

# Predicting the biotechnological potential of bacteria isolated from Antarctic soils, including the rhizosphere of vascular plants

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**Abstract** Although there is great interest in cultivable Antarctic microorganisms for potential biotechnology applications, little is known about the microbial diversity and the metabolic potential of samples from Antarctic environments. Two hundred and fifty bacterial isolates were obtained from Antarctic soil and from rhizosphere samples of the Antarctic plants *Colobanthus quitensis* and *Deschampsia antarctica*. The samples were screened using amplified ribosomal DNA restriction analysis (ARDRA) and identified by 16S rRNA sequencing. Their metabolic potential was predicted *in silico* using the Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUS) tool. Additionally, the strains were screened for the production of amylases, proteases, cellulases and chitinases at 4, 12 and 25 °C. The bacterial isolates obtained were classified into the phyla Proteobacteria, Arthrobacter, Firmicutes and Bacteroidetes. The species belonging to the genus *Pseudomonas* were predominant in all the soil and rhizosphere samples. Twenty-one different Operational Taxonomic Units (OTUs) were detected, and the functional profile of each OTU indicated that most of the predicted genes were related to metabolic functions. Among the OTUs, one affiliated with the genus *Rhodococcus* sp. (OTU 7) and one affiliated with the genus *Rhizobium* sp. (OTU 18) showed the largest numbers of predicted genes for amino acid and carbohydrate metabolism and for xenobiotics. The traditional enzyme screening indicated that only proteases were produced by all the

bacterial isolates and that the bacterial isolates with greatest predicted metabolic potential (OTUs 7 and 18) also presented the greatest diversity of enzyme production. The results suggested that gene prediction might reflect, at some level, the real metabolic plasticity of the microorganisms and provide a tool for screening promising biotechnological strains, including strains producing multiple enzymes.

**Keywords** Antarctic soils · PICRUS · Enzymes · 16S rRNA · Admiralty bay

## Introduction

The Antarctic Peninsula is one of the most densely populated regions of the Antarctic continent, and it has been the subject of several biodiversity surveys (Riffenburgh 1998; Curry et al. 2005; Teixeira et al. 2010, 2013). However, the microbial diversity of Antarctic soils is scarcely known (Fan et al. 2013). Because of the harsh environmental conditions, Antarctic soils are considered restrictive environments compared with soils worldwide. The microbial diversity in Antarctic soils has been assessed using both culture-dependent and culture-independent methods. García-Echauri and colleagues (2011) described, through culture-dependent methods, the dominance of Betaproteobacteria (35.2%), followed by Gammaproteobacteria (18.5%), Alphaproteobacteria (16.6%), Gram positive bacteria with high GC contents (13.0%), Cytophaga–Flavobacterium–Bacteroides (13.0%) and Gram positive bacteria with low GC contents (3.7%) in glacier sediment, sea-side mud, melted glacier ice, and *Deschampsia antarctica* rhizospheres from Collins Glacier, Antarctica. Teixeira et al. (2010) provided the first major sequencing effort for bacteria from Antarctic soils and revealed a high diversity

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in rhizosphere samples from vascular plants in Admiralty Bay, Antarctica. These authors also reported two possible main influences on maritime Antarctic soil microbiomes: birds and plants (Teixeira et al. 2013). However, Chong et al. (2012), using culture-independent methods [i.e., PCR, denaturing gradient gel electrophoresis (DGGE) and cloning], suggested that most of the Antarctic soil environments contained fewer bacterial species than expected, considering the large number of sequences recovered from Antarctic soil samples available in the databases.

Although culture-independent methods allow us to explore the broad microbial diversity of Antarctic environments, studies involving culture-dependent methods allow the description of the microbial physiology and are required for the taxonomic description of species. Some culture-dependent surveys have been conducted recently, including the description of the *Arthrobacter psychrochitiniphilus* species in ornithogenic soil in Ardley Island (Wang et al. 2009) and the description of new *Pseudomonas* species in soils from the South Shetland Islands (Bozal et al. 2007; Carrión et al. 2011). Culture-dependent and -independent techniques are complementary and can provide comprehensive information about the taxonomy and physiology of bacterial strains, contributing to the development of biotechnological products.

Currently, one of the main areas of interest in the study of extreme environments is prospecting for microbes with biotechnological uses, especially psychrophilic or psychrotolerant enzymes (Antranikian et al. 2005). These studies are especially important because psychrophilic microorganisms develop structural and functional adaptations to survive in harsh environments (D'Amico et al. 2006; Marx et al. 2007; Joshi and Satyanarayana 2013), which may represent biotechnological opportunities to be explored. As an example, psychrotolerant enzymes may have different industrial applications due to their capacity to work efficiently at low temperatures (Helmke and Weyland 2004; Tosi et al. 2010). Thus, the development and evaluation of new and efficient prospecting tools are welcome.

The main approach used to study bacterial diversity and taxonomy is based on the gene encoding the 16S rRNA, which is a common marker for phylogenetic analyses used extensively to characterize bacterial strains. Despite the capacity to establish phylogenetic affiliation, the 16S rRNA gene itself does not contain the information needed to infer metabolic function directly (Jiménez et al. 2014). Recently, a new approach was proposed to predict functional profiling of microbial communities using 16S rRNA gene sequences (Langille et al. 2013). The PICRUS pipeline (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) (Langille et al. 2013) enables one to infer the whole gene content of communities based on the taxonomical assignment of the population and the retrieval of

whole genome information of the nearest sequenced reference genome present in the Integrated Microbial Genomes (IMG) system. The program has been used to predict the gene content of different samples, including samples from soil and the human microbiome, with good correlation with metagenomic data (Langille et al. 2013; Liu et al. 2016). We propose that the program can be adapted to predict the gene content of single bacterial lineages after clustering them into OTUs.

