POLISH POLAR RESEARCH
vol. 37, no. 3, pp. 403–419, 2016 doi: 10.1515/popore-2016-0021

Isolation and characterization of an Antarctic *Flavobacterium* strain with agarase and alginate lyase activities

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Abstract: Several bacteria that are associated with macroalgae can use phycocolloids as a carbon source. Strain INACH002, isolated from decomposing *Porphyra* (Rhodophyta), in King George Island, Antarctica, was screened and characterized for the ability to produce agarase and alginate-lyase enzymatic activities. Our strain INACH002 was identified as a member of the genus *Flavobacterium*, closely related to *Flavobacterium faecale*, using 16S rRNA gene analysis. The INACH002 strain was characterized as psychrotrophic due to its optimal temperature (17 ºC) and maximum temperature (20°C) of growth. Agarase and alginate-lyase displayed enzymatic activities within a range of 10°C to 50°C, with differences in the optimal temperature to hydrolyze agar (50°C), agarose (50°C) and alginate (30°C) during the first 30 min of activity. Strain *Flavobacterium* INACH002 is a promising Antarctic biotechnological resource; however, further research is required to illustrate the structural and functional bases of the enzymatic performance observed during the degradation of different substrates at different temperatures.

Key words: Antarctic, King George Island, *Flavobacterium*, agarase, alginate-lyase.

Introduction

Antarctica is the most pristine and extreme continent with a diverse and adapted biodiversity (Lewis 1984). The geographic isolation of the Antarctic continent and the "extreme" physical conditions (mainly low temperatures) are selective pressures responsible for microorganisms (mainly bacteria and fungi) with unique metabolic pathways (Nichols *et al*. 2002). In Antarctic ecosystems, marine microorganisms are not only important because they play key roles in every marine ecological process (Martin *et al*. 2014), but also because they are a source of new and rare compounds and enzymes with putative biotechnological applications (Zhang and Kim 2010; Luna 2015).

Antarctic environments can sustain a large diversity of well-adapted microorganisms known as psychrophiles or psychrotrophs (Loperena *et al*. 2012). Compounds and enzymes produced by these microorganisms have the capability and advantage for technological use due to higher enzymatic activities at lower temperatures than their mesophilic and thermophilic counterparts (Loperena *et al*. 2012; Muffler *et al*. 2015).

Most of these compounds and enzymes are appealing to bioindustry because they are more stable and active than the corresponding compounds or enzymes found in plants or animals (Stach 2010; Sana 2015).

Proteases, amidases, lipases and polysaccharidases are among these enzymes used for biotechnological applications (Zhang and Kim 2010). More recently, protein engineering efforts have been taken to increase performance by optimizing their catalytic activity (Sarkar *et al*. 2010). For example, in marine ecosystems, hydrolytic microbial enzymes are vital due to their ability to catalyze the hydrolysis of structural sugars in seaweeds as phycocolloids (e.g. agar and alginates), producing biologically active oligosaccharides with putative applications in medicine, agriculture and bioethanol production (Delattre *et al*. 2011; Kim *et al*. 2012).

Bacterial communities have critical roles in marine nutrient cycles with specialized species containing a wide range of polysaccharide hydrolases that break down complex molecular structures. The specific set of polysaccharide hydrolases in individual microorganisms varies among species. In rare cases, these specialized species have the ability to degrade different complex polysaccharides of algal, vascular plant, fungal and animal origin (Hutcheson *et al*. 2011).

Some enzymes involved in the degradation of phycocolloids (such as: alginates, agar-agar and agarose) have been characterized in various mesophilic genera of bacteria, such as *Alteromonas*, *Bacillus*, *Flavobacterium*, *Pseudomonas*, *Pseudoalteromonas*, *Streptomyces*, *Janthinobacterium* and *Vibrio*, with optimal activity ranging from 30ºC to 55°C (Fu and Kim 2010). However, few studies have been conducted on cold-adapted microorganisms in extreme environments.

The genus *Flavobacterium* is found in freshwater, sea and soils of warm, temperate and polar environments (Bernardet and Bowman 2011). Some species in the genus *Flavobacterium* have previously been reported with the ability to hydrolyze agar or alginate (McCammon and Bowman 2000; Huan *et al*. 2013), and recently an Antarctic *Flavobacterium* was reported as an agarase-producing strain (Kim *et al*. 2014).

It is necessary to find new alternatives for biocatalysis, given that industrial processes that use enzyme-mediated reactions are usually energy-expensive. Thus, the search and characterization of new sources of hydrolytic microbial enzymes with high performance at low temperatures are a promising alternative for lowering costs in different bioprocesses and improve the overall efficiency (Georlette *et al*. 2004).

