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Characterizing proteases in an Antarctic *Janthinobacterium* sp. isolate: Evidence of a protease horizontal gene transfer event

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Abstract We report the isolation of a cold-adapted bacterium belonging to the genus *Janthinobacterium* (named AU11), from a water sample collected in Lake Uruguay (King George Island, South Shetlands). AU11 (growth between 4°C and 30°C) produces a single cold-active extracellular protease (ExPAU11), differentially expressed at low temperature. ExPAU11 was identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-ToF MS) as an alkaline metallo-protease (70% coverage with an extracellular protease of *Janthinobacterium* sp. PI12), and by protease-inhibitor screening identified as a serine-protease. To the best of our knowledge this is the first experimental evidence of a cold-active extracellular protease produced by *Janthinobacterium*. Furthermore, we identified a serine-protease gene (named JSP8A) showing 60% identity (98% query coverage) to subtilisin peptidases belonging to the S8 family (S8A subfamily) of many cyanobacteria. A phylogenetic analysis of the JSP8A protease, along with related bacterial protein sequences, confirms that JSP8A clusters with S8A subtilisin sequences from different cyanobacteria, and is clearly separated from S8A bacterial sequences of other phyla (including its own). An analysis of the genomic organization around JSP8A suggests that this protease gene was acquired in an event that duplicated a racemase gene involved in transforming L- to D-amino acids. Our results suggest that AU11 probably acquired this subtilisin-like protease gene by horizontal gene transfer (HGT) from a cyanobacterium. We discuss the relevance of a bacterial protease-HGT in the Antarctic environment in light of this hypothesis.

Keywords **Antarctic, cold-active protease, horizontal gene transfer, *Janthinobacterium*, subtilisin**

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1 Introduction

Bacteria belonging to the genus *Janthinobacterium* are Gram-negative Betaproteobacteria (order Burkholderiales, family Oxalobacteraceae), commonly found in soil and water, and in a variety of foods where they cause food spoilage[1]. Two species (*J. agaricidamnosum* and *J. lividum*) and many strains have been described. *Janthinobacterium* strains have been

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isolated from temperate environments, but many Arctic and Antarctic cold-tolerant isolates have also been reported[2-5].

The production of extracellular hydrolytic enzymes by isolates of this genus has been reported, *e.g*. production of chitinase[6], *β*-agarase[7], and chitosanase[8]. However, the production of extracellular proteases by *Janthinobacterium* strains is quite rare[1], in spite of reports to the contrary by Dainty et al.[9], Bach et al.[10], and Tomova et al.[11]. Proteases are ubiquitous endo- and exo-peptidases that catalyze the hydrolytic breakdown of proteins into peptides or amino acids. They are classified into families based on significant similarity in amino acid sequence, with each family identified by a letter representing the catalytic type (A, aspartic; C, cysteine; G, glutamic; M, metallo; N, asparagine; S, serine; T, threonine; U, unknown), and subfamilies based on evidence of very ancient evolutionary divergence.

Proteases have many industrial applications, and have been recognized as a key step in the degradation and utilization of proteinaceous polymers by bacteria[12-14]. Major interest exists in finding novel proteolytic enzymes with new properties, such as high performance at low-temperature, because of potential industrial applications. Many investigators have isolated proteolytic bacteria from extremely cold environments and the proteases have been characterized[15-17]. For an interesting review of proteases from psychrotrophs, see Kasana[14].

We reported previously the isolation of *Pseudomonas* and *Flavobacterium* strains that produce extracellular cold-active proteases, from water samples collected at Fildes Peninsula (King George Island, South Shetlands, Antarctica)[17]. Our current work aims to analyze the occurrence of serine-proteases in the cold-tolerant *Janthinobacterium* sp. AU11 isolate. Serine proteases are among the most important enzymes with industrial applications. During this work we found evidence that AU11 most likely acquired a serine-protease gene by horizontal gene transfer (HGT) from a cyanobacterium. Given this hypothesis, we discuss the relevance of a protease-HGT event in the Antarctic environment.

