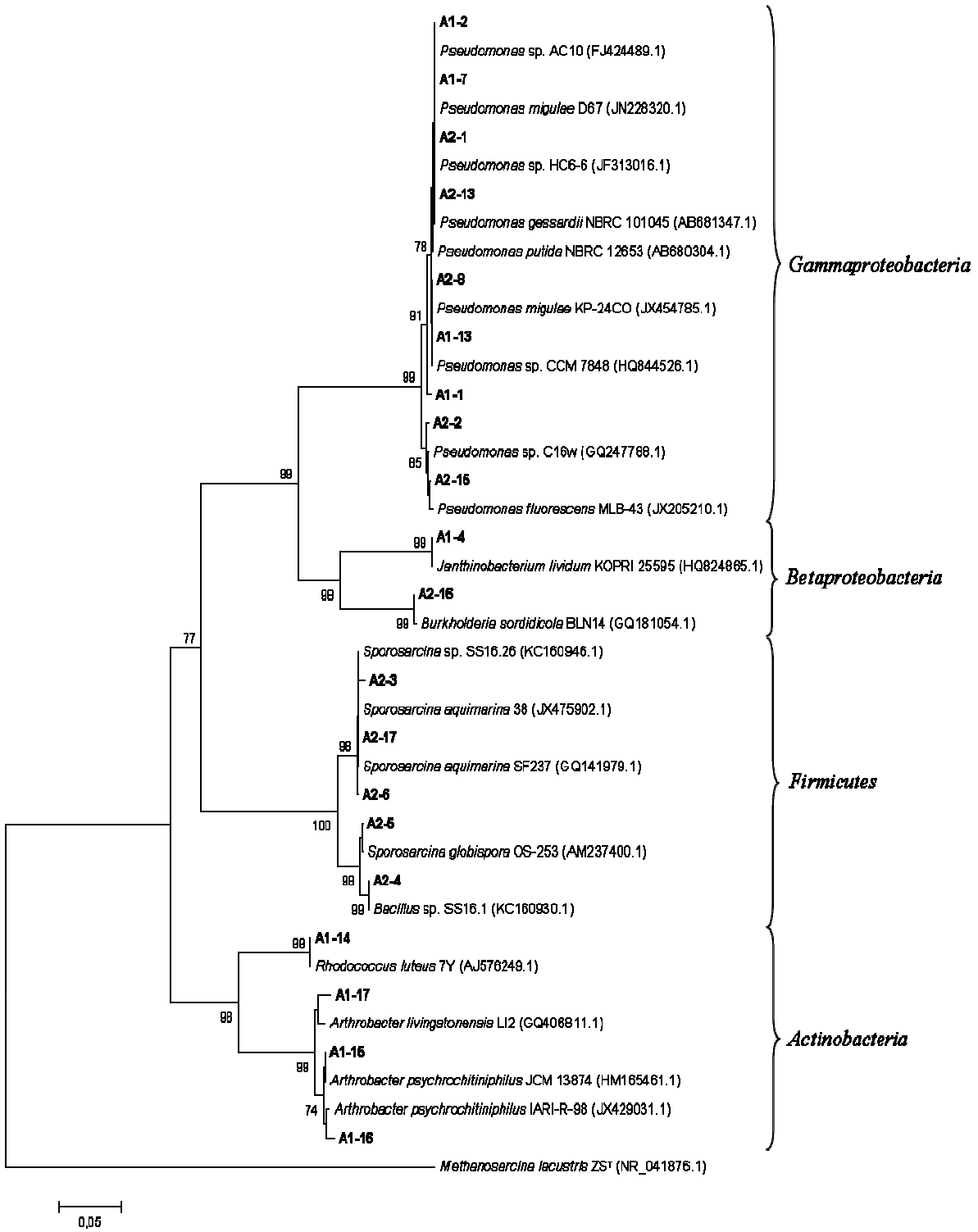

Figure 1: Neighbor-joining tree showing the phylogenetic position of the Antarctic isolates among their closest neighbors. Bar, 5% substitutions in nucleotide sequence. Bootstrap values greater than 70% confidences are shown at branching points. The archaeon *Methanosarcina lacustris* was used as an out-group. Accession numbers are given in parentheses

