MICROBIOLOGY

Mycobiota on exoskeleton debris of *Neohelice granulata* in an alkaline-sodic salt marsh: *in vitro* enzyme ability at different temperatures and pH

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Abstract: This study analysed the mycobiota on exoskeleton debris of the crab *Neohelice granulata* collected from an alkaline salt marsh and assessed the *in vitro* enzyme ability of selected isolates at different temperatures and pH. Exoskeleton fragments were incubated in moist chambers on paper and on agar medium. Growth and enzyme ability of selected fungi were also evaluated in agar media with 0.5 % casein, 1% Tween®20, and Chitin-Azure® by the production of a halo/growth ratio. We identified 22 fungal species using both methods. Since the two isolation methods added information to one another, both ones are necessary to recover the cultivable mycobiota associated with the exoskeleton debris. All fungi showed greater levels of enzyme activity in alkaline than acid medium with Tween®20. The halo diameter on casein and chitin varied according to the fungal isolate and pH. Most fungi had a larger halo at 4°C than at the other temperatures tested. *Clonostachys rosea* showed the greatest activity in all media at 4ºC. We conclude that exoskeletons of the *N. granulata* are a source of fungi able to produce enzyme activities that show differences upon incubation conditions to which they are cultivated such as ones including specific temperatures and pH values.

Key words: crab exoskeletons, diversity, enzyme activity, fungal decomposers.

INTRODUCTION

Extreme ecosystems are currently considered the most interesting places to investigate microorganisms and/or their roles and enzymes, mainly due to the stressful conditions in which they grow. For several years, our working group and other researchers at world level are analyzing in different stressful ecosystems specific organic materials as source of fungi to identify isolates with specific physiological behaviours and able to synthetize enzymes, that might be active under stressful conditions and therefore to be evaluated as potential tools for biotechnological applications (Saparrat et al. 2007, Franco et al. 2018). An example of this type of ecosystem, from which there is not any information available, is a brackish salt marsh called "cangrejal", which is located at Campos del Tuyú National Park, in the eastern part of the Salado Sedimentary Basin (36°21´00"S; 56°52´00"W). The "cangrejal" covers lowlands of less than 4 meters above sea level, with a large proportion of flooded lands and drainage channels. Due to the periodic flooding caused by the tides, there is a constant supply of brackish water in this area and therefore a high salt content (30.40 and 35.8 mS/cm, Carol et al. 2015), which generates saline-sodic soils with pH between 7.5 and 9 (Beade 1981). This area is characterized by the presence of high densities of the burrowing semiterrestrial crab *Neohelice granulata* (Dana 1851) (syn. *Chasmagnathus granulatus*), which is endemic to tropical and

subtropical estuaries of South America (Boschi 1964, Spivak 2010). The endo- and exocuticle of crustacean exoskeletons are constituted by several compounds, mainly proteins (15-40%), chitin (20-40%), lipids (1-17%), and different calcium, phosphorous, and magnesium salts, as well as β-carotene (Stevenson 1985, Lage-Yusty et al. 2011, Spivak 2016). Therefore, exoskeletons are one of the major animal sources of organic matter in soils of brackish salt marshes such as Campos del Tuyú National Park.

The structure of *N. granulata* populations, including features of their life history, reproduction, feeding behaviour, and ecological role, have been studied in numerous environments characterized by low or fluctuating salinity (Botto et al. 2006, Mendez-Casariego et al. 2011). *Neohelice granulata* is a semiterrestrial burrowing crab endemic from tropical and subtropical estuaries in South America. These species occur in the southwestern Atlantic Ocean in Brazil (from Río de Janeiro 22°57'S, 52°32'W, to Rio Grande do Sul, Uruguay and San José Gulf, Northern Patagonia, Argentina 42°25'S, 64°36'W) (Spivak 2010). Dense populations of *N. granulata* inhabit almost all zones of the intertidal, the soft bare sediments flats and areas with the cordgrass *Spartina densiflora*, *Spartina alterniflora* and *Sarcocornia perennis* (formerly known as *Salicornia ambigua*) (Botto et al. 2005, Bortolus 2006). This crab constructs vertical burrows of up to 10 cm of diameter that can reach up to 1 m depth in vegetated marshes. The burrows remain open during high tide periods and generally remain full of water during low tide. Furthermore, these burrows act as passive traps of sediment and detritus in open mud flats. Burrowing activity may also change sediment characteristics (Botto et al. 2006, Mendez-Casariego et al. 2011). It is estimated that organisms annually produce 100 to 200 billion tons of exoskeleton remains, constituting

a huge amount of biomass in lake waters and bottom sediments (Swiontek Brzezinska et al. 2008). Chitinolytic microorganisms play an important role in the degradation of the exoskeleton, being bacteria and fungi capable of producing chitinolytic enzymes, which degrades exoskeletons. However, the fate of *N. granulata* exoskeletons in these stressful environments and the microorganisms involved in their decomposition are still unknown.