In this study, we describe the isolation and identification of an Antarctic *Flavobacterium* strain, using a polyphasic taxonomic approach, and evaluate the ability of the cell-free supernatant of the culture to hydrolyze phycocolloids at different temperatures under controlled conditions.

Material and methods

Samples and study site. — Fronds from decomposing *Porphyra* (Rhodophyta) were collected from an intertidal zone in Fildes Bay, King George Island (Fig. 1) (62°12'14.7' S; 58°57'26.2'' W), South Shetlands, the Antarctic.

Bacterial isolation and culture condition. — Isolation was carried out by cutting small fractions (30 mm x 30 mm) of algae, which were spread on Marine Agar (MA) plates and incubated at 15°C in dark for 2 weeks. Strains with potential agarolytic activity were selected according to the intensity of agarolytic activity displayed on the MA plates. Target colonies of bacteria were isolated by picking those that formed depressions around themselves. These were then inoculated on new MA plates to obtain pure colonies and then transferred to pure cultures. The strains were cultured in marine broth under aerobic conditions at 10 or 20°C with shaking at 150 rpm. These strains were also maintained as a 20% glycerol suspension at -80°C. Twenty-four hour cultures at 10°C were used as an inoculum for most of the experiments. All the following assays and evaluations were conducted with the bacterial strain with the highest agarolytic activity named INACH002.

Morphological and biochemical characterization. — Strain INACH002 was characterized microscopically using Gram staining according to the protocol described by Freeman (1985). Biochemical assays were conducted using the commercially available API 20NE and API ZYM kits (Biomerieux), according to the manufacturer's instructions. Salt tolerance in the INACH002 strain was

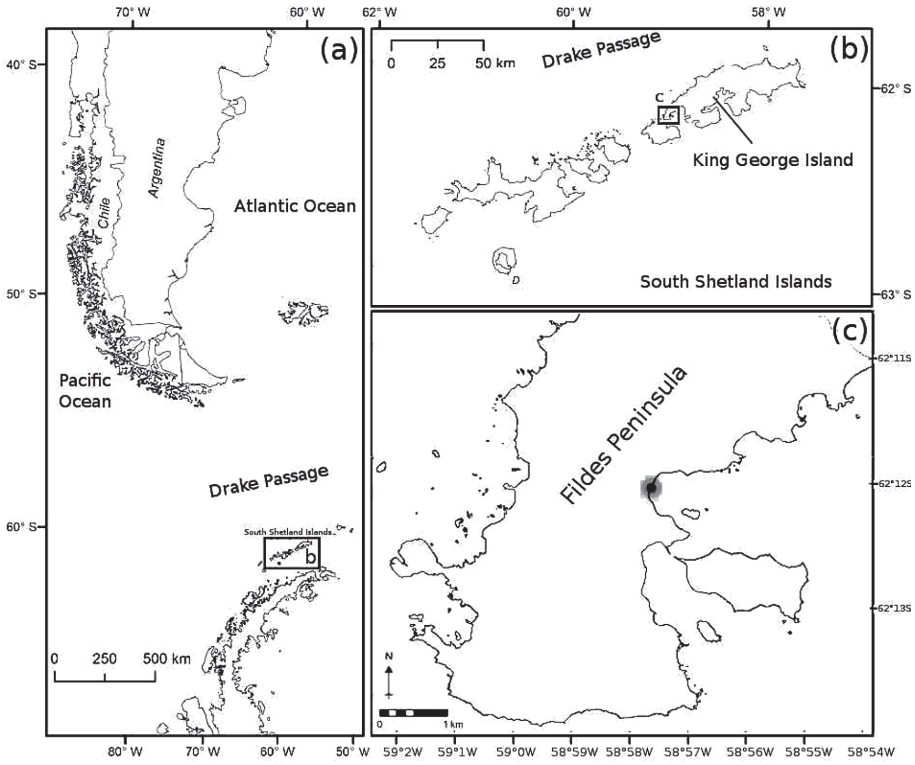

Fig. 1. Sampling site (62º12'14.7'' S – 58º57'26.2'' W) with the location of the Antarctic Peninsula (**a**), South Shetland Islands (**b**) and King George Island (**c**).

assayed under different levels of salt using different concentrations of NaCl [3, 4, 5, 6, and 7% (w/s)].

Growth rate estimation. — The growth rates were calculated at different temperatures using the formula $\mu = [\ln(N_t) - \ln(N_0)]/\ln 2\ (t - t_0)$ at 5, 15, 20, 25, 30 and 36°C; where N corresponded to the cell density at time t (expressed in days). The Ratkowsky square-root model $\sqrt{\mu} = b(T - T_{min})[1 - e^{c(T-T_{max})}]$ was used to estimate the potential optimal growth temperature and maximum temperature of growth (Ratkowsky *et al*. 1983).