Twelve isolates, designated A1, were retrieved from the sediment sample of Deception Island, and 12 isolates, designated A2, were recovered from the soil sample of Galindez

Island. Nine strains were isolated at 10 ± 2°C, and 15 strains were isolated at 18 ± 2°C. After amplification and restriction of the 16S rRNA gene of each isolate, their restriction profiles were compared and isolates were distributed into 12 groups. Gram staining and 16S rRNA gene sequence analysis indicated that Gram-negative bacteria dominated among isolates (15 strains), while nine Gram-positive strains were isolated (Table 1). Out of Gram-positive phylotypes, four belonged to the phylum *Actinobacteria*, represented by genera *Arthrobacter* and *Rhodococcus*, and five belong to the phylum *Firmicutes*, represented by genera *Bacillus* and *Sporosarcina*. All Gram-negative phylotypes belong to the phylum *Proteobacteria* (classes *Gamma-* and *Betaproteobacteria*). All *Gammaproteobacteria* isolates were members of the genus *Pseudomonas*, and *Betaproteobacteria* were presented by genera *Janthinobacterium* and *Burkholderia*. The constructed neighbor-joining tree shows the phylogenetic position of A1 and A2 isolates among their closest neighbors (Figure 1). *Proteobacteria* were presented in both A1 and A2 samples (66.6% and 50%, respectively), *Actinobacteria* were identified only in A1 sample (33.4%), while *Firmicutes* phylum was presented only in A2 sample (41.7%).

The observed domination of *Proteobacteria* in the samples was in agreement with previous reports of *Proteobacteria* as the most abundant phylum among the microbial communities in Antarctic environments (Yergeau et al., 2007; Spain et al., 2009). *Proteobacteria*, *Actinobacteria* and *Firmicutes* are among the major phyla that have most often encountered in Antarctica (Aislabie et al., 2006; Peeters et al., 2011). Predominance of pseudomonads in the samples was not surprising as they are characterized by high metabolic versatility, aerobic respiration and motility (Reddy et al., 2004). Culturable bacteria belonging to endospore-forming genera within the phylum *Firmicutes* (*Bacillus* and *Sporosarcina*) were readily isolated from ornithogenic soil sample from Galindez Island but not from Deception Island sample. *Firmicutes* phylum is favored by greater availability of nutrients such as ornithogenic soils (Aislabie et al., 2009). Spore forming is a well-known survival strategy of microorganisms inhabiting Polar Regions (Yukimura et al., 2009). The *Actinobacteria* is considered less capable of long-term dormancy than *Firmicutes*; presence of members of this phylum in the sample from Deception Island may indicate higher organic content in this biotope.

3.2 Growth patterns of the Antarctic isolates

Growth temperature range of the Antarctic isolates was tested in NB (pH 7.0). It was found (Table 1), that most of isolated Antarctic bacteria are psychrotolerant (psychrotrophs) determined by their ability to grow at both +4 ± 0.1°C and above 20°C, with highest growth rate observed between 20 and 30°C, and upper growth limits at 30 or 37 °C (Morita, 1975). Only A2-2 and A2-15 isolates could be considered to be psychrophilic in that they didn't grow or grew very poorly above 25°C. Our results confirmed previous findings that in cold ecosystems psychrotrophs are usually more abundant than obligate psychrophiles (Van Trappen et al., 2002). The predominance of psychrotolerant microorganisms is probably a response to the wide temperature fluctuations in Antarctic ecosystems that allows microorganisms to survive at these unstable conditions (Delille and Perret, 1989). Nine isolates (A1-14, A1-15, A1-16, A1-17, A2-2, A2-5, A2-7, A2-15, A2-16) showing slow growth and low population density in NB, can be considered facultative oligotrophs able to grow at both low and high concentration of organics. One possible reason for low growth of these isolates might be that they are adapted to low nutrient environments as Antarctic soils, or a required nutrient or "growth factor" was absent in the used medium.