During the microbial degradation of the organic compounds of *N. granulata* exoskeletons, enzyme complexes such as those catalysing lipolysis, proteolysis, and chitinolysis might be involved. Although fungi have been considered as key contributors to detritus-based food webs in salt marshes (Torzilli et al. 2006), according to Swiontek Brzezinska et al. (2013) chitinolytic fungi and bacteria play an important role in the degradation of shrimp waste. However, there is no information hitherto available on the fungi associated with *N. granulata* and their role in the degradation of the exoskeleton compounds. Therefore, our aims were to study the diversity of cultivable microfungi that grow on exoskeleton debris of this crab collected at Campos del Tuyú National Park and to analyse the ability of selected isolates to grow on agar media and produce lytic enzyme systems at different incubation temperatures and pH of the media. We hypothesized that crab exoskeleton debris found in brackish salt marshs from Campos del Tuyú National Park (Argentina) might be a source for the isolation of fungi able to produce enzymes highly active at specific temperatures or pH values, which are related to the environmental characteristics of fungal source and to environment where were collected as well as to fungal optima for enzymatic activity. These enzymes might be used as biotechnological tools for several industrial processes (Elíades et al. 2010, Franco et al. 2018).

MATERIALS AND METHODS

Study area and sampling

Exoskeletons of *N. granulata* were collected from an area of "cangrejales" in Bahía de Samborombón using a random sampling method. This methodology requires that each sample must have equal opportunity to be selected. The sampling was carried out randomly in three areas of 200 m^2 of different "cangrejales", where exoskeletons were collected on ground surface or on the vegetation cover. This area belongs to Campos del Tuyú National Park and is located in the southwestern region of La Plata River estuary, General Lavalle (36°21'S, 56°52'W), Buenos Aires, Argentina (Figure 1). The climate and characteristics of the study area were previously described by Volpedo et al. (2005). The physical and chemical features of the soil in the study area were determined according to

SAMLA (2004) and PROMAR-AACS (1991), and are shown in Table I.

Sampled exoskeletons were stored in plastic bags at 4°C until processing after four days (Dick et al. 1996). In the laboratory, they were fragmented into small pieces (approx. 5mm^2). Thereafter, exoskeletons were washed with sterile distilled water to remove soil traces and left to dry in a petri dish with filter paper, as shown in Figure 2a-b.

In vitro incubation of exoskeletons, fungal isolation and diversity analyses

The following methods were employed for fungal isolation: *(i)* moist chambers: five exoskeleton pieces per plate were incubated in Petri dishes (90 mm diam.) with moistened filter paper at 25ºC (n=60), and *(ii)* cultivation on solid media of exoskeletons pieces previously washed with sterile water and fragmented as described above (Parkinson & Williams 1961). For the latter
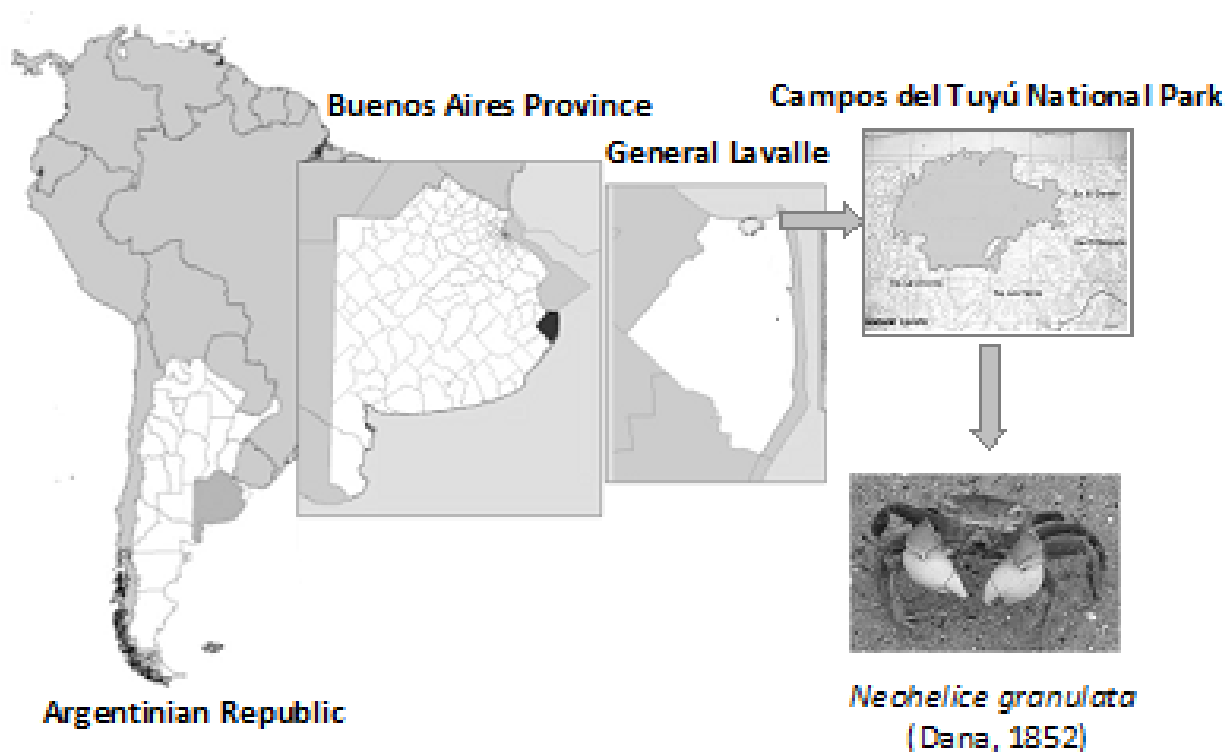