2 Materials and methods

2.1 Isolation and identification of an extracellular protease-producing bacterium

An extracellular protease-producing bacterium was isolated from a water sample collected from Lake Uruguay (near the Uruguayan Antarctic Scientific Base; 62°11′4″S, 58°51′7″W; King George Island, South Shetlands), and identified by sequencing a 1 500 bp 16S rDNA fragment as described by Martinez-Rosales and Castro-Sowinski[17].

2.2 Growth rate and protease production

The ability of AU11 to grow and produce extracellular proteases at different temperatures (4°C, 18°C, and 30°C) was analyzed in Luria-Bertani (LB; 10 g·L^{-1} tryptone, 5 g·L^{-1} yeast extract, 10 g·L^{-1} NaCl) and MM (5% LB and 5% skim milk) liquid medium, as described by Martinez-Rosales and Castro-Sowinski[17]. Proteolytic activity was determined in the cell-free supernatant of MM grown cells, using azocasein as a substrate[18], when clarification of the medium (milk coagulation) was evident. Cell-free supernatant was obtained after centrifugation and filtration (0.45 μm Millipore filters) of grown culture medium. One unit of enzyme activity (U) was defined as the amount of cell-free supernatant required to increase absorbance by one unit at 340 nm under the assay conditions.

2.3 Zymography and serine protease inhibitor effects on extracellular protease activity

Protease profiles were analyzed using zymogram gels with gelatin as the copolymerized substrate and 8% acrylamide as the resolving gel (5% for the stacking gel). Cell-free supernatant samples were obtained after clarification of the MM medium at 4°C. In parallel, zymograms were run with copolymerized gels supplemented with 2 mM phenylmethilesulfonyl fluoride (PMSF; serine protease inhibitor). Methods were as described by Martinez-Rosales and Castro-Sowinski[17].

2.4 Extracellular protease identification via proteomics

Cell-free supernatant of MM grown cells was used to identify the extracellular protease by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-ToF MS). The cell-free supernatant was analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (12% acrylamide for the resolving gel and 5% for the stacking gel), and then the proteins were visualized by Coomassie staining. In-gel digestion and MALDI-ToF MS of the prominent band was done as described by Piñeyro et al.[19] in the Analytical Biochemistry and Proteomics Unit of the Institute Pasteur (Montevideo, Uruguay). The protein was identified by database searching at NCBI (National Center for Biotechnology Information; http://www.ncbi.nlm.nih.gov[20]) with peptide m/z values (mass divided by charge) using the MASCOT software (www.matrixscience.com[21]).

2.5 Amplification of protease genes

Genomic DNA was extracted using the phenol-chloroform procedure described by Sambrook et al.[22] and used as template for the Polymerase Chain Reaction (PCR). For protease gene amplification the consensus-degenerate hybrid primers designed by Acevedo et al.[23] (B2F: 5′GGC CAC GGC ACC CAY GTB GCS GG 3′ and B2R: 5′CGT GAG GGG TGG CCA TRS WDG T 3′) were used. Then, specific primers (PROTJF: 5′GGT TTG ATC CTG CCC ATC TTT GC 3′ and PROTJR: 5′CAG TTG CTG TTG GGC ATG GGA G 3′) were designed and used to amplify the protease gene fragment. The entire coding sequence (CDS) and genomic context was obtained by inverse PCR

(iPCR) using the outward primers OUT1 (5′GC AAA GAT GGG CAG GAT CAA ACC 3′) and OUT2 (5′TCC CAT GCC CAA CAG CAA CTG 3′) (both primers are the reverse complement of specific primers PROTJF and PROTJR). Purified PCR products were cloned into the pTZ57R/T vector (InsTAclone PCR Cloning Kit; Thermo Scientific), and sequenced by Macrogen Inc. (http://www.macrogen.com/; Korea) using the dideoxy chain-termination method. DNA sequences were analyzed using BLASTx and BLASTn searches at the NCBI web site (http://www.ncbi.nlm.nih.gov/[20]) and at the peptidase database MEROPS (http://merops.sanger.ac.uk/[24]). The nucleotide sequence for the amplified serine protease was named JSP8A.