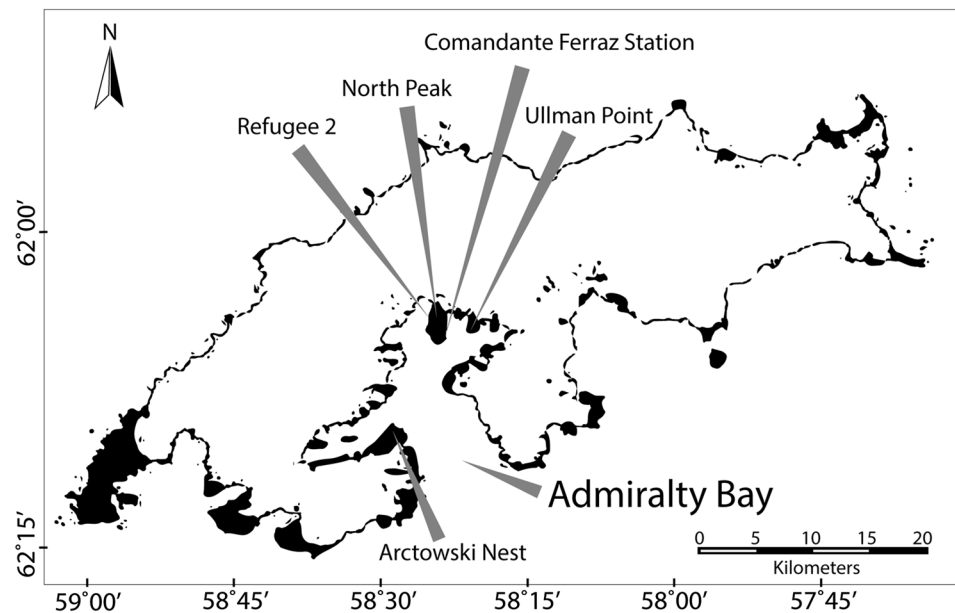
Therefore, this work aims to (1) make a phylogenetic analysis of bacterial isolates from different soils from the Maritime Antarctica; (2) propose the use of PICRUS for predicting the functional capacity of bacterial isolates; and (3) evaluate the production of some enzymes of industrial interest.

## Materials and methods

### Site description, sampling and soil analyses

Admiralty Bay is located on King George Island, South Shetland Islands. The temperatures in Admiralty Bay range from  $-3$  to  $8$  °C in summer. Glaciers cover 90% of the island, but ice-free areas are present in the coastal zone during freezing and thawing climate cycles. Composite soil (from 0 to 5 cm depth) and rhizosphere samples were taken from different areas, sampling points, on King George Island, to increase the coverage of microorganisms with distinct biotechnological potential. Sampling was performed during the austral summer of 2006/2007 Antarctic Research Expedition from Brazil. Soil samples were taken from the following places (Fig. 1): (1) under mosses at North Peak ( $62^{\circ}04'849''\text{S}$ ,  $58^{\circ}24'024''\text{W}$ ); (2) in “Refugee 2” ( $62^{\circ}04'21''\text{S}$ ,  $58^{\circ}25'335''\text{W}$ ), where the so-called “yellow soil” was visibly yellowish and similar to acid sulfate soil (Simas et al. 2008); (3) on “Ullmann Point” ( $62^{\circ}05'015''\text{S}$ ,  $58^{\circ}23'987''\text{W}$ ) from the rhizosphere of *Deschampsia antarctica*; (4) at Comandante Ferraz Scientific Station ( $62^{\circ}05'06''\text{S}$ ,  $58^{\circ}24'12''\text{W}$ ) from the rhizosphere of *Colobanthus quitensis*; (5) on “Arctowski Nest” ( $62^{\circ}09'790''\text{S}$ ,  $58^{\circ}29'687''\text{W}$ ) from ornithogenic soil. With the exception of the samples collected at North Peak and Refugee 2, all the other samples were collected at sea level between 500 m and 1 km from the coast. The North Peak, Ullmann Point and Comandante Ferraz Station sites are ice-free areas during the austral summer. These areas are reported to contain andesitic/basaltic soil features (Simas et al. 2008). The ornithogenic soil was collected close to the Arctowski Polish Station near an Adelie penguin nest. Rhizosphere soil was separated from bulk soil when the rhizosphere samples were taken. Approximately 500 g of soil was collected from each sampling point. The samples

**Fig. 1** Map of the sample sites on King George Island, South Shetland archipelago, Antarctica. Sample sites are indicated by *long arrowheads*. Adapted from Simões and colleagues (2004)



were placed in sterile plastic bags and immediately transferred to the laboratory, where they were stored at  $-20\text{ }^{\circ}\text{C}$ .

The metals in the soil samples were dissolved by the procedure described by Nadkarni (1984). Briefly, aqua regia was added to each sample and the mixture was heated in a microwave oven for 2 min at a power setting of 625 watts. The mixture was transferred to a fume hood and  $\text{H}_3\text{BO}_3$  was added' would better convey the intended meaning here. Please consider indicating the amounts of aqua regia and  $\text{H}_3\text{BO}_3$  used. Ions were analyzed by atomic absorption spectroscopy using Perkin Elmer equipment. The pH was analyzed in water using the Tecnopon MPA 210 potentiometer as described by APHA (1992). Organic carbon and sulfur were analyzed by the method described and adapted by Verardo et al. (1990) and Hedges and Stern (1984) using the CNHS EA 1110 analyzer from Carlo Erba Instruments. The results are included in Table 1.

### Isolation of bacterial strains

The samples were homogenized, and 5 g of each soil or rhizosphere sample was added to 45 mL of saline solution (0.85%). Serial ten-fold dilutions were prepared in saline, and 0.1 mL of each dilution was spread on the surface of lysogeny agar (tryptone 10 g/L, yeast extract 5 g/L, NaCl 10 g/L) (Schleif and Wensink 1981) in Petri dishes. The plates were incubated for 4 weeks at  $4\text{ }^{\circ}\text{C}$ . After incubation, different morphotypes were characterized, and representative colonies were transferred to new plates. Successive transfers were performed to obtain pure cultures. The purity of each colony was checked visually and by microscopy. Pure strains were then preserved at  $-80\text{ }^{\circ}\text{C}$  in lysogeny broth with 20% v/v glycerol.

### Identification of bacterial isolates

Two hundred and fifty bacterial isolates preserved at  $-80\text{ }^{\circ}\text{C}$  were activated and cultured overnight. The genomic DNA was extracted using the Wizard® Genomic DNA Purification Kit (Promega) in accordance with the manufacturer's instructions. A fragment of approximately 1450 bp of the 16S rRNA gene was then amplified using the universal primers 27F (5'-AGA-GTT-TGA-TCM-TGG-CTC-AG-3') and 1492R (5'-TAC-GGY-TAC-CTT-GTT-ACG-ACT-T-3') (Lane 1991). The PCR reaction mixture (50  $\mu\text{L}$ ) contained 1X buffer, 2.0 mM  $\text{MgCl}_2$ , 0.2 mM dNTP, 5  $\mu\text{M}$  of each primer, 50 ng of DNA, and 2.5 U of Taq DNA polymerase (Invitrogen (now Life Technologies), Carlsbad, CA, USA). The amplification was performed in a thermocycler (Mastercycler Gradient, Eppendorf, Hamburg, Germany) with an initial denaturation step at  $94\text{ }^{\circ}\text{C}$  for 3 min, followed by 35 cycles of denaturation at  $94\text{ }^{\circ}\text{C}$  for 1 min, annealing at  $55\text{ }^{\circ}\text{C}$  for 1 min and extension at  $72\text{ }^{\circ}\text{C}$  for 2 min, with a final extension at  $72\text{ }^{\circ}\text{C}$  for 10 min. The PCR products were examined by electrophoresis on 1% agarose gels stained with SYBR® Safe DNA Gel Stain (Life Technologies) and visualized under UV light.