Figure 1. Location of the sampling site in Campos del Tuyú National Park, General Lavalle, Buenos Aires, Argentina.

method, pieces were left to dry for 24 h on filter paper discs to avoid bacterial and yeast growth and then plated according to Widden & Parkinson (1973). Sixty pieces were inoculated onto 12 Petri dishes containing cornmeal, yeast, glucose agar (CMYG) with 0.5 % streptomycin and 0.25 % chloramphenicol (washing and particle technique). Plates were incubated at 25°C until the development of fungal colonies. For microscopic observation of morphological featuresof fungi grown in both methods, Malt Extract Agar (MEA) slide cultures were prepared and mounted with lactophenol cotton blue. Fungi were identified according to Carmichael et al. 1980, Domsch et al. 1993, and Ellis 1971 (Table II), and deposited in the culture collection of "Instituto Spegazzini" (LPSC). Stock cultures were maintained on 2% (w v⁻¹) MEA slants at 4°C.

In the case of the moist chamber technique, each exoskeleton piece was considered a sampling unit. The percentage frequency of each fungal species was calculated as:

$$\frac{\text{Number of exoskeleton pieces colonized by a fungal species}}{\text{Total number of pieces analysed}} \times 100$$

The frequency was calculated based on the number of times a species occurred on the exoskeleton pieces. We also estimated the species richness (S) and evenness (J) of the mycobiota and he diversity index of Shannon-Weiner (H´) using the frequency of each fungal species (Magurran 1988, Cabello & Arambarri 2002). The data were analysed with a one-way analysis of variance (ANOVA), followed by Tukey´s test (P <0.05).

Growth and enzyme activity of selected isolates and their response to different incubation temperatures and pH of the media

Agar plugs (6 mm diam.) obtained from each fungus growing on Oxoid CMA with 0.2 % glucose were inoculated onto Petri dishes with agar media supplemented with 0.5 % casein, 1%, Tween®20 (polyoxyethylene sorbitan monolaurate Merck®), and 0.08% Chitin-Azure® (Sigma®, C3020) according to Hankin & Anagnostakis 1975, Howard et al. 2003, and Koneman & Roberts 1987, respectively. Three replicate plates of each culture medium per isolate were prepared. Dishes were incubated in the dark at 4 ± 1°C, 16 ± 1°C and 26 ± 1°C for 7 or 20 days in the case of cultures on chitin medium. In addition, the effect of pH was evaluated using three replicates of each medium mentioned above with pH 5.0, 8.0, and 10, which were incubated in the dark at 26 ± 1°C for 7 or 20 days in the case of cultures on chitin medium. The pH was adjusted after autoclaving using buffer solutions (Nagai et al. 1998). For each treatment, fungal growth was estimated by measuring the diameter (cm) of each colony. The hydrolytic enzyme activity was estimated qualitatively by

Table I. Soil physical and chemical features.

Characteristic	Value[a]
pH	8.1±0.14
Organic C %	1.24±0.11
Organic matter %	2.14±0.18
Total N %	0.13±0.01
Total P mg/kg	559.67±17.21
Electric conductivity (dS/m)	52.51±10.87
K⁺ (meq/l)	0.58±0.09
Ca²⁺ + Mg²⁺ (meq/l)	327±73.88
Na⁺ (meq/l)	844.23±249.68
RAS (sodium adsorption ratio)	64.34±13.62
Texture	clayey/argillaceous

**[a]Values are means of three representative replicate samples ± standard deviation.**

the presence of a clearance/precipitation halo due to hydrolase action. Results were expressed as mean values ± standard deviation (SD) of the ratio between the hydrolytic halo and the colony diameter (Saparrat et al. 2008). The fungal growth and enzyme activity were compared with ANOVA and the differences between means were discriminated by Fisher's least-significant-difference multiple-range test (P <0.05), using InfoStat software.