2.6 Phylogenetic affiliation of the protease (JSP8A) from AU11

BLASTp was used to search for protein sequences homologous to JSP8A in the NCBI and MEROPS databases. Only sequences with a minimum identity of 28% and higher were considered. Multiple sequence alignments were created with MUSCLE software[25]. The LG+G model was inferred as the most probable model by means of the "modelgenerator" program[26]. A phylogenetic tree was inferred using the maximum likelihood method with Phyml version 3.0[25], and five random starting trees. The default Shimodaira-Hasegawa-like (SH-like) test[27] was used to evaluate branch supports.

2.7 Analysis of putative horizontal gene transfer events in *Janthinobacterium*

Putative HGT events in *Janthinobacterium* sp. strain Marseille (the only strain of the genus with a complete genome available at the moment) were investigated comparing the species tree (based on 16S rDNA; data not shown) and the homologous gene tree. Each gene in *Janthinobacterium* sp. strain Marseille was 'blasted' against all completely sequenced bacterial genomes available as of this writing. Following the coverage and identity criteria described above, up to 20 homologous genes were selected for each gene. A phylogenetic tree for each set of homologous proteins and the 16S rDNA were inferred using the maximum likelihood method as implemented in Phyml version 3.0[28]. The default SH-like test was used to evaluate branch supports. Phylogenetic reconstruction was made with an amino acid LG+G model for homologous proteins and with a nucleotide GTR+G model for the 16S rDNA. Ranger-DTLsoftware[29] was used to compare the homology-based 16S and protein sequence phylogenetic trees. Results were confirmed by visual inspection.

2.8 GenBank accession numbers

The partial 16S rDNA and protease gene sequences of AU11 were deposited in the GenBank database and the following accession numbers were assigned: JN416568 and JN416569 for the 16S rDNA and the protease JSP8A gene, respectively.

3 Results

A cold-active extracellular protease producing bacterium (AU11) was isolated based on its ability to clarify MM-agar around the colonies at 4°C, indicating caseinolytic activity. AU11 was identified by sequencing of a 16S rDNA gene fragment. The isolate had 100% 16S rDNA identity, excluding gaps, with several polar *Janthinobacterium lividum* strains (KF993615 and HQ824865, among others). The isolate was designated *Janthinobacterium* sp. AU11 awaiting conclusive identification through a polyphasic approach.

Bacterial growth at different temperatures (30°C, 18°C and 4°C) was monitored in LB liquid medium. AU11 was able to grow at all the tested temperatures, showing a doubling-time of 3, 2, and 15 h at 30°C, 18°C and 4°C, respectively (data not shown). Independent of the growth temperature, the isolate always reached a similar optical density (OD) at stationary phase (0.75 ± 0.5 OD).

Proteolytic activity was evident after reaching the stationary phase in liquid medium by coagulation of milk proteins and clarification of the milky white MM medium at 18°C and 4°C. At this point, extracellular proteolytic activity was 1.20 ± 0.02 and 1.39 ± 0.01 $U \cdot mL^{-1}$ of cell-free cultures, respectively. Although growth was detected (measured by counting colony forming units CFU), coagulation and proteolytic activity were not detected in liquid cultures of AU11 at 30°C, even after two weeks of incubation.

To analyze whether AU11 produces different extracellular proteases, zymography was performed as described above (data not shown). The analysis revealed the presence of only one band with activity (hydrolysis of gelatin). Zymograms were also created adding PMSF (an inhibitor of serine-proteases) to the polyacrylamide gels, and results showed a decrease in band intensity. The results suggested that AU11 produces a single extracellular protease, potentially inhibited by PMSF.

The extracellular protease (ExPAU11) produced in MM medium was identified by SDS-PAGE (Figure 1a) and subsequent analysis by MALDI ToF-MS (Figure 1b). ExPAU11 is a 49 kDa protein highly similar to an alkaline-protease and a Zn-dependent metallo-serralysin like-AprX protease, both from *Pseudomonas fluorescens* (Acc. AAC38255 and ABY65932, respectively).