DNA extraction, PCR amplification and gene sequencing. — INACH002 was grown at 10°C for seven days with agitation in 50 ml of Marine Agar medium. After incubation, biomass was harvested by centrifugation at 4,000 rpm for 20 min. DNA was extracted from a liquid culture according to the basic protocol described by Wilson (1987). The 16S rRNA gene was amplified using the universal bacterial primers 27F (5' AGAGTTTGATCMTGGCTCAG 3') and

1492R (5' TACGGYTACCTTGTTACGACTT 3') (Jiang *et al*. 2006). The PCR reaction mixture consisted of 50 ng of bacterial genomic DNA, 5 µl of 10X PCR buffer (with 15 mM MgCl2), 1 µl of 10 mM dNTPs, 1 µl of forward primer (10 µM), 1 µl of reverse primer (10 µM), and 0.8 units of Taq polymerase (Promega, USA) and ultrapure water was added to a final volume of 50 µl. The PCR reaction included an initial denaturation step: 1 cycle of 5 min at 95°C, followed by 28 cycles of 95°C for 1 min, 60°C for 30 sec and 72°C for 2 min, with a final extension step at 72°C for 10 min. The amplified PCR product (~1.5 kb) was separated with a 1% (w/v) agarose gel, stained with ethidium bromide, and visualized under ultraviolet light. The PCR product was purified with PCR Cleanup kit (Axygen) and outsourced for sequencing using the Sequencer AB-13730 (Macrogen Inc., Seoul, Korea). The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of the strain is KP715246.

Phylogenetic analysis. — The 16S rRNA sequence was analyzed using BLASTn (Altschul *et al*. 1990) and phylogenetic analysis was performed using the software Bosque (Ramírez-Flandes and Ulloa 2008). The MUSCLE 3.6 algorithm (Edgar 2004) in the Bosque package was used to align the 16S rRNA gene sequences obtained from Genbank (http://www.ncbi.nlm.nih.gov/genbank/). Aligned DNA sequences were trimmed to eliminate poorly aligned regions. The analysis involved 33 nucleotide sequences with a total of 1433 positions in the final dataset. For construction of the phylogenetic tree, a substitution model was generated using MEGA 5 (Tamura *et al*. 2011). The HKY85 model (Hasegawa *et al*. 1985) was used for the maximum likelihood method through PhyML (Guindon and Gascuel 2003), and the neighbor-joining method using the NJ Phylip (Felsenstein 2005) program. Statistical evaluation of tree topologies was performed with 1000 bootstrap repetitions.

Extracellular extract and hydrolysis assay. — INACH002 was incubated for two weeks at 10°C in minimal medium Marine Agar (MA), containing 0.15% agar. Cells were separated from the liquid medium by centrifugation at 8,500 rpm for 20 minutes at 4°C. After that, the supernatant was filtered through a 0.22 µm filter, in order to remove all liquid medium, and then lyophilized in a lyophilizer Vis Tris 2K (SP Industries, USA). The freeze-dried extract was resuspended in phosphate buffered saline (PBS) to obtain a 40X concentrated stock solution with respect to the initial culture volume.

The dinitrosalicylic acid method described by Miller (1959) was used to measure the amount of reducing sugars released as a product of hydrolysis of agarose and alginate. Solutions of agarose or alginate (1%, w/v) were used as substrate in hydrolysis reactions. The reactions were performed using three independent replicates by adding 50 µl of concentrated extract (40X) to 500 µl of substrate. Incubation was undertaken in a water bath at 10, 30 and 50°C,

for 30, 120 and 240 min. For quantification of reducing sugar a standard curve was performed with galactose.

Statistical analysis. — The comparison of the lyase activity was carried out using analysis of variance (ANOVA) to test significant differences among reducing sugar production at different temperatures and substrate. We used a Tukey multiple comparison test as an *a posteriori* test. Both tests were performed using the statistical software GraphPad Prism version 4.0 for Windows (GraphPad Software, San Diego California USA, www.graphpad.com).

Results

Morphological and biochemical characterization. — The INACH002 strain displayed a yellow color surrounded by a depression on MA plates. The bacteria were rod-shaped, non-spore-forming, gram-negative, and with non-motile characteristics. The strain grew in MA in 3–4% (w/v) NaCl but not at 5%. Starch, agar and alginate were degraded, but DNA, casein, gelatin, CM-cellulose and tyrosine were not. The API 20NE kit assay was positive for reduction of nitrates to nitrogen, hydrolysis of esculin, Beta-galactosidase activity, assimilation of D-glucose, D-mannose, D-mannitol and maltose. Cytochrome oxidase was positive, but negative for reduction of nitrates to nitrites, indole production, D-glucose fermentation, arginine dihydrolase and urease activities, hydrolysis of gelatin, assimilation of arabinose, n-acetyl-glucosamine, potassium gluconate, capric acid, adipic acid, malate, trisodium citrate, and phenylacetic acid.