RESULTS

Twenty-two fungal taxa were recorded from the exoskeleton rests; 14 using the moist chamber technique and 17 from the cultivation of washed exoskeleton pieces on agar media (Table II). Nine taxa were recovered using both methods, being *F. solani* (moist chamber: 20.83 % and cultivation on agar media: 35.83 %) and *A. alternata* (moist chamber: 13.33 % and cultivation on agar media:

35.83 %) the most frequent ones. Five species were recorded exclusively on exoskeleton pieces incubated in moist chambers, in which a dematiaceous sterile mycelium was dominant. Eight taxa, including *F. equiseti*, were detected only from washed exoskeleton pieces cultivated on agar media. However, there were no significant differences between diversity and evenness indexes of the mycobiota obtained from exoskeleton pieces incubated in moist chambers (H´:1.86; E: 0.48) and from washed exoskeletons cultivated on agar media (H´: 2.13, E: 0.51; P> 0.001).

All isolates grew on the three culture media at the incubation temperatures and pH tested, however, some strains showed a differential growth at certain incubation temperatures and/or pH (Figure 3). The alkalization of the medium containing Tween 20 did not reduce the growth of *F. equiseti* LPSC 1423 and *F. solani* LPSC 1422. A similar response was observed for *A. alternata* LPSC 1244, *F. solani* LPSC 1422, and *S. brevicaulis*
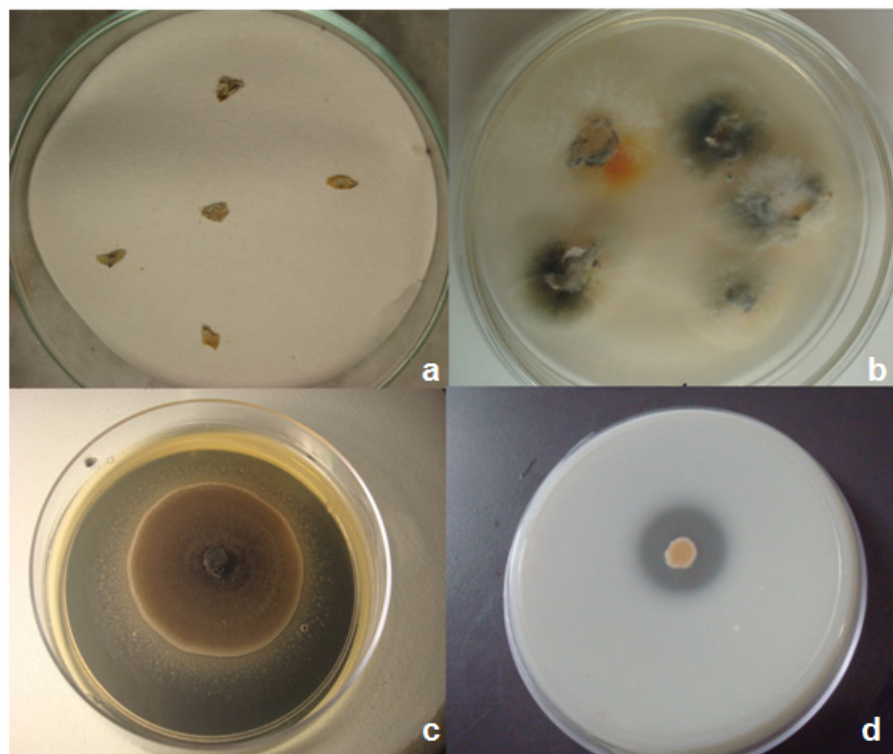
Figure 2. a-b. Fungal isolation from exoskeleton debris of the crab *Neohelice granulata*. a. Development of fungal colonies on filter paper in moist chambers. b. Fungal colonies from washed exoskeleton pieces on CMYG culture medium. c. Lipolytic activity in agar media. d. Proteolytic activity in agar media.

LPSC 1362 on casein. However, the latter fungus showed a greater colony diameter on chitin azure at pH 8 than under both acid and strong alkaline conditions. On the other hand, all fungi tested on three types of media revealed a greater colony diameter with the increase in incubation temperature (Figure 4).

The enzyme ability of the selected fungal species on specific media at three different pH values and incubation temperatures is shown in Tables III and IV, respectively. All fungi tested showed higher activity on Tween 20 medium under alkaline than acidic pH values. *Clonostachys rosea* LPSC 1236 and *S. brevicaulis* LPSC 1236 showed the greatest halo/growth ratio at pH 10. However, some isolates were not able to hydrolyse Tween 20 at pH 5.0. *Fusarium solani* LPSC 1422 was the only fungus that

Table II. Frequency of the fungal taxa on exoskeleton debris according to the isolation method and their contribution to the Shannon-Weaver diversity index (H´).