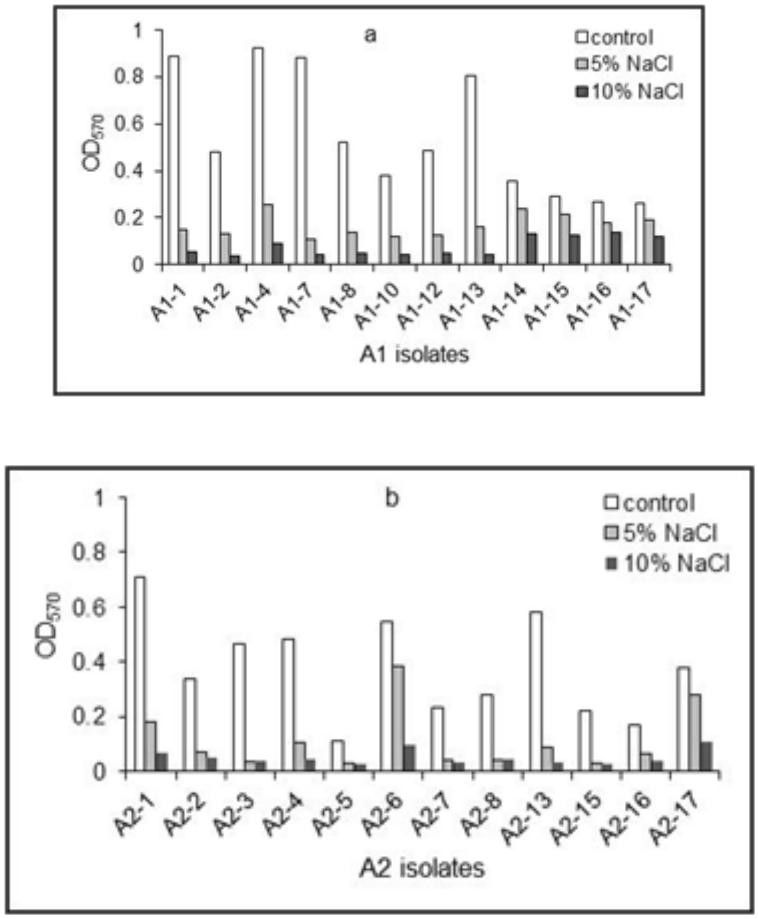

Figure 2: The effect of salt concentration on growth of the Antarctic bacteria

Salt tolerance of the Antarctic isolates was tested by their ability to grow in NB containing 5 and 10% NaCl (pH 7.0 and 20°C). Of A1 isolates, three tolerate up to 5% NaCl in the growth medium, and eight tolerate up to 10% NaCl (Figure 2a). Of A2 isolates, two tolerate up to 5% NaCl, and five - up to 10% NaCl (Figure 2b). Isolates able to tolerate up to 5% NaCl (about 21% of all isolates) can be considered slightly halotolerant, and isolates able to tolerate up to 10% NaCl (54% of all isolates) - moderately halotolerant. It could suggest that halotolerance of microbial isolates is due to permanent impact of high salinity sea water during formation of ground microcenoses in maritime Antarctica.