To reduce the redundancy among the isolates, the PCR products were analyzed by the amplified ribosomal DNA restriction analysis (ARDRA) technique using the restriction enzymes AluI, HinfI and HaeIII (Thermo Scientific). Amplified products were digested with the individual restriction enzymes, and the fragments obtained were separated by electrophoresis on 1.5% agarose gels in TAE buffer. The gels were stained and visualized as previously described. The ARDRA profiles in different gels were normalized using a 100 bp ladder (Invitrogen). The isolates

**Table 1** Mean of soil characteristics for surface soil cores (0–5 cm) collected in five sites at Admiralty Bay, King George Island

Local-sample	Soil depth (0–5 cm)	Na (mg/kg)	Ca (mg/kg)	Mg (mg/kg)	K (mg/kg)	Al (mg/kg)	S (mg/kg)	P (mg/kg)	Fe (mg/kg)	Cu (mg/kg)	Zn (mg/kg)	Mn (mg/kg)	Pb (mg/kg)	pH, 1:2.5 water solution	Corg.
Refugee 2-yellow soil		48.31	4.000	1320.5	11.70	0.00	1.257.0	514.0	25.105	85.88	35.53	622.2	12.23	3.6	0.45
C. Ferraz chemical module-Coloban-thus quitensis	0–5	70.84	3.760	1344.4	11.70	0.00	499.2	620.0	21.542	71.78	35.38	337.7	10.83	6.6	2.00
North Peak-soil under mosses	0–5	71.30	1.600	600.0	31.20	918.0	220.8	626.0	35.603	51.63	42.18	396.7	30.73	6.6	0.78
Ullmann Point-Des-champsia antarctica	0–5	74.29	2.440	360.0	31.20	1.822.0	486.4	190.5	30.223	59.18	49.18	749.7	33.03	8.8	2.28
Pinguiner Arctow-isky-ornithogenic soil	0–5	113.39	4.800	168.0	8.97	2.586.0	624.0	1.005.0	30.833	42.98	82.38	582.7	37.68	4.3	9.70

were grouped into phylotypes according to their ARDRA profiles by visual comparison. We considered that two isolates belonged to the same phylotype if they shared the AluI, HinfI and HaeIII restriction profiles. After screening, two or more bacterial isolates from each phylotype were selected for 16S rRNA gene amplification followed by product purification using Illustra GFX PCR DNA and Gel Band Purification Kit (GE) in accordance with the manufacturer's instructions. The purified PCR products were sent to Macrogen Inc. (Geumcheon-gu, Seoul, Korea) for DNA sequencing. Products were sequenced on an Applied Biosystems ABI 3730XL automated sequencer using the primers 27F (5'-AGA-GTT-TGA-TCM-TGG-CTC-AG-3'), 1492R (5'-TAC-GGY-TAC-CTT-GTT-ACG-ACT-T-3'), 518F (5'-CCA-GCA-GCC-GCG-GTA-ATA-CG-3') and 800R (5'-TAC-CAG-GGT-ATC-TAA-TCC-3').

### Sequence analysis

Partial 16S rRNA gene sequences were trimmed using the RDP II Sanger pipeline (<http://rdp.cme.msu.edu/>) to eliminate low quality reads ( $Q < 30$ ) and to remove the primers. The full amplicon of the 16S rRNA gene was reconstructed by assembling the partial fragments into contigs, using Bioedit software (<http://www.mbio.ncsu.edu/BioEdit/bioedit.html>) (Hall 1999). The sequences were deposited at the GenBank under the reference numbers KX354837-KX354932.

Sequences were grouped into a single file, aligned against the Silva bacterial reference database and clustered into OTUs at 97% similarity using Mothur (v.1.33.0). A representative sequence of each OTU was selected and compared with the Ribosomal Database Project Release 11 database using the SeqMatch tools (Cole et al. 2009) to select related organisms to construct the phylogenetic tree. For OTUs with more than one sequence, the representative sequence was chosen randomly. We selected for each representative sequence of three microorganisms, two cultured and one uncultured. The representative sequences and their related sequences were aligned with each other using MEGA 5.2.2 (Tamura et al. 2011), and the tree was constructed using the maximum likelihood method (Jukes and Cantor 1969) and neighbor joining clustering (Gascuel and Steel 2006). *Desulfurococcus fermentans* was used as the outgroup.

### Functional inference of the isolates

One representative sequence from each OTU was treated as a separate sample and was taxonomically classified with the Greengenes database using Mothur. The resulting file was used as an input to the PICRUS tool

to predict whole gene content as described by Langille et al. (2013). To evaluate the accuracy of the prediction, we observed the nearest sequenced taxon index (NSTI) obtained from each prediction. The NSTI is an indicator of the availability of nearby genome representatives for each sample and is negatively correlated with the prediction accuracy.