In a second approach addressed to identify protease genes, degenerate primers designed from consensus sequences[23] were used in PCR reactions using AU11 DNA as the template. An amplification fragment of approximately 500 bp was obtained. The nucleotide sequence was analyzed using BLASTx, and revealed high identity (62%) to the subtilisin peptidase S8 family in cyanobacteria, *e.g. Planktothrix agardhii* (Acc. 4H6W_A), *Microcystis aeruginosa* (Acc. WP_002792090.1), *Nodularia spumigena* (Acc. WP_006196081.1), and *Calothrix parietina* (Acc. WP_015197715.1).

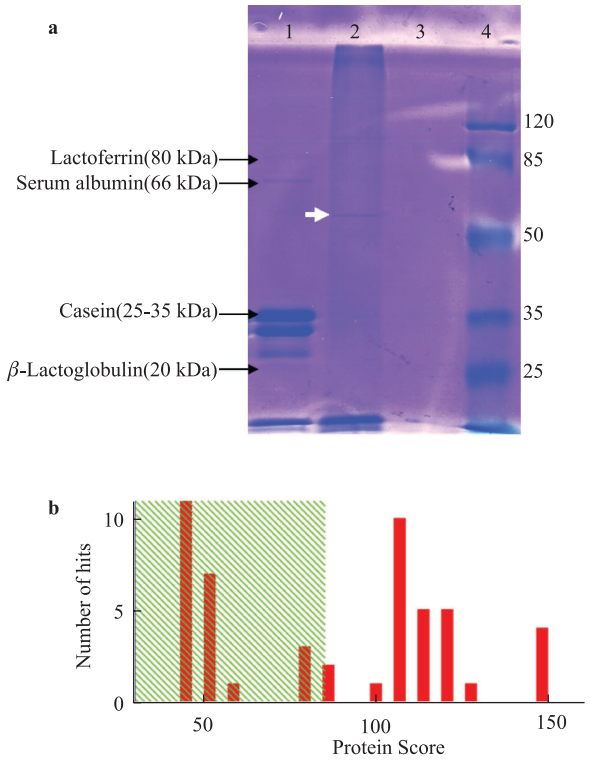

Figure 1 SDS-PAGE analysis of cell-free supernatant from *Janthinobacterium* sp. AU11 and MALDI-ToF MS analysis of the prominent protein band. **a**, SDS-PAGE. Lane 1: 5% skim milk; lane 2: cell-free supernatant of AU11 grown in MM medium at 4°C (the arrow indicates the extracellular protease identified by MALDI ToF-MS); lane 3: cell-free supernatant of AU11 grown in LB (1/20 diluted) at 4°C; lane 4: protein molecular marker (values at right are in kDa). **b**, Screenshot of MALDI-ToF MS results, showing that over 30 proteins matched ExPAU11 with scores greater than 85 (p<0.05). Al least eight peptides were identified within five proteins with scores of 150.

Outward specific primers were also designed and used to identify the complete CDS by iPCR. The CDS belongs to the subfamily 8A of serine proteases, with the subtilisin named Carlsberg being the model protease of the subfamily. The sequence was named JSP8A.

JSP8A is a 620 amino acid protein similar to the S8/S53 subtilisin kexin sedolisins cyanobacterial proteases, including *Calothrix* sp. PCC 6303 (50% identity, 80% query coverage; Acc YP_007137041), *Cylindrospermum stagnale* (48% identity, 80% query coverage; Acc YP_007146141), and *Gloeocapsa* sp. PCC 7428 (50% identity, 80% query coverage; Acc YP_007130541). BLAST analyses at MEROPS showed that JSP8A could belong to the S8/S53 subtilisin kexin sedolisin family (by PFAM search[30]), or to the cyanobactin maturation protease PatA/PatG family (by TIGRFAM search; http://www.jcvi.org/cgi-bin/tigrfams/index.cgi). The active site of the subtilisin-like clan of proteases contains a catalytic triad with the amino acid order Asp, His, Ser.