3.3 Hydrolytic enzymes activity

The Antarctic isolates were screened for production of hydrolytic enzymes and the results are summarized in Table 2. The size of halos and colonies were used to compare the enzyme activities and colony growth. A1 isolates showed higher potential to produce hydrolytic enzymes than A2 isolates exhibiting three to nine of the tested enzyme activities, while ten A2 isolates displayed one to five of the enzyme activities. Percentage distribution of the Antarctic isolates producing the indicated enzymes is presented in Figure 3. Protease activity (caseinase and gelatinase) was exhibited by 75% of A1 isolates and 42% of A2 isolates. Arbutin, PGA and phytate were hydrolyzed by 58, 50 and 42% of A1 isolates, respectively; 33% showed β-GAL and 25% RNAse activity. Of A2 isolates, PGAse and β-GAL activity was observed in 33% and 25% of isolates, respectively, about 16% hydrolyzed arbutin and cmc, and 8% - RNA. The AMY, HE-CEL and XYL activity was detected only in A1-7

isolate, and LIP activity in A2-16 isolate. No hydrolysis of DNA and AZCL-Chitosan was observed by any of isolates.

Table 2: Hydrolytic enzymes activity of bacterial isolates from two Antarctic islands

Isolate code	PRO (sm)	PRO (gel)	LIP	AMY	RNAse	CEL (cmc)	CEL HE-cell	XYL	Ure	PGAse	PHY	β-GLU	β-GAL
A1-1	+++	+++	-	-	-	-	-	-	-	++	-	+++	-
A1-2	+	+++	-	-	-	-	-	-	-	+	-	-	-
A1-4	+	+++	-	-	+	-	-	-	+++	-	+	+++	+
A1-7	+	+++	-	+	++	-	+++	+++	+	-	+	±	-
A1-8	+	+++	-	-	-	-	-	-	-	-	-	-	-
A1-10	+	+++	-	-	++	-	-	-	-	-	-	-	-
A1-12	++	++	-	-	-	-	-	-	-	++	-	-	-
A1-13	++	+++	-	-	-	-	-	-	-	+	-	++	-
A1-14	-	-	-	-	-	-	-	-	+++	+	-	-	-
A1-15	-	-	-	-	-	-	-	-	+++	-	++	+	++
A1-16	++	-	-	-	-	-	-	-	+++	++	++	+	+
A1-17	-	-	-	-	-	-	-	-	+++	-	+	+++	+
A2-1	+	-	-	-	+++	-	-	-	+	-	-	-	-
A2-2	-	-	-	-	-	+	-	-	±	++	-	+++	-
A2-3	-	-	-	-	-	-	-	-	-	-	-	-	-
A2-4	-	-	-	-	-	-	-	-	+++	-	-	-	+
A2-5	+	-	-	-	-	+	-	-	+++	-	-	-	-
A2-6	-	-	-	-	-	-	-	-	+++	++	-	-	-
A2-7	-	-	-	-	-	-	-	-	-	-	-	-	-
A2-8	+++	+	-	-	-	-	-	-	++	-	-	-	-
A2-13	++	+++	-	-	-	-	-	-	-	-	-	-	-
A2-15	-	-	-	-	-	-	-	-	-	+	-	-	-
A2-16	++	-	++	-	-	-	-	-	-	+	-	++++	+
A2-17	-	-	-	-	-	-	-	-	-	-	-	-	++

Symbols: ±, zone < 1 mm; +, zone 1 to 2 mm; + +, zone 3 to 4 mm; + + +, zone 5 to 9 mm; + + + +, zone ≥ 10 mm; -, no enzyme activity detected. The β-GAL activity was measured by the intensity of blue-colored colonies. DNAse and CSN activities were negative for all tested isolates and are not included in the Table. Abbreviations of the enzymes are included in Materials and Methods section

Heterotrophic bacteria and their enzymes play a key ecological role in their natural habitats for nutrient cycling, litter degradation and many other processes. The activity of the enzymes at low temperatures is one of the essential mechanisms for adaptation of microorganisms to cold environments. Patterns of enzyme activities in environmental samples are a useful tool for assessing the functional diversity of microbial communities and organic matter turnover (Ferrer et al., 2007). They could also be applied especially in cold regions as indicators of biogeochemical changes (Konieczna et al., 2011). Diverse cold-adapted bacteria have been isolated from Antarctic environments able to degrade a wide range of polymeric substances producing enzymes like proteases, amylases, cellulases, xylanases, lipases, pectinases, and chitinases (Kumar et al., 2011; Loperena et al., 2012; Gesheva and Vasileva-Tonkova, 2012).