### Screening for extracellular hydrolytic enzyme production

One isolate from each OTU was used for enzyme screening and was tested for the production of hydrolytic enzymes. The detection assays, based on growth on solid media with single substrates as carbon sources, were conducted to evaluate the production of extracellular hydrolytic enzymes by the bacterial isolates. The microorganisms were cultured on agar plates (yeast extract 1.5 g/L, meat extract 1.2 g/L, glycerol 12 mL; tryptone 3 g/L, bacteriological agar 22.5 g/L, pH 7.3) supplemented with starch (1%) for amylase tests. Gelatin 0.4% was used for protease tests. Carboxymethyl cellulose in 10 g/L,  $\text{KH}_2\text{PO}_4$  4 g/L,  $\text{Na}_2\text{HPO}_4$  4 g/L, tryptone 2 g/L,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  0.2 g/L,  $\text{CaCl}_2$  0.001 g/L,  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  0.004 g/L, agar 15 g/L, pH 7, was used for cellulase tests (Bairagi et al. 2002). For the chitinase tests, medium containing  $\text{K}_2\text{HPO}_4$  0.7 g/L,  $\text{KH}_2\text{PO}_4$  0.3 g/L,  $\text{MgSO}_4 \cdot 5\text{H}_2\text{O}$  0.5 g/L,  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  0.01 g/L,  $\text{ZnSO}_4$  0.001 g/L,  $\text{MnCl}_2$  0.001 g/L, agar 20 g/L, was used. The colloidal chitin was prepared as described by Murthy and Bleakley (2012). The incubation was carried out for 3 days for microorganisms at 25 °C, 7 days for microorganisms at 12 °C and 15 days for microorganisms at 4 °C. The different times of incubation were necessary because of the differences in the microbial growth rates under different temperatures. This was set to evaluate the enzymatic production after the observed growth. For all the tested enzymes, a clear halo around the colony after incubation was considered to be a positive reaction. The clear zones of hydrolysis in media containing CM-cellulose were developed by flooding the agar surface with an aqueous solution of Congo Red dye (1 mg/mL) for 15 min at room temperature (Teather and Wood 1982). In the case of starch, the hydrolysis zones were observed by flooding the agar media with an iodine solution (Sunnotel and Nigam 2002; Brizzio et al. 2007). For gelatin plates, a mercury chloride solution ( $\text{HgCl}_2$  15 g, HCl 20 mL, 100 mL distilled water) was used. Keratinolytic activity was measured in vials with 5 mL of solution ( $\text{Na}_2\text{HPO}_4$  11.876 g,  $\text{KH}_2\text{PO}_4$  9.072 g, one feather, distilled water 1,000 mL, pH 7.2). The microbial pellet was incubated at 4, 12 and 25 °C for 2 weeks at 150 rpm.

## Results

### Bacterial identification

Two hundred and fifty bacterial isolates were obtained from the different soil samples. A morphological diversity was observed, but circular, white and opaque colonies, with entire edges, and a diameter of 0.4 mm predominated.

The ARDRA profiles from the 250 isolates were clustered into 42 phylotypes, from which two or more bacterial isolates were selected for 16S rRNA gene sequencing, resulting in 96 sequenced bacteria. After a quality check, the sequences were clustered using a cutoff of 3% of dissimilarity, which grouped all the sequences into 21 different operational taxonomic units (OTUs) (Table 2), showing that the resolution of the ARDRA was more stringent than the cluster at 3%. Therefore, it is very unlikely that the initial screening grouped different species into the same phylotype.

The phylogenetic affiliation of each OTU showed that the bacterial isolates belonged to four different phyla, Proteobacteria, Firmicutes, Actinobacteria and Bacteroidetes. The representative sequences are summarized in Table 2.

The Proteobacteria phylum (Fig. 2) was predominant in all the sampled soils, representing up to 71.7% of all the bacterial isolates recovered. In the rhizosphere of *Colobanthus quitensis*, this phylum accounted for 60% of

the recovered isolates, 66.7% in the rhizosphere of *Deschampsia antarctica*, 63.6% in the soil under mosses, 76.7% in ornithogenic soil and 83.3% in yellow soil. The Proteobacteria phylum is represented by OTUs 1, 3, 4, 5, 14, and 18. The genus *Pseudomonas* (OTU 1) was the predominant genus found, and it included 54 isolates found in ornithogenic soil, soil under mosses, yellow soil and the rhizospheres of *Deschampsia antarctica* and *Colobanthus quitensis*.

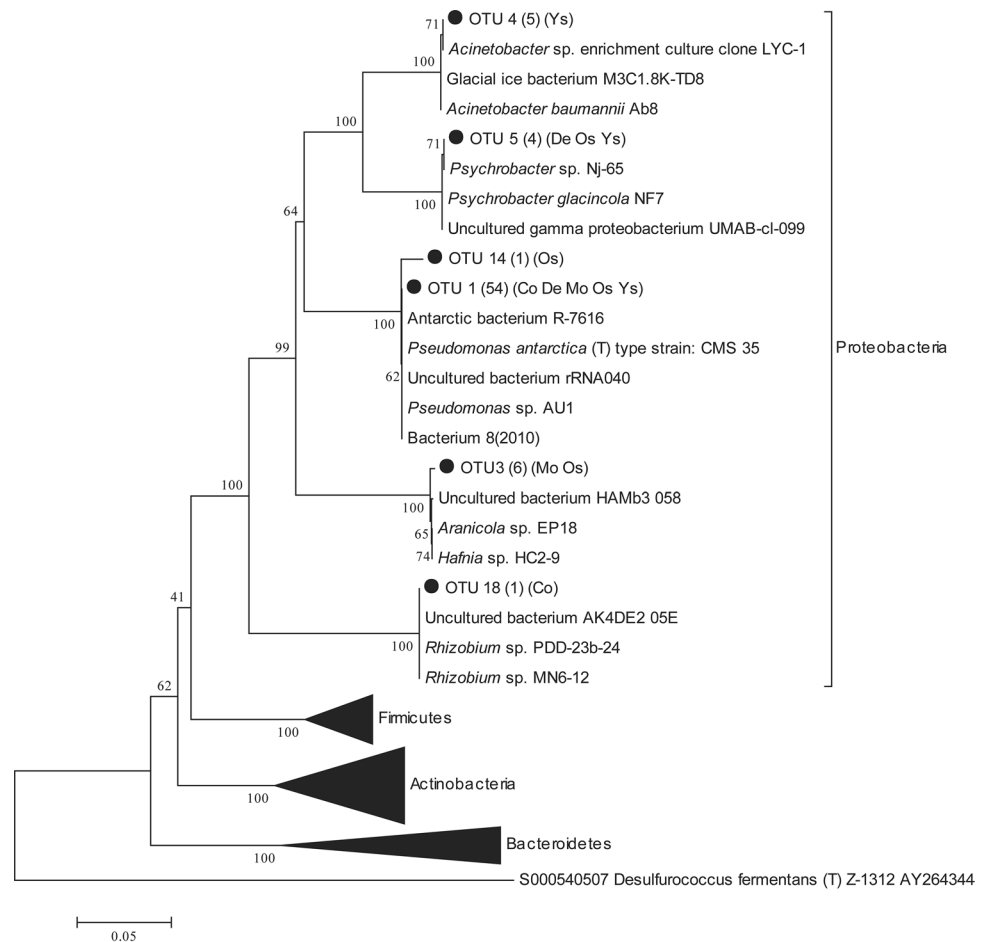
The second most frequently represented phylum in the analyzed samples (represented by OTUs 2, 7, 11, 13, 17, 19 and 21) was Actinobacteria (Fig. 3), represented in all the soils, and it corresponded to 17.2% of the recovered bacterial samples. In the rhizospheres of *Colobanthus quitensis* and *Deschampsia antarctica*, the relative abundances were 26.7 and 22.2%, respectively. In soils under mosses, ornithogenic soil and yellow soil, the values were 18.2, 11.6 and 16.7%, respectively.