Taxa	Isolation source			
	Moist chambers		Agar media	
	Frequency	Contribution to H ($-pi \log_2 pi$)	Frequency	Contribution to H ($-pi \log_2 pi$)
Ascomycota				
Fusarium solani	**20.83**	0.33	**35.83**	0.34
Alternaria alternata	**13.33**	0.28	**35.83**	0.34
Cladosporium cladosporioides	1.67	0.07	1.67	0.05
Fusarium sp	1.67	0.07	10.00	0.18
Purpureocillium lilacinum	1.67	0.07	4.17	0.10
Scopulariopsis brevicaulis	1.67	0.07		
Acremonium kiliense	0.83	0.04		
Chaetomium globosum	0.83	0.04		
Fusarium equiseti			24.17	0.30
Fusarium graminearum			10.00	0.18
Clonostachys rosea			4.17	0.10
Penicillium sp	0.83	0.04	4.17	0.10
Aspergillus sp			0.83	0.03
Beauveria sp			0.83	0.03
Penicillium rubrum			0.83	0.03
Phoma sp			0.83	0.03
Mucoromycota				
Mucor sp	1.67	0.07	0.83	0.03
Rhizopus stolonifer			4.17	0.10
Mycelia sterilia				
Dematiaceous sterile mycelium 1	34.17	0.37		
Dematiaceous sterile mycelium 2	2.50	0.10	0.83	0.03
Hyaline sterile mycelium 1	12.50	0.27		
Hyaline sterile mycelium 2	0.83	0.04	7.50	0.15

was not able to hydrolyse casein at the three pH values analysed. The halo/growth ratio on casein varied depending on the fungal isolate and pH value. *Alternaria alternata* LPSC 1244 showed proteolytic activity at pH 5.0, whereas *P. lilacinum* LPSC 1236 and *S. brevicaulis* LPSC 1362 at alkaline pH. While *F. equiseti* LPSC 1422