Growth rate estimation. — INACH002 was characterized as psychrotrophic based on the optimum growth temperature (17ºC) predicted by the Ratkowsky equation: $\sqrt{\mu} = 0.2022(T + 5.529)\ [1 - e^{0.2358(T-25.07)}]$; $R^2 = 0.9839$. The growth rate value estimated at the optimal temperature was (μ) = 3.87. The estimation of the maximum growth temperature was 25.06°C with a growth rate of (μ) = 0.015. Slow growth was observed at 5 and 25°C with an estimated growth rate of (μ) = 2.11 and 0.10, respectively. No growth was recorded at 30°C and 36°C (Fig. 2).

Phylogenetic Analysis. — BLAST results of the almost complete 16S rRNA gene sequence (1419 nt) from INACH002 had 99% identity with *Flavobacterium faecalis* (KF214259). The sequence was aligned with representative members of the genus *Flavobacterium* used in the species description of *Flavobacterium faecalis* from Kim *et al*. (2014). The similarity analysis using the alignment of 1433 sites revealed that INACH002 was most similar to *Flavobacterium faecale* (99.8%), followed by *Flavobacterium algicola* (97.6%) and *Flavobacterium* S20

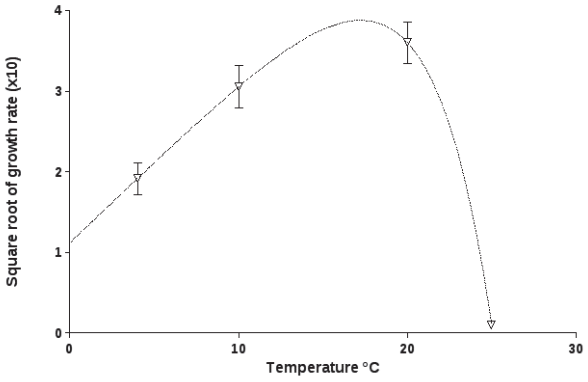

Fig. 2. Growth curve rate of INACH002 versus incubation temperature. Triangles indicate the square root of the specific growth rate (±SD) calculated from growth curves at each incubation temperature. The line indicates the fitted of square root of specific growth rate *vs.* temperature function, $\sqrt{\mu} = 0.2022(T + 5.529)\ [1 - e^{0.2358(T-25.07)}]$.

(95.6%). Phylogenetic analyses with 32 sequences of the genus *Flavobacterium*, using maximum likelihood and neighbor joining methods, resulted in identical topologies. Analyses showed that strain INACH002 forms a monophyletic group with *Flavobacterium faecale*, *Flavobacterium algicola* and *Flavobacterium* S20. A well-supported subclade with *Flavobacterium algicola, Flavobacterium faecale* and INACH002 is shown in Fig. 3.

Hydrolytic activity assay. — Crude extract of INACH002 had different hydrolytic activity at different temperatures, times and substrates. As Fig. 4 shows, there were different values of reducing sugar with the use of different substrates. During the first 30 minutes of the reaction, the highest value of reducing sugars was produced at 50ºC using agarose (0.613± 0.005 mg/ml) and agar (0.553± 0.011 mg/ml) as substrate. Significant differences between values of reducing sugars were recorded with agarose at 10, 30 and 50°C ($p < 0.0001$, $F = 17141$, $n = 3$). Similar results were achieved when agar was used as the substrate ($p < 0.0001$, $F = 244.7$, $n = 3$). When alginate was used as a substrate, the mean values at 10, 30 and 50°C were 0.170 (± 0.001), 0.218 (± 0.031) and 0.207 (± 0.009) mg/ml, respectively. In the case of alginate, no differences were recorded at different temperatures ($p = 0.0502$, $F = 5.132$, $n = 3$) after 30 minutes.

No extract reached the saturation point of reducing sugar production after incubation for 4 hours at 10°C. The value of reducing sugar produced at this temperature did not exceed 0.375 ± 0.004, 0.199 ± 0.004 and 0.322 ± 0.076 mg/ml to agar, agarose and alginate, respectively.