The Firmicutes phylum (Fig. 4) (OTUs 6, 10, 15, 16 and 20) represented 13.3% of the sequenced bacterial isolates of the rhizosphere of *Colobanthus quitensis*, 18.2% of bacterial isolates from soils under mosses, 4.6% from ornithogenic soil and 11.1% from the *Deschampsia antarctica* rhizosphere. Bacterial isolates belonging to the Firmicutes phylum were not detected in yellow soil samples. The phylum Bacteroidetes (Fig. 5) was only

**Table 2** Taxonomic affiliation of representative sequences of each OTU

OTU	Representative microorganism for each OTU	Base pairs	Identity (%)	Accession number
1	<i>Pseudomonas</i> sp. SAP724.1	1463	99	JX067675.1
2	<i>Arthrobacter cryoconiti</i> strain Cr6-08	1430	99	NR_108846.1
3	<i>Hafnia</i> sp. HC4-16	1397	99	JF313002.1
4	<i>Acinetobacter calcoaceticus</i> strain N7	1383	99	KU041598.1
5	<i>Psychrobacter</i> sp. Nj-65	1433	99	AM491467.1
6	<i>Bacillus thuringiensis</i> strain AS09	1445	99	KX101236.1
7	<i>Rhodococcus</i> sp. EL2	1390	99	FJ517615.1
8	<i>Algoriphagus antarcticus</i> strain 64	1340	100	JX475901.1
9	Bacterium HAB 15	1376	100	KF593843.1
10	<i>Planococcus kocurii</i> strain ATCC 43,650	1429	99	CP013661.2
11	<i>Arthrobacter</i> sp. THG-S12	1380	99	KJ810590.1
12	<i>Chryseobacterium antarcticum</i> strain AT1013	1439	99	AY553293.2
13	<i>Arthrobacter</i> sp. KAR53	1407	99	KR055014.1
14	<i>Pseudomonas</i> sp. SCS1-O4	1340	98	KR023914.1
15	<i>Bacillus methylophilus</i> strain LD34	1384	99	KR855694.1
16	<i>Staphylococcus epidermidis</i> strain LOCK 1005	1397	99	KT728836.1
17	<i>Arthrobacter antarcticus</i> strain 27	1383	99	KF923437.1
18	<i>Rhizobium</i> sp. MN6-12	1333	99	JQ396566.1
19	<i>Leifsonia kafniensis</i> strain KFC-22	1408	99	NR_042669.1
20	<i>Sporosarcina aquimarina</i> strain 38	1450	100	JX475902.1
21	<i>Clavibacter michiganensis</i> subsp. <i>phaseoli</i> strain LPPA 982	1418	99	NR_133729.1

**Fig. 2** A phylogenetic tree of the Proteobacteria phylum, including microorganisms sampled in Antarctic soils, obtained from similar microorganisms using the RDP pipeline of SeqMatch and edited in the MEGA 5.2 program using the Jukes–Cantor method of maximum likelihood and a bootstrap value of 1000



detected in ornithogenic soil, and it corresponded to 7% of the sequenced isolates.

### Functional inference of the isolates

The prediction of functional genes was investigated using the PICRUS tool for the 21 OTUs obtained from the Antarctic soil and rhizosphere samples. With the exception of OTU 9, which presented an NSTI of 0.154, all the other OTUs showed a very low NSTI, ranging from 0.001 to 0.062.

The overview of the functional prediction is shown in Fig. 6. Regardless of the bacterial isolate, metabolism was the most abundant functional category found, with almost 1700 different genes predicted on average in each OTU. Environmental and genetic information processing showed, on average, 500 different genes each, and cellular processes showed approximately 100 different genes. The OTUs 7 (*Rhodococcus* sp.) and 18 (*Rhizobium* sp.) showed the largest number of predicted functional genes involved

in metabolism, with 2715 and 3826 different genes, respectively.

Analyzing the metabolism class (Fig. 7), we observed that the relative abundance of the sub-classes was very homogeneous among the isolates. However, the OTUs 7 and 18 (*Rhodococcus* sp. and *Rhizobium* sp.) showed more genes associated with each subclass, highlighting the predicted functional genes for the metabolism of amino acids, carbohydrates, and for xenobiotic metabolism. The large number of genes may suggest that these isolates have high metabolic capacities.

### Screening for extracellular hydrolytic enzyme production

Table 3 shows the results of the enzymatic screening using a representative isolate of each OTU. In general, the observed hydrolytic activity was similar at all temperatures. Keratinase production was not detected in any OTU. The production of amylase was detected in 50% of the OTUs, including OTUs 1, 2, 3, 6, 7, 14, 15, 17, 18 and 20 at all

**Fig. 3** A phylogenetic tree of the Actinobacteria phylum, including microorganisms sampled in Antarctic soils, obtained from similar microorganisms using the RDP pipeline of SeqMatch and edited in the MEGA 5.2 program using the Jukes-Cantor method of maximum likelihood and a bootstrap value of 1000



temperatures. The production of proteases was detected in all the OTUs except number 8 at temperatures of 12 and 25 °C. The OTUs 9, 10 and 12 did not show protease activity at 4 °C. The production of cellulase was detected only in OTUs 7 and 18 at all temperatures, and chitinase was detected only in OTU 7.

