BIOLOGICAL SCIENCES

Evaluation of wood degradation rates by Teredinidae (Mollusca: Bivalvia) in two ecologically distinct areas, and temperature and salinity influences on the cellulolytic activity of associated bacteria

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Abstract: Teredinidae (shipworms) is a family of marine wood-boring bivalves that has an important role in the degradation of wood through its symbiotic relationship with cellulolytic bacteria. To evaluate the rate of degradation of wood by teredinids in two sites with different oceanographic conditions in Rio de Janeiro State, Brazil, artificial structures composed of pine wood sheets were immersed in the ocean for three months at Arraial do Cabo in an area under the influence of upwelling, and at Ilha Grande Bay under tropical and oligotrophic influences. After the immersion period, teredinids were removed from the collectors, identified, and counted. Wood consumption by the teredinids was quantified by comparing the dry weights of the collectors before and after immersion. Associated bacteria were isolated and their cellulolytic activities evaluated at different temperatures and salinities. Two Teredinidae species were recorded: *Bankia gouldi* and *Lyrodus floridanus.* The highest wood degradation rate and enzymatic activities of the isolated bacterial strains were recorded at Arraial do Cabo, suggesting that upwelling influenced the activities of those species.

Key words: Associated bacteria, cellulose, shipworms, teredinids, upwelling, wood-boring bivalves.

INTRODUCTION

The bivalve molluscs of the Teredinidae family have specialized in perforating and degrading wood (cellulose) in marine environments. The main factors affecting the distributions and activities of those organisms are wood availability (cellulose) and oceanographic characteristics (principally temperature and salinity) (Turner 1966, Borges et al. 2014, Pati et al. 2014). Those environmental variables also influence the survival and activities of their endosymbiotic bacteria (Distel et al. 2002a, Trindade-Silva et al.

2009, Horak & Montoya 2014), thus affecting the rate and quantity of wood consumption.

The unusual and highly destructive wood degradation behaviour of those Teredinidae is directly related to their economic importance and ecological roles in food chains. The damage to woody structures in marine and estuarine environments (such as boats, fishing equipment, piers, and other man-made structures) caused by those invertebrates results in losses estimated at millions of Euros per year in Europe (Borges et al. 2014). On the other hand, like other wood-boring animals from the Pholadina suborder, teredinids play a crucial role in carbon

recycling degrading large amounts of cellulose in marine environments and maintaining fish stocks by remineralizing carbon and making organic matter available to other organisms (Turner 1966, Turner & Johnson 1971, Hutchings & Saenger 1987). According to Robertson (1991), more than 90% of the wood mass of *Rhizophora* sp. decomposed in mangrove swamps during the first four years of decay is the result of the actions of those animals.

The ability of Teredinidae to metabolize cellulose and other complex polysaccharides that compose the majority of wood material is due to their symbiotic relationship with the cellulolytic nitrogen-fixing bacteria *Teredinibacter turnerae* (Waterbury et al. 1983, Distel et al. 2002b). The bacteria are found at their highest concentrations in specialized intracellular structures (bacteriocytes) located in the gills, called Deshayes glands (Betcher et al. 2012, Sipe et al. 2000, Waterbury et al. 1983), which was first confirmed by Popham & Dickson (1973) using electron microscopy.

Studies of symbionts isolated from 24 Teredinidae species from different geographic regions demonstrated the presence of identical 16S rRNA sequences (or typically intraspecific variations), suggesting the existence of a single symbiont (*Teredinibacter turnerae*) for the entire Teredinidae family (Distel et al. 1991, 2002b).

Teredinibacter turnerae has stimulated great biotechnological interest due to its capacity to transform cellulose (the most abundant compound in nature) into biomass, organic acids, or other by-products without the addition of any nitrogen source. The development and optimization of processes for the use or transformation of those materials is quite relevant to finding promising substitutes (such as biofuels) for traditional energy sources (Imam et al. 1993).

In addition to secreting cellulase(s), protease(s), and organic acids, that bacteria was the first Pseudomonadaceae described having the ability to fix nitrogen (Distel et al. 1991, 2002a). That bacterial family includes organisms of both medical and environmental importance related to the production of compounds with diverse biotechnological applications (Distel et al. 1991, 2002a, Imam et al. 1993).

Despite their ecological and biotechnological importance, there have been very few recent works focusing on the family Teredinidae in Brazil (Maldonado & Skinner 2016, Junqueira et al. 1989, Junqueira & Silva 1991), and none have measured wood degradation rates.

The objectives of the present study were therefore to evaluate the rate of degradation of wood by teredinids in two localities with different temperature regimes, and to evaluate the influence of both salinity and temperature on the enzymatic activity associated with the cellulose degrading bacteria obtained from each Teredinidae species.

MATERIALS AND METHODS

Study areas

Artificial wooden structures were immersed between December 2016 and May 2017 in two coastal regions of Rio de Janeiro State, Brazil: Arraial do Cabo (AC - 22°58'22"S x 42°00'49"W) and Ilha Grande Bay (IGB - 23°03'09"S x 44°14'37"W), representing two distinct oceanographic environments in relation to nutrient concentrations and, especially, sea surface temperatures (Valentin 2001, Creed et al. 2007) (Figure 1).