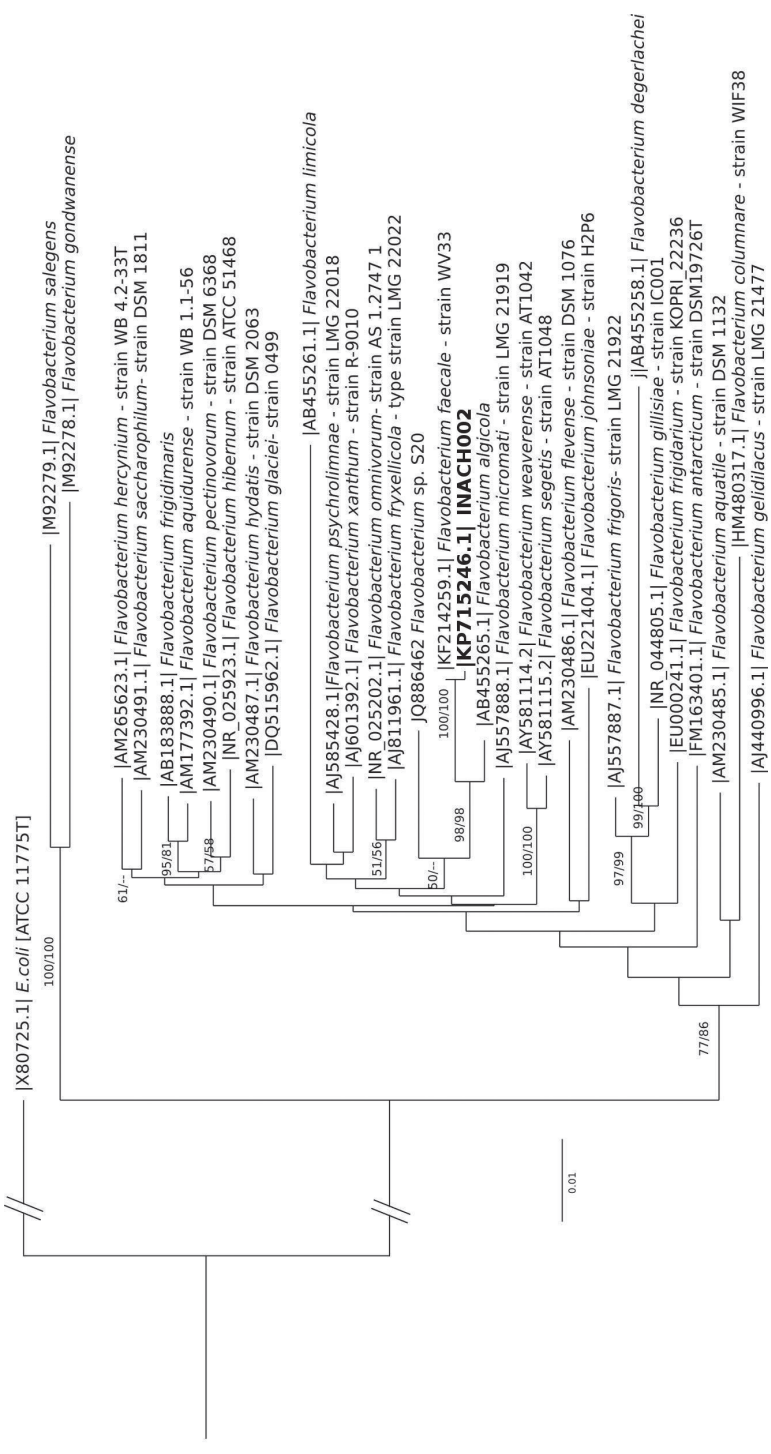

Fig. 3. Phylogenetic tree of 16S rRNA gene sequences inferred by maximum likelihood and distance methods (using an alignment of 1433 sites). Numbers above branches indicate bootstrap values of ML and NJ, respectively. Sequences used are presented with GenBank accession numbers followed by the name of the strain. The sequence reported in this paper is in bold.