## Discussion

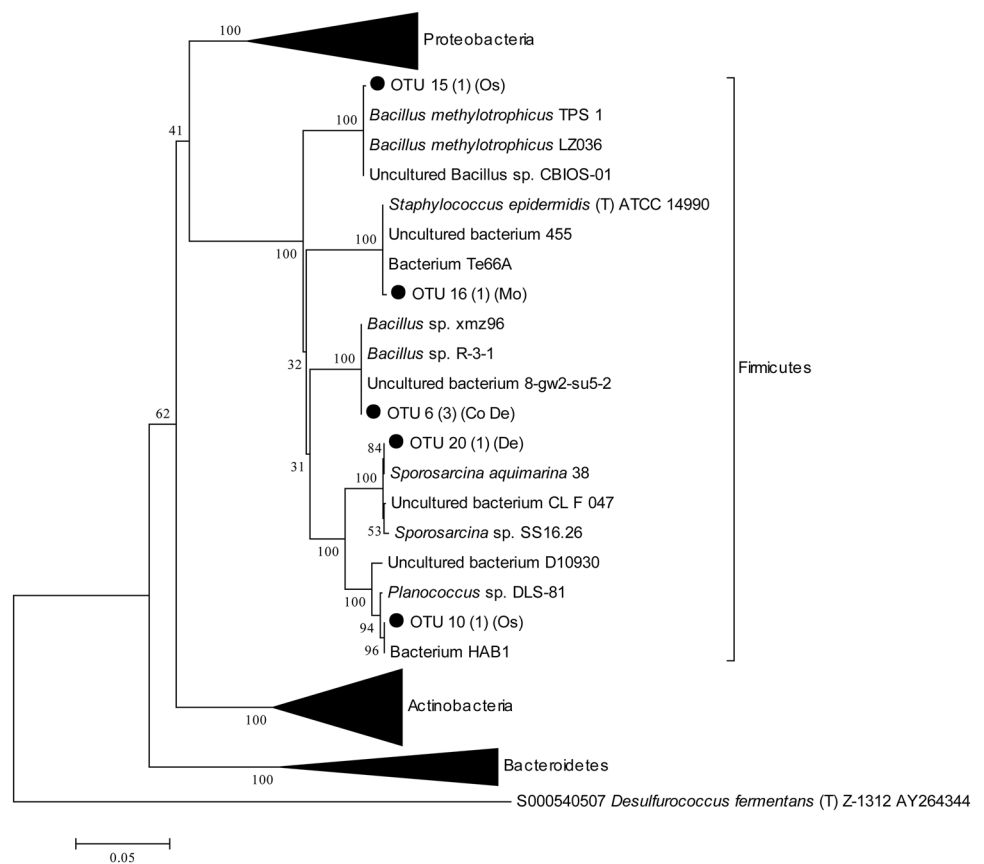
We analyzed part of the culturable microbial community present in some places that had not been assessed previously. The preliminary results of the use of PICRUS to select strains with higher biotechnological potential

indicates that this is a potential biotechnological screening approach, and the results for the traditional screening for enzymatic production at different temperatures selected promising strains for different biotechnological applications, including strains that produced multiple enzymes.

The functional inference data were evaluated to generate an overview of the prediction of functional genes for each microorganism sampled. With the exception of OTU 9, all the OTUs showed a very low NSTI, ranging from 0.001 to 0.062, which means that the prediction was based on a very close representative genome (Langille et al. 2013), and therefore, it indicates a high correlation with real data. OTU 9 showed an NSTI of 0.154, which indicates a lower correlation with the whole genome



**Fig. 4** A phylogenetic tree of the Firmicutes phylum, including microorganisms sampled in Antarctic soils, obtained from similar microorganisms using the RDP pipeline of SeqMatch and edited in the MEGA 5.2 program using the Jukes–Cantor method of maximum likelihood and a bootstrap value of 1000



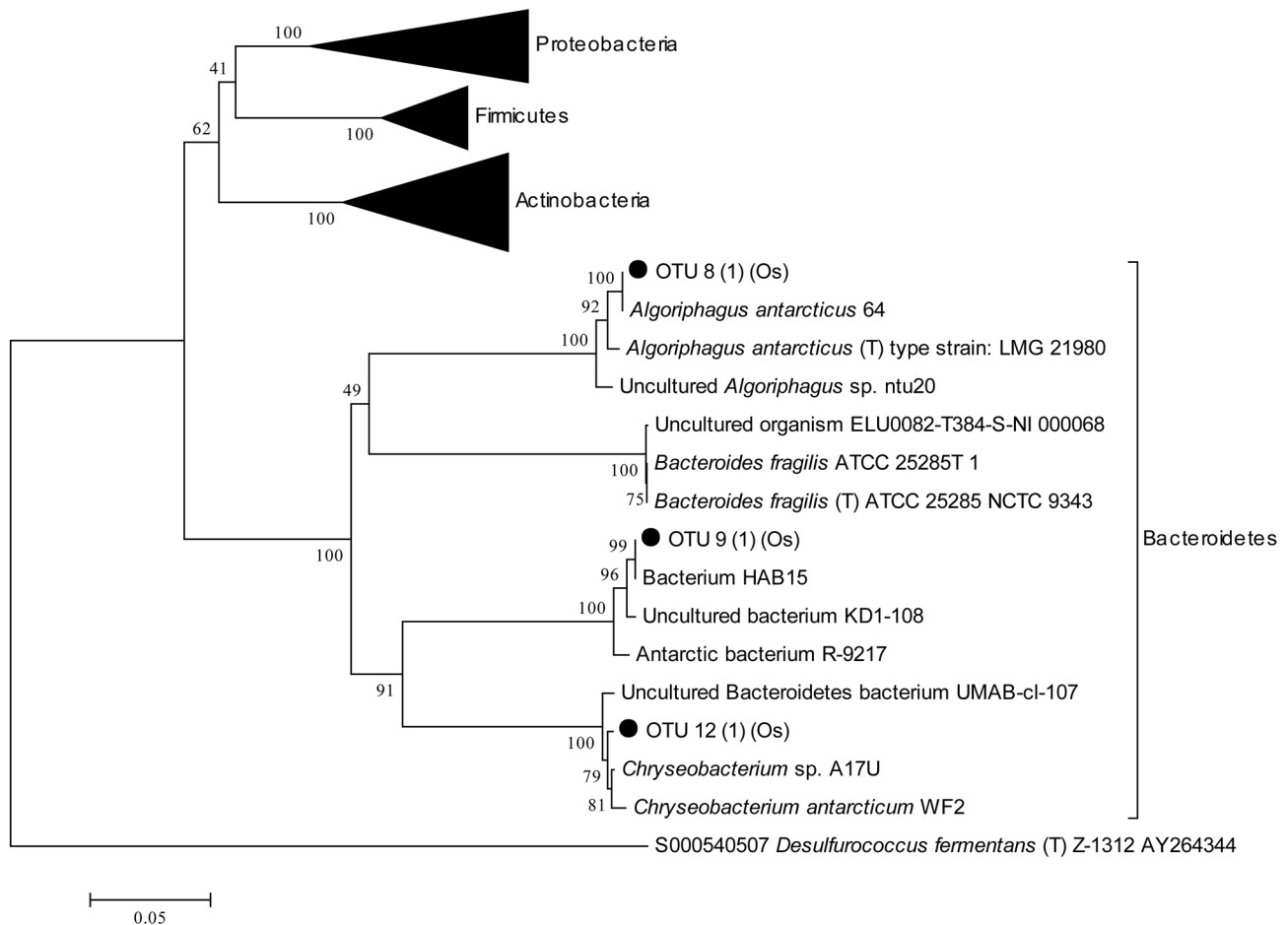
sequencing. This result may be related to the fact that this OTU did not cluster with any known lineage of bacteria and may represent a novel species. Therefore, it has no close members in the database, and the whole genome content cannot be reliably predicted.