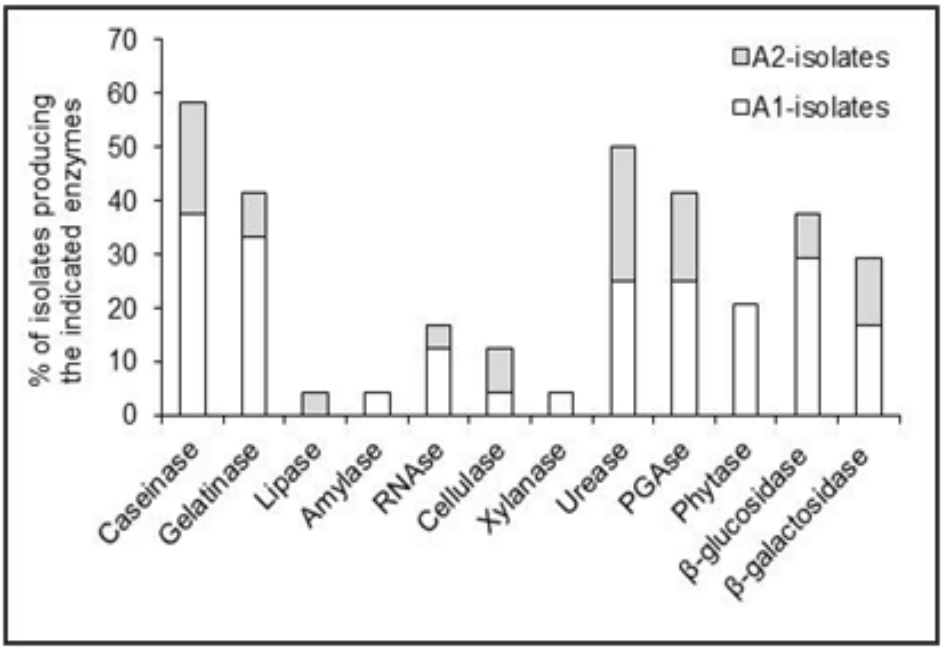

Figure 3: Distribution (%) of the Antarctic bacteria producing the indicated hydrolytic enzymes

Few studies have reported on ribonuclease and phytase production by Antarctic bacteria (Reddy et al., 1994; Park and Cho, 2011). Relatively high number of protease (73%), phytase and β-glucosidase (50%), and urease (46%) producing bacteria in the sample from Deception Island could be related to the specific habitat from which microorganisms were isolated. The presence of a variety of biopolymer-degrading enzymes detected in isolates from Deception Island (9 enzyme activities of the 15 tested) suggest significant contribution of bacteria to the hydrolysis of the major organic constituents (proteins, polysaccharides, polyribonucleotides), therefore, their important role in carbon and nitrogen cycling in the relevant habitat. In this study, we detected several promising psychrotolerant bacteria that could be applied for production of industrially important enzymes.

Of particular interest are the following psychrotolerant bacteria: A1-1 (producing protease, PGAse, phytase and β-glucosidase), A1-4 (protease, urease and β-glucosidase), A1-7 (protease, RNAse, cellulase and phytase), A1-13 (proteases, PGAse and β-glucosidase), A1-15 and A2-2 (β-glucosidase), A1-17 (phytase, β-glucosidase and β-galactosidase), salt-tolerant strains A2-6 and A2-17 (PGAse and β-galactosidase, respectively), A2-16 (protease, RNAse, PGAse and β-glucosidase). To the best of our knowledge, this is the first report for polygalacturonase production by Antarctic bacteria, and β-glucosidase production by culturable Antarctic *Burkholderia* strain. The results obtained in this study contribute to better understanding of the diversity of culturable heterotrophic bacteria in Antarctic terrestrial habitats and their potential for hydrolytic enzymes production, allowing detecting promising psychrotolerant producers of industrially important enzymes.