only revealed chitinolytic activity at pH 10, *A. alternata* LPSC 1244 failed to decolorize the medium supplemented with chitin azure at the three pH values tested. The halo/growth ratios of the other fungi did not differ considerably at pH 8. On the other hand, growth and enzyme activity showed a different response
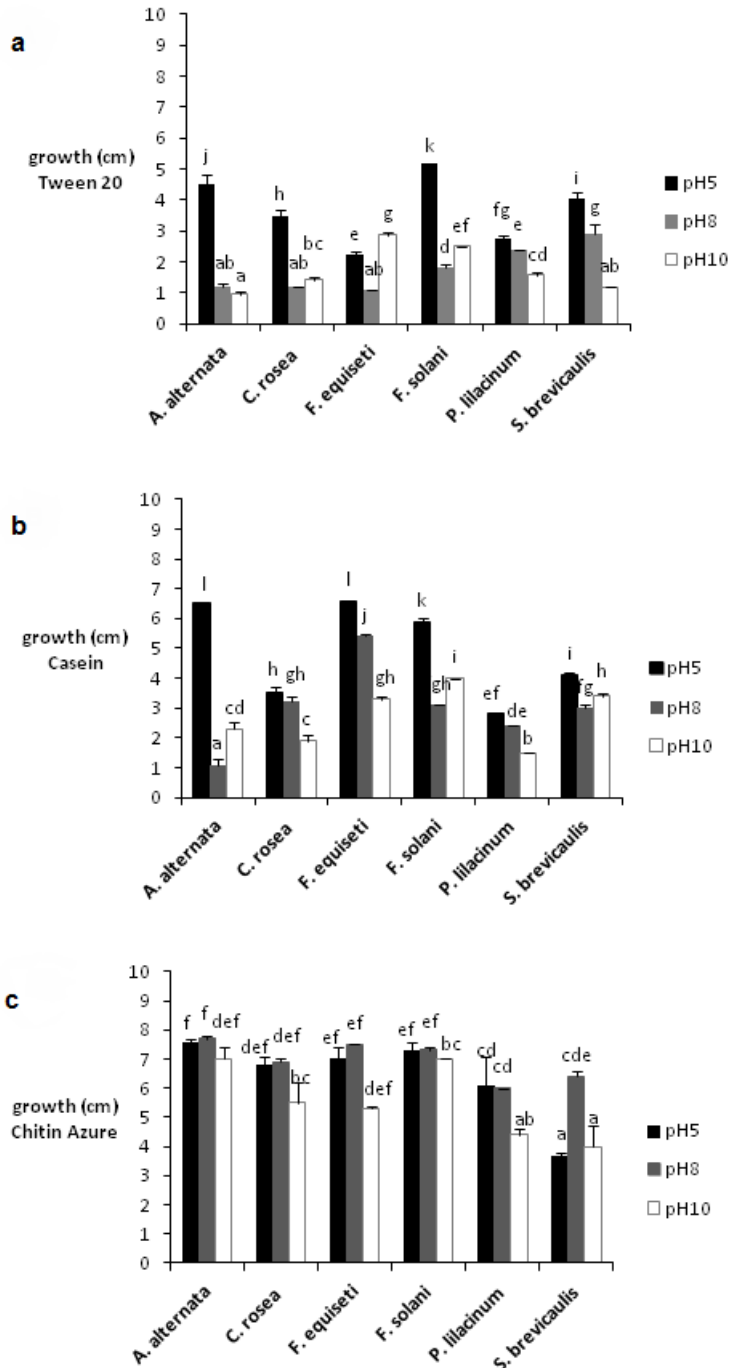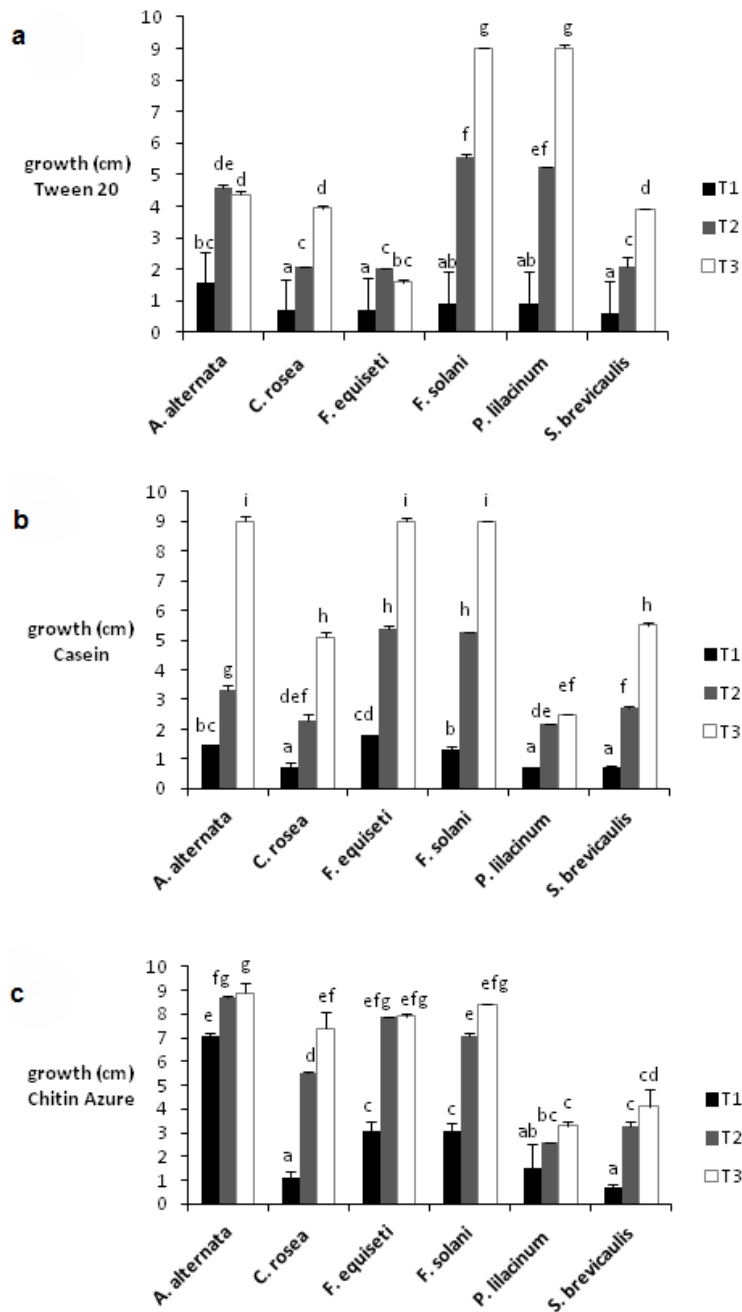

Figure 3. Colony growth of the selected fungal species on specific substrates (a, Tween®20; b, casein; c, Chitin-Azure®) at 26°C and pH 5 (black bars), pH 8 (grey bars), and pH 10 (white bars). The data are means of three replicates ± SD; the same letters above the bars indicate non-significant differences (multiple-range-Test; P ≤ 0.05).

to temperature. All isolates varied their halo/ growth ratios in the three substrates analysed according to the incubation temperature, being in most fungi greater at 4°C than at the other incubation temperatures tested (Table IV).