In addition to the JSP8A gene, the flanking regions were also sequenced, eventually yielding 3 600 bp that include the protease gene flanked by intergenic regions and two Asp/Glu racemases with 69%–70% identity to many burkholderial racemases (Figure 2).

Figure 2 Genetic organization around JSP8A protease gene. From left to right: intergenic region (IR1, 61 bp), Asp/Glu racemase (705 bp), intergenic region (IR2, 304 bp), protease (JSP8A gene, 1 863 bp), intergenic region (IR3, 120 bp), and Asp/Glu racemase 547 bp).

A phylogenetic tree of the AU11 JSP8A protease along with those most closely related bacterial protein sequences is shown in Figure 3. JSP8A clusters with S8A subtilisin sequences from different cyanobacteria, and is clearly separated from S8A bacterial sequences of other phyla (including its own). This strongly suggests an HGT event of the JSP8A gene from a cyanobacterium to *Janthinobacterium* sp. AU11 (Betaproteobacteria).

A closer study of the phylogeny also suggests that an earlier HGT event may have occurred from an Alphaproteobacterium to a cyanobacterium (Figure 3). Considering the lack of a strong phylogenetic signal at some nodes, as reflected by the SH-like test values, it is premature to speculate on early HGT events; however, this phenomenon may be considered a reasonable explanation for the observed patterns. Furthermore, among all available completely sequenced genomes from the order Burkholderiales, including the closely related *Janthinobacterium* sp. strain Marseille and *Herminiimonas arsenicoxydans*, no significantly similar sequences to JSP8A (with an identity > 40%) were found. This result further supports the hypothesis that *Janthinobacterium* sp. AU11 acquired JSP8A through an HGT event.

With these results on hand, we addressed the following question: how frequent are the HGT events, in particular protease-gene transfer, in *Janthinobacerium*? First, we studied the distribution of protease and non-protease homologous proteins in the genome of members of the family *Oxalobacteraceae*. The result showed that despite obvious variations in the number of proteases per genome, the relative frequency of each family and subfamily had remained remarkably stable among all analyzed microorganisms (Supplementary Figure 1S), suggesting a similar pattern of proteases among the members of *Oxalobacteraceae* (high correlation among distribution of subfamilies; $0.56 < r^2 < 0.87$). This result validated the use of *Janthinobacterium* sp. Marseille as reference instead of AU11 genome. In addition, results showed that S8A subtilisins-like proteases appeared underrepresented, suggesting that the acquisition of a S8A protease might have an important impact on protease genomic composition.