Acknowledgement

The authors thank the National Fund for Scientific Research of the Bulgarian Ministry of Education, Youth and Science, for financial support of this work (Contract DNTS Ukraine 01/1 - 2012). This article is dedicated to the memory of Dr. Victoria Gesheva gratefully

4. References

1. Aislabie, J.M., Chhour, K.L., Saul, D.J., Miyauchi, S., Ayton, J., Paetzold, R.F., and Balks, M.R., (2006), Dominant bacteria in soils of Marble Point and Wright Valley, Victoria Land, Antarctica, Soil biology and biochemistry, 38(10), pp 3041-3056.

2. Aislabie, J., Jordan, S., Ayton, J., Klassen, J.L., Barker, G.M., and Turner, S., (2009), Bacterial diversity associated with ornithogenic soil of the Ross Sea region, Antarctica, Canadian journal of microbiology, 55(1), pp 21-36.

3. Cavicchioli, R., Charlton, T., Ertan, H., Mohd Omar, S., Siddiqui, K.S., and Williams, T.J., (2011), Biotechnological uses of enzymes from psychrophiles, Microbial biotechnology, 4(4), pp 449-460.

4. Delille, D., and Perret, E., (1989), Influence of temperature on the growth potential of southern polar marine bacteria, Microbial ecology, 18(2), pp 117-123.

5. Feller, G., and Gerday, C., (2003), Psychrophilic enzymes: hot topics in cold adaptation, Nature reviews microbiology, 1(3), pp 200-208.

6. Ferrer, M., Golyshina, O., Beloqui, A., and Golyshin, P.N., (2007), Mining enzymes from extreme environments, Current opinion in microbiology, 10(3), pp 207-214.

7. Gesheva, V., (2009), Distribution of psychrophilic microorganisms in soils of Terra Nova Bay and Edmonson Point, Victoria land and their biosynthetic capabilities, Polar biology, 32(9), pp 1287-1291.

8. Gesheva, V., and Negoita, T., (2012), Psychrotrophic microorganism communities in soils of Haswell Island, Antarctica, and their biosynthetic potential, Polar biology, 35(2), pp 291-297.

9. Gesheva, V., and Vasileva-Tonkova, E., (2012), Production of enzymes and antimicrobial compounds by halophilic Antarctic Nocardioides sp. grown on different carbon sources, World journal of microbiology and biotechnology, 28(5), pp 2069-2076.

10. Karasová, P., Spiwok, V., Malá, Š., Králová, B., and Russel, N.J., (2002), Beta-galactosidase activity in psychrotrophic microorganisms and their potential use in food industry, Czech journal of food sciences, 20(2), pp 43-47.

11. Konieczna, I., Wojtasik, B., Kwinkowski, M., Burska, D., Nowinski, K., Zarnowiec P., and Wiesław Kaca, W., (2011), Analysis of cultivable aerobic bacteria isolated from bottom sediments in the Wijdefjorden region, Spitsbergen, Polish polar research, 32(2), pp 181-195.

12. Kumar, L., Awasthi, G., and Singh, B., (2011), Extremophiles: A novel source of industrially important enzymes, Biotechnology, 10(2), pp 121-135.

13. Loperena, L., Soria, V., Varela, H., Lupo, S., Bergalli, A., Guigou, M., Pellegrino, A., Bernardo, A., Calvino, A., Riva, F., and Batista, S., (2012), Extracellular enzymes produced by microorganisms isolated from maritime Antarctica, World journal of microbiology and biotechnology, 28(5), pp 2249-2256.