Analyzing the metabolism prediction, we found more predicted genes for the *Rhodococcus* sp. OUT 7 and the *Rhizobium* sp. OUT 18, especially genes related to metabolism, with more predictions for the *Rhodococcus* sp. OTU 7 than for the *Rhizobium* sp. OUT 18. Interestingly, these same bacterial isolates with higher numbers of predicted genes, showed more metabolic plasticity, considering the conditions tested, when analyzed for enzymatic activity. The *Rhodococcus* sp. OTU 7 was the only one capable of producing 4 different types of enzymes (amylase, protease, cellulase and chitinase). The *Rhizobium* sp. OTU 18 was capable of producing three different types of enzymes (amylase, protease and cellulase). All the other bacterial isolates only showed positive results for two or less enzyme tests. Therefore, gene prediction may represent at some level the real metabolic plasticity of the microorganisms. However, a more comprehensive study should be performed to confirm this hypothesis. Although it is a tool that

allows a deeper look into the physiology of the isolates, the gene prediction methodology and interpretation proposed by Langille et al. (2013) requires care, and should be accompanied by an evaluation of the impact of horizontal gene transfer in microbial communities. In addition, the results are dependent on the annotations of genomic data (Jiménez et al. 2014).

Only proteases, which hydrolyze the peptide bonds of proteins to generate polypeptides or free amino acids (Alnahdi 2012), were produced by all the bacterial isolates. This is an interesting result considering the economic importance of proteases in the global industrial market. The alkaline proteases, for example, constitute approximately 60–65% of this market (Pant et al. 2015). Other hydrolytic activities (amylase, cellulase and chitinase) were observed in specific bacterial isolates under specific conditions; however, keratinase production was not detected. The use of microbial diversity for biotechnological prospecting allows the exploration of different metabolisms that can meet a wide range of industrial applications (Manivasagan et al. 2014).

The enzyme tests also revealed that increasing temperatures did not increase the enzymatic activity. In fact, the



**Fig. 5** A phylogenetic tree of the Bacteroidetes phylum, including microorganisms sampled in Antarctic soils, obtained from similar microorganisms using the RDP pipeline of SeqMatch and edited in

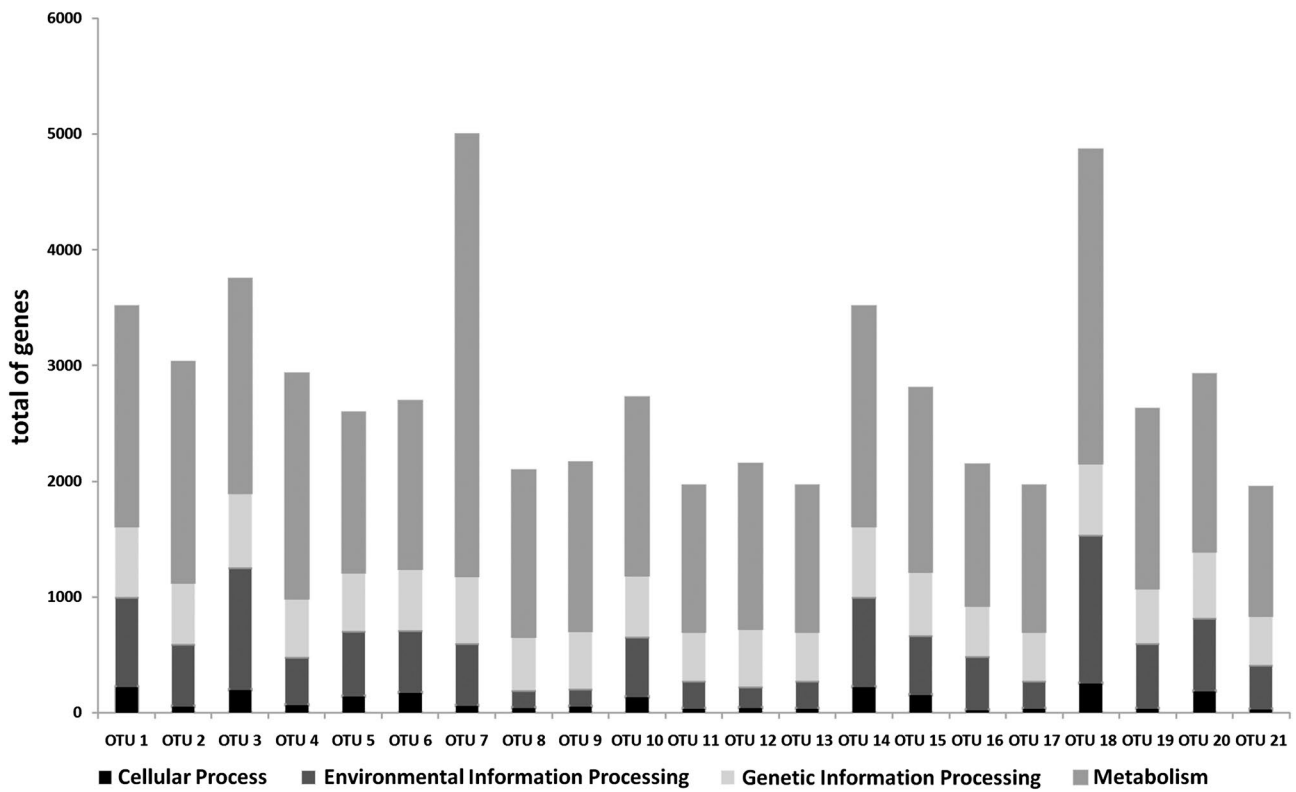
the MEGA 5.2 program using the Jukes–Cantor method of maximum likelihood and a bootstrap value of 1000

profiles of the enzymatic activities at different temperatures were similar. This result is consistent with the presence of psychrotrophic bacteria with the ability to grow at temperatures below 15 °C. Similar results were previously described (Feller and Gerday 2003; Selbmann et al. 2010), indicating that psychrotrophs can be more abundant than obligate psychrophiles in cold ecosystems. However, we cannot infer that the isolates are actually psychrotrophs based on this behavior because psychrotrophic microorganisms have the ability to grow at low temperatures, although they grow better above 20 °C. The optimum enzyme activity could be different, and more experiments are needed to confirm the psychrotrophic profiles of the isolated bacteria.