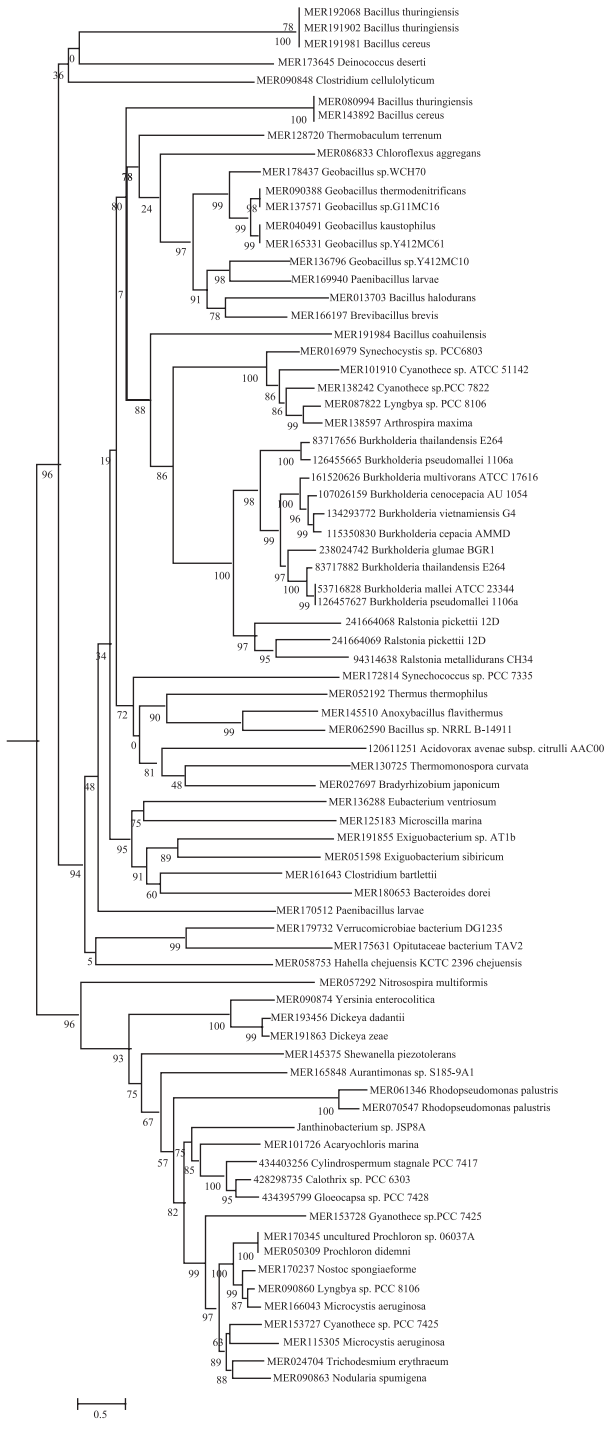

Figure 3 An inference by maximum likelihood of JSP8A's phylogeny. Reconstruction is based on the amino acid sequence of the amplified region of JSP8A gene (alignment length = 260 sites). The numbers in branches represent nodal support values. Phylogenetic analysis was performed by maximum likelihood using PhyML and the SH-like test for branch support with five random starting trees. JSP8A groups with proteases belonging to the phylum cyanobacteria and a few proteases from the phylum Proteobacteria. The tree was arbitrarily rooted using the mid-point rooting method.

A closer analysis of *Janthinobacterium* sp. Marseille genome showed 270 putative events of HGT (8% total gene content). Interestingly, only 9 out of 270 corresponded to proteases (four genes) or non-protease homologous (five genes) genes (Supplementary Table 1s), suggesting a low frequency of protease-gene transfer in this genus. Indeed, only the gamma-glutamyltranspeptidase 2 might represent a recent gene acquisition of this species.

4 Discussion

We isolated a cold-adapted, extracellular-protease producing *Janthinobacterium* strain (AU11), which probably acquired a serine protease-gene by HGT from a cyanobacterium.

Using a proteomic approach, we demonstrated that AU11 produces a single alkaline metallo-protease that is active and stable at 4°C and 30°C, when grown either at 4°C or 18°C (production was not detected at 30°C), as reported for a Zn-dependent metallo-protease in *Flavobacterium psychrophilum*[31]. Antarctic bacterial isolates that produce thermo-stable extracellular proteases when growing in a skim milk supplemented liquid medium have been previously identified[32-33].

Interestingly, during the identification of AU11 protease genes by PCR, a CDS with high identity to cyanobacterial subtilisin peptidases belonging to the S8 family (subfamily S8A) was found, suggesting an event of protease-gene transfer. The genetic organization around this protease gene also suggests that an HGT event was involved in JSP8A acquisition. The presence of direct repeats in intergenic regions IR1 and IR3, and the duplication of racemase genes also reinforce the HGT hypothesis. Racemases are enzymes involved in amino acid transport and metabolism. They transform L-amino acids to D-amino acids by racemization[34]. Bacterial D-amino acids serve important functions as building blocks of peptidoglycan (PG), teichoic acids, and poly-gama-glutamate biopolymers. PG is the major component of the bacterial cell wall, and D-amino acids play a major role in the alteration of PG structure and synthesis. D-amino acids also act as a modulator of bacterial growth and persistence, and their incorporation into PG is involved in bacterial adjustment to changes in environmental conditions[34]. Our bioinformatics analysis suggests that this is a rare event of protease-gene transfer in *Janthinobacterium* (Figure 1, Supplementary Table 1s).