14. Margesin, R., and Miteva, V., (2011), Diversity and ecology of psychrophilic microorganisms, Research in microbiology, 162(3), pp 346-361.

15. Morita, R.Y., (1975), Psychrophilic bacteria, Bacteriological reviews, 39(2), pp 144-167.

16. Niederberger, T.D., McDonald, I.R., Hacker, A.L., Soo, R.M., Barrett, J.E., Wall, D.H., and Cary, S.C., (2008), Microbial community composition in soils of Northern Victoria Land, Antarctica, Environmental microbiology, 10(7), pp 1713-1724.

17. Park, I., and Cho, J., (2011), The phytase from antarctic bacterial isolate, *Pseudomonas* sp. JPK1 as a potential tool for animal agriculture to reduce manure phosphorus excretion, African journal of agricultural research, 6(6), pp 1398-1406.

18. Pedersen, M., Hollensted, M., Lange, L., and Andersen, B., (2009), Screening for cellulose and hemicellulose degrading enzymes from the fungal genus *Ulocladium*, International biodeterioration and biodegradation, 63(4), pp 484-489.

19. Peeters, K., Ertz, D., and Willems, A., (2011), Culturable bacterial diversity at the Princess Elisabeth Station (Utsteinen, Sør Rondane Mountains, East Antarctica) harbours many new taxa, Systematic and applied microbiology, 34(5), pp 360-367.

20. Reddy, G.S.N., Matsumoto, G.I., Schumann, P., Stackebrandt, E., and Shivaji, S., (2004), Psychrophilic pseudomonads from Antarctica: Pseudomonas antarctica sp. nov., Pseudomonas meridiana sp. nov. and Pseudomonas proteolytica sp. nov., International journal of systematic and evolutionary microbiology, 54(Pt 3), pp 713-719.

21. Reddy, G.S.N., Rajagopalan, G., and Shivaji, S., (1994), Thermolabile ribonucleases from antarctic psychrotrophic bacteria: detection of the enzyme in various bacteria and purification from Pseudomonas fluorescens, FEMS microbiology letters, 122(3), pp 211-216.

22. Russell, N.J., (2000), Toward a molecular understanding of cold-activity of enzymes from psychrophiles, Extremophiles, 4(2), pp 83-90.

23. Spain, A.M., Krumholz, L.R., and Elshahed, M.S., (2009), Abundance, composition, diversity and novelty of soil proteobacteria, The ISME journal, 3(8), pp 992-1000.

24. Tomova, I., Lazarkevich, I., Tomova, A., Kambourova, M., and Vasileva-Tonkova, E., (2013), Diversity and biosynthetic potential of culturable aerobic heterotrophic bacteria isolated from Magura Cave, Bulgaria, International journal of speleology, 42(1), 65-76.

25. Van den Burg, B., (2003), Extremophiles as a source for novel enzymes, Current opinion in microbiology, 6(3), pp 213-218.

26. Van Trappen, S., Mergaert, J., Van Eygen, S., Dawyndt, P., Cnockaert, M.C., and Swings, J., (2002), Diversity of 746 heterotrophic bacteria isolated from microbial mats from ten Antarctic lakes, Systematic and applied microbiology, 25(4), pp 603-610.

27. Yukimura, K., Nakai, R., Kohshima, S., Uetake, J., Kanda, H., and Naganuma, T., (2009), Spore-forming halophilic bacteria isolated from Arctic terrains: Implications for long-range transportation of microorganisms, Polar science, 3(3), pp 163-169.

28. Weisburg, W.G., Barns, S.M., Pelletier, D.A., and Lane, D.J., (1991), 16S ribosomal DNA amplification for phylogenetic study, Journal of bacteriology, 173(2), pp 697-703.

29. Yergeau, E., Newsham, K.K., Pearce, D.A., and Kowalchuk, G.A., (2007), Patterns of bacterial diversity across a range of Antarctic terrestrial habitats, Environmental microbiology, 9(11), pp 2670-2682.