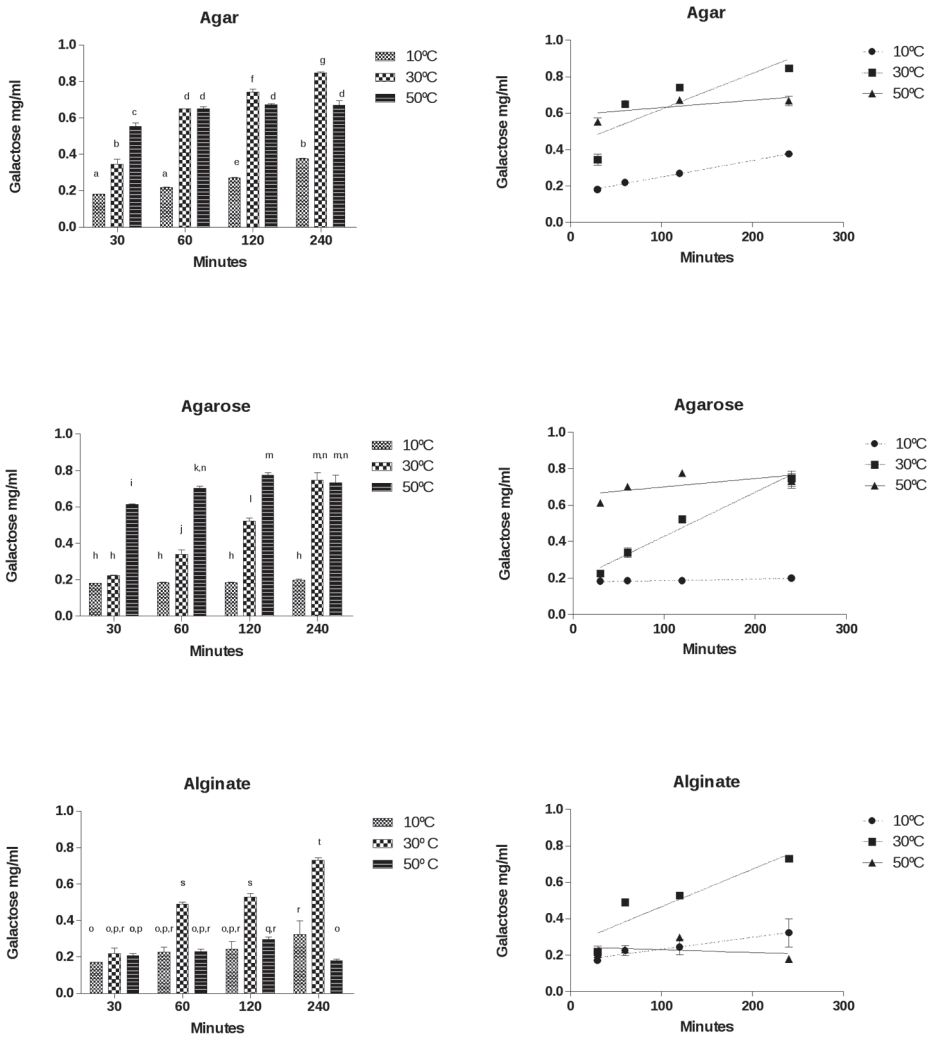

Fig. 4. Reducing sugar release (measured as galactose production) after hydrolysis reactions at three different reaction temperatures (10, 30 and 50ºC), using three different substrates: agar, agarose and alginate (left side) and effect of temperatures on galactose production over time. The data are means ± SD (n = 3). Statistical analyses were done using 1-way ANOVA with Tukey's multiple comparison test.

The slope of the reaction after the first 30 minutes was higher at 30°C. Additionally, extracts subjected to 30°C had higher values of reducing sugars after 4 hours. The maximum value was recorded in agar (0.847 ± 0.005 mg/ml), followed by agarose (0.747 ± 0.041 mg/ml) and alginate (0.730 ± 0.014 mg/ml). When agarose was used as a substrate, the highest value was first achieved after two hours at 50°C (0.776 ± 0.013 mg/ml), but no significant differences of sugar reduction values were found between 30 and 50°C after 4 hours.

Discussion

Enzymes from marine microorganisms, especially those derived from microorganisms adapted to extreme environments, have become increasingly important for potential biotechnological applications (Georlette *et al*. 2004). For example, the ability to degrade complex sugars by specific enzymes has been reported as a characteristic of some algae-associated epiphytic bacteria (Hosoda *et al*. 2003; Fu and Kim 2010). In literature, several examples can be found, like the marine bacterium *Saccharophagus degradans*, which was isolated from decomposing *Spartina alterniflora*, found in estuaries (Ekborg *et al*. 2005). This microorganism has the ability to completely decompose plant material due to a multi-enzyme system, which allows at least ten different polysaccharides, including agar, to degrade the plant to its elemental or sugar derivatives (Shin *et al*. 2009). Most of the isolated and characterized agarolytic enzymes have a maximum activity at temperatures above 30°C (Hosoda *et al*. 2003; Fu and Kim, 2010). *Pseudoalteromonas antarctica* with agarolytic activity was described by Vera *et al*. (1998) from decomposing algae found on the coast of Valdivia (Chile). These authors found that the bacterial colonies had a very active agarolytic ability to degrade agar 48h after inoculation in the culture. This *P. antarctica* was more active than *Paenibacillus* spp. described by Hosoda *et al*. (2003), which begins to degrade agar after 10 days of cultivation. In this case, both bacteria have a maximum agarolytic activity at temperatures above 20°C. The strain INACH002 described in our study and reported as belonging to the genus *Flavobacterium* (Fig. 3), was able to hydrolyze agar in a range of temperatures from 10 to 50ºC, agarose between 30ºC and 50°C, and alginate between 10ºC and 30ºC (Fig. 4). The enzymatic activity is strongly dependent on temperature. The specific activity of the psychrophilic enzymes is very high at low temperatures, but never higher than that of the mesophilic enzyme at 37°C (Feller 2013). The weak stability of the psychrophilic enzymes and their inactivation at moderate temperatures could explain the slopes of the activities recorded to assays at 50°C. However, the high hydrolytic capability and reducing sugar release during the first 30 min at 50ºC from agar and agarose suggest more thermostable enzymes than the alginate lyase. Although the presence of thermostable enzymes in psychrotophic bacteria results are perplex, other enzymes, such as aldehyde dehydrogenase and aspartase have previously been described in the psychrophilic *Cytophaga* isolated from the Antarctic (Kazuoka *et al*. 2003; Oikawa *et al*. 2003).