Members of the S8 family, such as JSP8A, are proteases involved in nutrition. Thus, our result opens a discussion of the relevance of lateral transfer events of nutritionally involved genes, such as hydrolytic enzymes, in extreme environments. Additionally, the subtilisin peptidase was transferred adjacent to a racemase, in an event that probably duplicated the racemase gene. This proximity, protease-racemase, might contribute to bacterial fitness in a cold and oligotrophic environment like the Antarctic lakes. The up-regulation of Asp/Glu racemase genes as part of the cold-adaptation repertoire has been previously reported[35]. In a possible scenario, L-amino acids hydrolyzed by JSP8A

protease (and its homologues), are then available to be racemized to D-amino acids, which can then be incorporated into PG, perhaps an overall cold-adaptation of bacterial membranes, that *Janthinobacterium* AU11 was able to acquire and retain.

HGT events are common in the lifestyle of prokaryotes, and an important force driving genomic evolution in the microbial world[33]. Environmental conditions on the Antarctic continent are severe, and its habitats are regarded as ecosystems of low productivity because of their oligotrophy and the low temperatures to which they are exposed. Although the environment is inhospitable, it contains a highly diverse microbial population[36]. Cyanobacterial mats are a major feature of water bodies, and are a well-established endemic microbiota of Antarctica[37]. Therefore, the exchange of genetic material from cyanobacteria to other bacteria is quite feasible. Slimy substances secreted by cyanobacterial mats hold them together and create different internal chemical environments. In this situation, similar to a microbial biofilm, HGT is favored and gene-exchange between microorganisms constitutes a major factor in their genetic adaptation and evolution[38].

Gene exchange among organisms in extremely cold environments has been reported by some authors[33, 39]. In this work we present evidence that suggests a protease-gene transfer may have occurred from a cyanobacterium to *Janthinobacterium* AU11 in an extreme environment. In addition to virulence factors such as toxins, transport systems, adhesins, and antibiotic-resistance factors, the dissemination of protease sequences (involved in pathogenesis) has been reported[40-41]. However, it is very likely that the acquisition of the protease gene, shown during this work, plays a role in fitness and low temperature adaptation. During the Antarctic winter, the level of energy supplies decreases dramatically, but during the summer season, when biological C- and N-fixation rates are maximal, carbon and nitrogen supplies increase. In this situation the acquisition of an important metabolic trait, such as the hydrolysis of proteins to amino acids and oligopeptides, contributes to competitiveness in a unique environment like the Antarctic. The emergence of the new phenotypic property furnishes several nutritional advantages in this low-productivity ecosystem.

Future work will deal with AU11 genome sequencing, and the analysis of the occurrence of horizontal gene transfer events in this bacterium, among others.

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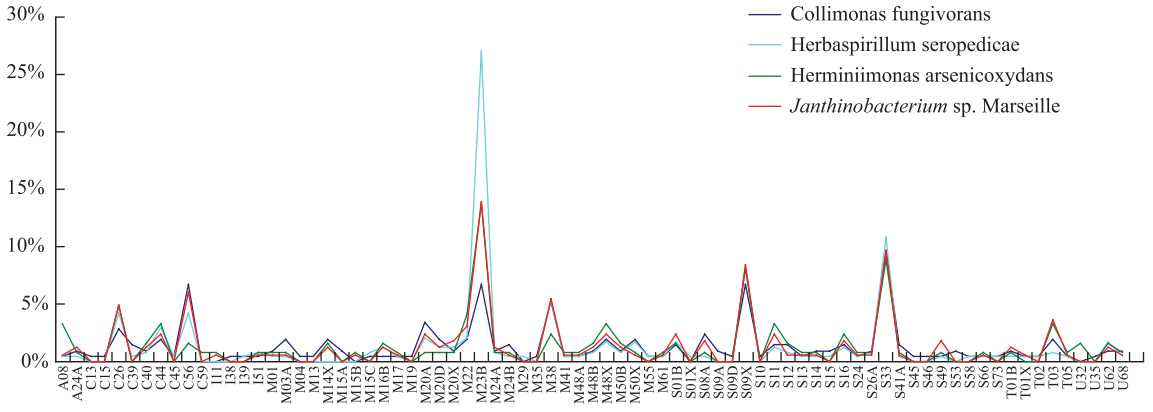