DISCUSSION

Two isolation methods were used to recover the cultivable mycobiota associated with the moult of *N. granulata* inhabiting a brackish salt marsh in Campos del Tuyú National Park (Argentina), which were complementary because they revealed specific taxa. This fact can be closely

Figure 4. Colony growth of the selected fungal species on agar medium with specific substrates (a, Tween®20; b, casein; c, Chitin-Azure®) at pH 7 and three different temperatures: 4°C (black bars), 16°C (grey bars), and 26°C (white bars). The data are means of three replicates ± SD; the same letters above the bars indicate non-significant differences (multiple-range-Test; P ≤ 0.05).

related to the existence of specific limitations for each isolation method, being physiological requirements (including nutritional ones) the critical aspects such as suggested by Mallo et al. (2017). In this sense, the washing technique has been suggested be far more efficient to isolate actively growing microfungi on isolation source (Cabello & Arambarri 2002). Oppositely, moist chamber technique is further dependent on the spores available and adhered on the isolation source, which must be viable and can germinate on the incubation conditions as well as their hyphae grow using cellulosic materials such as filter paper, which is highly different to the chemical nature of the exoskeleton debris of *N. granulata*. Nayar & Jothish (2013) reported that some propagules such as ones from Ascomycota show specific difficulties to germinate. Therefore, since a relationship between isolation sources of the fungi and their metabolic pathways might exist (Franco et al. 2018), it is probable that the fungi recovered from exoskeleton debris applying each isolation method can be different. This suggests that it is important to use multiple isolation methods to recover fungi from exoskeleton debris of *N. granulata*. However, the estimated fungal richness and

diversity did not differ between the isolation methods used, which is in accordance with other previous finding analyzing the same parameters in alkali-tolerant mycobiota of *Distichlis spicata* (Poaceae) litter in alkaline soils from Buenos Aires Province (Elíades et al. 2014). Specifically, *Purpureocillium lilacinum, Clonostachys rosea, Acremonium kiliense* were previously reported also from those samples of a saline-sodic soil (Elíades et al. 2014) with similar characteristics to the soil found in Campos del Tuyú National Park.

All fungi isolated from *N. granulata* debris were able to decompose the specific carbon substrates that are representative of the structural components of the crab exoskeleton, and showed proteolytic, lipolytic, and chitinolytic activity in agar media. Previous findings suggest that metabolic abilities of the fungi, including synthesis of lytic enzymes such as proteases, lipases and chitinases, are adaptive and of ecological significance, which also are highly dependent upon each isolate and so revealing intraspecific physiological variability in different taxa according their isolation source (Fenice et al. 1997, Franco et al. 2018, Barrera et al. 2019). Also, the behaviour of each fungal strain in

Table III. *In vitro* extracellular ability of six selected fungi grown on agar media supplemented with Tween®20, casein and Chitin-Azure® at three different pH and 26°C. The same letters indicate non-significant differences (P ≤ 0.05).

	Enzyme ability (halo/growth)								
	Tween 20			Casein			Chitin Azure		
	pH 5	pH 8	pH 10	pH 5	pH 8	pH 10	pH 5	pH 8	pH 10
A. alternata	0 a	1 b	1.7 h	1.1 c	0 a	0 a	0 a	0 a	0 a
C. rosea	1.1 c	1.7 ih	2.7 j	1.1 c	1.1 c	0 a	1 def	1 def	0.8 c
F. equiseti	1,1 c	1.8 i	1.27 e	1 b	1.1 c	0 a	0 a	0 a	1.7 h
F. solani	0 a	1.37 gf	1.5 g	0 a	0 a	0 a	0.9 cd	1 def	1.3 g
P. lilacinum	0 a	1.1 c	1.2 d	0 a	1.1 c	1.2 d	0.9 cd	0.93 de	0.67 b
S. brevicaulis	1.17 d	1.2	2.8 k	0 a	1.2 d	1.3 e	1.1 f	1.03 ef	0.7 h

growth and enzyme activity is conditioned by the medium type, its pH and/or incubation temperature on which is cultivated, since environmental factors modulate the fungal response. In the three media analysed, fungal growth was favoured at the highest incubation temperature tested (25°C), however, most fungi showed higher levels of enzyme activity at 4°C than at 15 and 25°C. This profile of enzymatic activity upon incubation temperature can be related to a behaviour type for these fungi associated to exoskeleton debris of *N. granulata* as cold-tolerant mesophiles in the sense of Onofri et al. (2004). As other extremophilic microfungi such as Antarctic ones, strains isolated from *N. granulata* debris might to be adapted to live in unstable microenvironments with notorious thermal variations by the effect of climate and possibly by their exposition to natural water level fluctuations on littoral zones of brackish salt marshs where their isolate source (exoskeleton debris) are accumulated in Campos del Tuyú National Park (Argentina) due to hydrologic regimes dependent of environmental conditions. The probable biochemical adaptation of these fungi with higher levels of enzyme