In agreement with previous reports for *Flavobacterium*, the morphological characteristics of INACH002, typically rod-shaped cells and yellow-pigmented colonies were similar to *Flavobacterium* strains isolated from a variety of Antarctic habitats (McCammon and Bowman 2000). The molecular analysis of the 16S rRNA gene sequence confirmed that strain INACH002 belongs to the genus *Flavobacterium* and had the highest identity (99.8%) with *F. faecale,* although

differing in physiological characteristics related to the use of carbon source and alginate lyase activity.

The phylogenetic analysis using related sequences of the genus *Flavobacterium*, clustered strain INACH002 with *F. faecale* and *F. algicola* in a well-supported subclade, which was distinguishable from other phylogenetically-related sequences. Strain INACH002 was isolated from *Porphyra* (Rhodophyta) at the Escudero Station. This Station is located in Fildes Bay, close to the sampling site of *F. faecale* at *King Sejong* Station on King George Island, the Antarctic (Kim *et al*. 2014). Nevertheless, it is well known that phylogeny derived solely based on the 16S rRNA gene is not adequate in separating closely related species in *Flavobacterium* as gyrB (Peeters and Willems 2011), and, as far as we know, information about gyrB in *F. faecale* is still not available in GenBank. Despite this inconvenience, results of API ZYM analyses showed some physiological differences that can be found between these two closely related strains suggesting possible differences at the species level (Table 1). Characteristics that distinguish strain INACH002 from *F. faecale* were: the ability to assimilate D-glucose, maltose, degrade alginate, reduce nitrate to nitrogen, hydrolyze esculin, assimilate glucose, mannitol and maltose and presence of the activity of valine aminopeptidase, acid phosphatase, esterase lipase (C8) but not lipase (C14) and β-galactosidase.

The agar lyase or alginate lyase activity can be found in several species of the genus *Flavobacterium* (Thomas *et al*. 2012; Huang *et al*. 2013; Kim *et al*. 2014). In the subcluster composed by strain INACH002, *F. faecale*, *F. algicola* and *Flavobacterium* sp. S20, the agar lyase activity is present only in strain INACH002 and *F. faecale*, while the alginate lyase is present in all taxa except for *F. faecale*. It is well known that the phylum Bacteroidetes can obtain the capability to degrade algal carbohydrates by lateral gene transfer (LGT) from other marine bacteria (Thomas *et al*. 2011; Touchon *et al*. 2011; Hehemann *et al*. 2012; Thomas *et al*. 2012). Genomes from this phylum appear to be highly plastic with frequent genetic rearrangements that suggest their adaptation to distinct and specific environmental conditions (Thomas *et al*. 2011). Thus, similar to the acquisition of genes coding for porphyranases and agarases by some human gut bacteria as reported by Hehemann *et al*. (2010, 2012), this could also happen in flavobacteria associated with algal surfaces. Nevertheless, the presence of agar or alginate lyase activity *per se* cannot be used as a phylogenetic character in this group. The presence of this enzyme in some clades could exemplify the bacterial plasticity to evolve and use new carbohydrate sources.

In most studies, the agarase activity is observed when microorganisms are grown in a medium with low nutrient concentration and using agar as a unique carbon source, suggesting that enzyme synthesis is strictly dependent with the suitable inducer. The same phenomenon is also observed in strain INACH002, suggesting that agarolytic enzymes could be under control of a common regulatory

Table 1

Different characteristics of INACH002 with closely related members of the genus *Flavobacterium*. Strains: 1, INACH002; 2, *Flavobacterium faecale*; 3, *F. algicola* NBRC 102673 T. Data are from this study. +, Positive; W weakly positive; -, negative; ND, data either not determined or unreliable.