Supplementary Figure 1S Relative frequency (%) of protease subfamilies among members of the family *Oxalobacteraceae*. The distribution of classes is highly correlated ($0.56 < r^2 < 0.87$).

Supplementary Table 1S Putative protease and non-protease gene transfer events in *Janthinobacterium* sp. Marseille

***Janthinobacterium* sp. Marseille (ptt file)**								
No.	Location	Strand	Length	PID	Gene	Synonym	COG	Product
1	89265..90971	-	568	152981433	*tar3*	mma_0080	COG0840NT	methyl-accepting chemotaxis protein
2	647835..648734	-	299	152981665	*penP*	mma_0570	COG2367V	Beta-lactamase class A
3	683569..685320	+	583	152981219	*ggt2*	mma_0601	COG0405E	gamma-glutamyltranspeptidase
4	1032907..1034628	+	573	152983038	*tar8*	mma_0910	COG0840NT	methyl-accepting chemotaxis protein
5	2046122..2047822	+	566	152980166	*ureC*	mma_1814	COG0804E	urease subunit alpha
6	3174543..3176246	-	567	152981448	*tar13*	mma_2826	COG0840NT	methyl-accepting chemotaxis protein
7	3449378..3450346	+	322	152981075	-	mma_3081	COG4977K	AraC family transcriptional regulator
8	3451436..3452257	-	273	152982503	-	mma_3083	COG0657I	hypothetical protein
9	3692385..3692903	-	172	152983320	*cpaA*	mma_3319	COG4960OU	A24A family peptidase

Merops database best hit

Merops (code)	**Definition**	**Type**
MER193396	subfamily M23B non-peptidase homologues (*Dickeya dadantii*) [M23.UNB]	M23B
MER095167	family S11 unassigned peptidases (*Janthinobacterium* sp. Marseille) [S11.UPW]	S11
MER095170	gamma-glutamyltransferase 2 {bacterial} (*Janthinobacterium* sp. Marseille) [T03.014]	T03
MER187249	subfamily M23B non-peptidase homologues (*Comamonas testosteroni*) [M23.UNB]	M23B
MER095230	family M38 unassigned peptidases (*Janthinobacterium* sp. Marseille) [M38.UPW]	M38
MER131087	subfamily M23B non-peptidase homologues (*Burkholderia cenocepacia*) [M23.UNB]	M23B
MER095280	family C56 non-peptidase homologues (*Janthinobacterium* sp. Marseille) [C56.UNW]	C56
MER061071	family S9 non-peptidase homologues (*Methylobacterium* sp. 4-46) [S09.UNW]	S09X
MER095295	subfamily A24A unassigned peptidases (*Janthinobacterium* sp. Marseille) [A24.UPA]	A24A

Putative organisms involved in the lateral gene transfer event

From	**To**
Oxalobacteraceae family	*Thiobacillus denitrificans*
Oxalobacteraceae family	Enterobacteriaceae family
Gammaproteobacteria or Epsilon/delta subdivision	*Janthinobacterium* sp. Marseille
Oxalobacteraceae family	*Thiobacillus denitrificans*
Oxalobacteraceae family	Nitrosomonadaceae family
Burkholderiales Order	Methylophilaceae family
Ralstonia	*Pseudomona*
Rhizobiales Order	Burkholderiales Order
Oxalobacteraceae family[a]	*Stenotrophomonas maltophilia*[a]

Note: [a] The direction of this transfer event could not be clearly determined.