activity at lower temperatures might be related to a wide enzymatic competence, that increases survival chances in unfavorable conditions and being able to produce enzymes which are active over a very wide range of temperatures, including low temperatures (Fenice et al. 1998). Also, an increase in enzyme activity from fungal culturesat low temperature might be related to thermal activation processes. Tibbett et al. 1999 observed the activation of certain enzymes at low temperatures in some species of *Hebeloma* (Basidiomycota), which they also attributed to the characteristics of the habitat from which the fungi were isolated. Elíades et al. 2015 also found that different strains of *Humicolopsis cephalosporioides* (Ascomycota) isolated from the soil of an Andean-Patagonian forest and exposed to different temperatures showed higher levels of enzyme activity at low temperatures. Romero Núñez 2012 suggested that the activation of fungal enzymes at low temperatures responds to the increase in enzyme catalytic efficiency, which is a physiological adjustment required to achieving their functional action under incubation conditions in which reaction rates are reduced.

Table IV. *In vitro* extracellular ability of six selected fungi grown on agar media supplemented with Tween®20, casein and Chitin-Azure® at pH 7 and three different incubation temperatures. The same letters indicate non-significant differences (P ≤ 0.05).

	Enzyme ability (halo/growth)								
	Tween 20			Casein			Chitin Azure		
	26° C	16° C	4° C	26° C	16° C	4° C	26° C	16° C	4° C
A. alternata	1.3 bc	1.1 b	1.8 d	0.9 b	1.23 d	1.1 bcd	0 a	0 a	0.2 ab
C. rosea	1.3 bc	1.7 d	3.9 f	1.03 bcd	1.17 cd	2.1 f	0.3 abc	0.2 ab	0.8 ab
F. equiseti	0 a	0 a	1.5 d	0 a	1.1 bcd	1.1 bcd	0.2 ab	0 a	0,9 d
F. solani	0 a	0 a	1.6 d	0 a	1 bc	1.2 cd	0.3 abc	0.4 abc	0.7 bc
P. lilacinum	1 b	1 b	3.9 fe	1.07 bcd	1.1 bcd	1.8 e	0.6 e	0 a	0 a
S. brevicaulis	1.5 cd	1.7 d	2.9 e	1 bc	1.1 bcd	2.2 f	0.53 abc	0.2 ab	0.3 abc

On the other hand, the fungal response to pH varied according to the isolate and type of specific substrate in the medium. *Fusarium solani* LPSC 1422 did not show a negative response to alkaline pH when grown in tween 20 and casein media, whereas in chitin media, this fungus grew better at pH 8 than at 5 and 10. Other isolates tested also showed larger halos of enzymatic activity at alkaline than acidic pH, which could be related to the soil pH conditions where the crabs live. Since alkalinization of the medium can facilitate the availability of certain metals that interfere in enzyme induction, activity and stability mainly because of the effect that H^+/OH^- concentration has on the availability and ionic forms of nutrients, the ionization of the specific chemical groups of the substrate and the enzymes active sites, as well as on protein denaturation (Saparrat et al. 2007), a link between enzymatic activity and external pH can be inferred. Saparrat et al. 2007 found a direct relationship between the degree of activity of cellulolytic enzymes and the pH of the reaction they catalyse, which were parameters also related to the alkaline substrates of the source environment of the fungi. Since the pH of the brackish salt marsh in Campos del Tuyú National Park (Argentina), where exoskeleton debris of *N. granulata* were collected, is a key factor in determining the structure of this edaphic ecosystem (Beade 1981), our results suggest that the enzyme systems of many fungi colonizing exoskeleton debris of the crab might be highly active at alkaline pH. In conclusion, although our results are preliminary, this is the first study that analysed the mycobiota associated with exoskeletons of *N. granulata*. These organic materials dominant in brackish salt marshs in Campos del Tuyú National Park (Argentina) are a source of fungi able to produce enzyme activities that show differences upon incubation conditions to which they are cultivated such as

ones including specific temperatures and pH values. However, further research will deepen our understanding of the physiology and role of the fungi isolated in the mineralization of *N. granulata* exoskeleton debris.

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NAF: participated actively in the samplings, data acquisition in the field, species identification, and statistical analyses. TML, LAE, and LR: identified the species, analysed and interpreted the data, and wrote the manuscript draft. MCNS: contributed to the conception and design of assays, analysed the data, and contributed to manuscript drafting. MNC: coordinated the research and contributed to species identification. MNC and MCNS: participated in the final edition of the text and reviewed critically the final version of the text adding a relevant intellectual contribution to the draft.