Characteristic	INACH002	*F. faecale*	*F. algicola*
Assimilation of (API 20NE):			
D -Glucose	+	-	+
Maltose	+	-	+
D -Mannose	w	-	+
Degradation of:			
Starch	+	+	+
Gelatin	-	-	-
Alginate	+	-	+
Agar	+	+	-
DNA	-	+	-
Casein	-	-	-
Tyrosine	-	-	-
API 20 NE			
Reaction/enzymes			
Reduction of nitrates to nitrites	-	-	-
Reduction of nitrates to nitrogen	+	-	-
Indole production (TRyptoPhane)	-	nd	nd
Fermentation (glucose)	-	-	nd
Arginine dihydrolase	-	-	+
Urease	-	-	nd
Hydrolysis (B-glucosidase : Esculin)	+	-	+
Hydrolysis (Protease : Gelatin)	-	-	-
β-galactosidase (Para-NitroPhenyl-ßD-Galactopyranosidase)	+	+	-
Assimilation Glucose	+	-	+
Assimilation Arabinose	-	-	-
Assimilation Mannose	+/-	-	+
Assimilation Mannitol	+	-	+

Table 1 – continued

Characteristic	INACH002	*F. faecale*	*F. algicola*
Assimilation N-Acetyl-Glucosamine	-	-	+
Assimilation Maltose	+	-	+
Assimilation Potassium Gluconate	-	-	-
Assimilation Capric acid	-	-	-
Assimilation Adipic acid	-	-	-
Assimilation Malate	-	-	-
Assimilation Trisodium citrate	-	-	-
Assimilation Phenylacetic acid	-	-	-
Cytochrome oxidase	+	+	+
API ZYM			
Alkaline Phosphatase	+	+	+
Esterase (C4)	-	+	-
Esterase lipase (C8)	+	-	-
Lipase (C14)	-	+	-
Leucine aminopeptidase	+	+	+
Valine aminopeptidase	+	-	-
Cystine aminopeptidase	-	-	-
Trypsin	-	-	-
alpha-quimotrypsin	-	-	-
Acid phosphatase	+	-	+
naftol-AS-BI-phosphohydrolase	+	+	+
α-galactosidase	-	-	-
β-galactosidase	-	+	-
β-glucuronidase	-	-	-
α-glucosidase	-	-	-
β-glucosidase	-	-	-
N-Acetyl-glucosamine	-	-	+
alpha-Mannosidase	-	-	-
alpha-fucosidase	-	-	-

agent (Shin *et al*. 2010; Yun *et al*. 2011). A similar phenomenon occurs with alginate lyase expression, which can be induced when a strain is cultured in the presence of alginate, as in *Alginovibrio aquatilis* (Stevens and Levin 1977), and some other marine bacteria associated with *Sargassum* (Romeo and Preston 1986; Brown and Presto 1991). However, in some cases, such as alginate lyase production in *Pseudomonas alginovora*, this is constitutive (Boyen *et al*. 1990). The pH optimum for most of the enzymes is between 7.5 and 8.5, with optimum temperature ranging from 25 to 50°C (Wong *et al*. 2000). Even when strain INACH002 was grown in a medium where the sole carbon source corresponded to agar, the cell-free supernatant was able to hydrolyze alginate, suggesting a possible constitutive expression of other phycocolloid-degrading enzymes. Similar expression has been observed in other bacteria, such as *Saccharophagus degradans*, isolated from saltwater marsh grasses, when cultivated in agarose, glucosamine, starch, xylan or xylose as the sole carbon source (Hutcheson *et al*. 2011). In addition, the presence of carbohydrase systems capable to degrade multiple complex polysaccharides (agar, alginate, chitin, cellulose, fucoidan, laminarin, pectin, pullulan, starch and xylan) had previously been reported for this species (Hutcheson *et al*. 2011). However, it is important to mention that there are limited reports assessing the combined capacity to degrade agar and alginate in the same study. There is no other strain of *Flavobacterium*, from the Antarctic or elsewhere that has been reported to degrade these two polysaccharides, as in the INACH002 strain.

Considering the Antarctic origin of our strain and its psychrotrophic conditions, future studies with isolated enzymes from this strain should aim to elucidate the observed difference in the ability to degrade various substrates at different temperatures. This information, in addition to possessing biotechnological value, can be extrapolated to an ecological context allowing a greater understanding of the carbon cycling processes of algal biomass stranded in the Antarctic. The Antarctic is becoming an important source of bio-resources with many current and potential applications, and should be protected and further explored as such. Characterization of agarase and alginate lyases will enhance and expand the use of these enzymes to engineer novel alginate polymers for applications in various industrial, agricultural, and medical fields

Acknowledgements. — Paris Lavin was supported by the grant Nº 78111301 from Inserción de Capital humano – CONICYT, Instituto Antártico Chileno (INACH), Chile. The authors would like to thank personnel at INACH for their logistic support in Antarctica.

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Received 2 October 2015
Accepted 20 May 2016