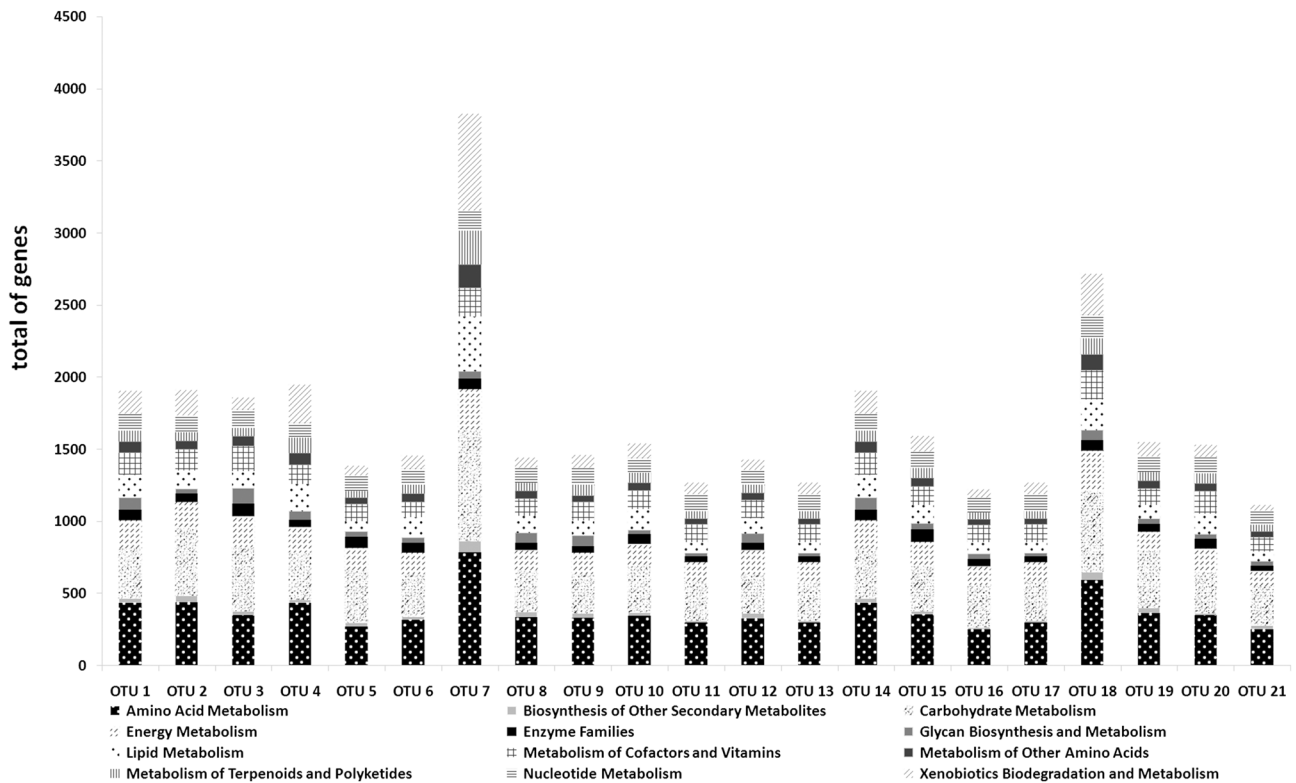
Regarding the taxonomic affiliation of the isolated bacterial samples, all the samples obtained were classified in 4 phyla (Proteobacteria, Firmicutes, Actinobacteria and Bacteroidetes). The Proteobacteria predominated in all the sampled soils, representing 71.7% of all the sequenced samples. Several studies in the vicinity of the Chinese

Great Wilson Station on King George Island (Pan et al. 2013) showed Proteobacteria and Actinobacteria as the dominant phyla in maritime Antarctic soils. The results of other studies on King George Island are in agreement with these results (Xiao et al. 2007). Teixeira et al. (2010) performed an NGS (next generation sequencing) survey, and they observed Firmicutes, Proteobacteria and Actinobacteria as the dominant phyla detected in *Colobanthus quitensis* and *Deschampsia antarctica* rhizosphere samples from maritime Antarctica.

Among all the isolates studied, the bacterial isolates identified as *Rhizobium* sp., *Sporosarcina* sp., *Rhodococcus* sp., *Arthrobacter* sp., *Pseudomonas* sp., *Psychrobacter* sp. and *Bacillus* sp., presented the most biotechnological potential, producing more than one hydrolytic enzyme. Further studies focusing on these genera have significant possibilities of generating positive results.



**Fig. 6** The total predicted functional genes for 21 OTUs isolated from Antarctic soils according to the categories of cellular processes, environmental information processing, genetic information processing and metabolism



**Fig. 7** The predicted functional genes for 21 OTUs isolated from Antarctic soils according to the metabolism class

**Table 3** Enzymatic production profiles of 21 OTUs obtained from Antarctic soil and rhizosphere samples

OTU	Sampling local	Presumed tax- onomy	Temperature (°C)														
			Amylase			Protease			Cellulase			Chitinase					
			4	12	25	4	12	25	4	12	25	4	12	25			
1	Ornithogenic soil Soil under mosses Yellow soil Rhizosphere of <i>D. antarctica</i> Rhizosphere of <i>C. quitensis</i>	<i>Pseudomonas</i> sp.	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
2	Ornithogenic soil Soil under mosses Rhizosphere of <i>D. antarctica</i> Rhizosphere of <i>C. quitensis</i>	<i>Arthrobacter</i> sp.	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
3	Ornithogenic soil Soil under mosses	Enterobacteriaceae	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
4	Yellow soil	<i>Acinetobacter</i> sp.	-	-	-	+	+	+	+	+	+	-	-	-	-	-	-
5	Ornithogenic soil Yellow soil Rhizosphere of <i>D. antarctica</i>	<i>Psychrobacter</i> sp.	-	-	-	+	+	+	+	+	+	-	-	-	-	-	-
6	Rhizosphere of <i>D. antarctica</i> Rhizosphere of <i>C. quitensis</i>	<i>Bacillus</i> sp.	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
7	Yellow soil Rhizosphere of <i>C. quitensis</i>	<i>Rhodococcus</i> sp.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8	Ornithogenic soil	<i>Algoriphagus</i> sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	Ornithogenic soil	Phylum Bacteroidetes	-	-	-	+	+	+	+	+	+	-	-	-	-	-	-



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