Arraial do Cabo is located on the eastern coast of Rio de Janeiro State where oceanographic upwelling is a significant feature. The upwelling Southeast Atlantic Central Water (SACW) is characterized by temperatures below
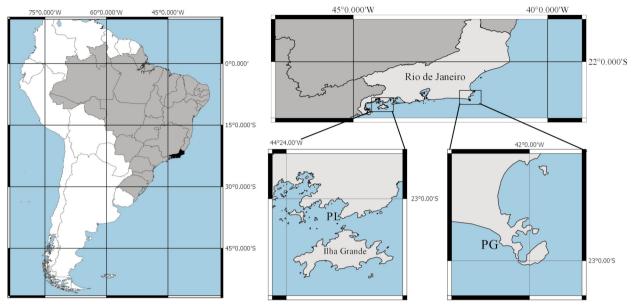
Figure 1. Map of the studied areas on the coast of Rio de Janeiro State. (PG = Praia Grande, in Arraial do Cabo); PL = Ponta Leste, in Ilha Grande Bay). Map generated by QGis V. 2.10.1.

20°C and a rich nutrient content (Valentin 2001). The surface emergence of those waters increases primary productivity, favouring mainly benthic life (Fernandes et al. 2017). Average annual rainfall is ca. 815 mm.year^{-1}, with a dry season during the Austral winter and a rainy summer (Guimaraens & Coutinho 2000, Skinner et al. 2007, 2011).

Ilha Grande Bay is located in the southern region of Rio de Janeiro State, and is characterized by coves, small bays, and numerous islands. The entire region is surrounded by the Serra do Mar mountain chain and Atlantic Forest vegetation. Annual rainfall reaches up to 2,700 mm.year^{-1}, with a rainy season in the Austral spring-summer and dry season during autumn-winter (Creed et al. 2007, Mayer-Pinto & Junqueira 2003, Skinner et al. 2016).

Sampling

Commercial pine *Pinus elliottii* Engelm laminates, a low-cost and easily attackable wood, was used to test wood degradation and to collect teredinids (Silva et al. 1988). Each of the collectors consisted of ten thin juxtaposed pine sheets (0.08 cm thick), each measuring 10.0 x 10.0 cm, pressed between Formica plates (12.0 x 8.0 cm).

That type of collector facilitates the removal and identification of wood-boring organisms (as opposed to solid substrate collectors), as the use thin wood sheets allows the extraction of whole organisms without any damage to their pallets (the calcareous structures used as a taxonomic character) (Junqueira et al. 1991, Barreto et al. 1993).

Five collectors were immersed (one meter deep) at each locality for three months, the ideal time period for achieving a maximum density of living animals (Junqueira et al. 1991). One collector was lost during the immersion period at Ilha Grande. Sea surface temperatures (SST) were recorded using iButton® sensors programmed to record values each hour at Ilha Grande and every two hours at AC.

The collectors were removed from the water after three months, placed inside thermal boxes with local sea water under aeration, and transported to the laboratory. The collector sheets were subsequently opened one by one

in the laboratory and Teredinidae individuals removed and identified to the species level (using a stereoscopic microscope) according to the morphology of their pallets, as described by Turner (1966).

To isolate their associated bacteria, the shells and pallets of the collected individuals were aseptically removed (after Teredinidae identification) and the remainder of the body washed five times in 1 mL of sterile distilled water to remove external contaminants. Three collectors from each locality were used for those analyses.

Isolation and cellulolytic activity of bacteria associated with teredinids

After washing, each set of teredinid species was macerated separately with the aid of a Polytetrafluoroethylene homogenizer and diluted 10x in sterile saline solution (0.9%). Aliquots of 0.1mL of this dilution, and decimal dilutions (10^{-2} and 10^{-3}), were seeded onto Carboxymethylcellulose (CMC) agar containing: CMC 10g; KH_2PO_4 4g; Na_2HPO_4 4g; triptone 2g; $MgSO_4.7H_2O$ 0.2g; $CaCl_2$ 0.001g; $FeSO_4.7H2O$, 0.004g; Agar 15g; pH7. Triplicates were made of each dilution.

After 48 hours of incubation at room temperature (between 25 and 28°C), morphologically distinct colonies on each plate were isolated for cellulose degradation tests. All colonies received an identification code consisting of the number of the isolated strain, species, and the locality where it was isolated.

A methodology adapted from Bairagi et al. (2002) was used to detect cellulolytic activity. Aliquots of the strains to be tested were inoculated (in duplicate, arranged at equidistant points) on two plates containing CMC agar and then incubated at 25°C. Colonies that demonstrated halo/colony ratios (called the enzymatic index - EI) ≥ 2.0 were considered

as potentially producing cellulase (Lealem & Gashe 1994). Visualization of the halo was achieved through the addition of a 0.1% congo red solution.

To verify cellulolytic activity at different temperatures and salinities, the bacterial strains (EI ≥ 2.0) were inoculated onto CMC agar containing different salt (NaCl) concentrations (0, 17 and 35 ppt). Greene & Freer (1986) determined that *T. turnerae* shows optimum growth at 17 ppt, while 35 ppt mimics seawater conditions. Tests at 170 ppt and 350 ppt of salt were performed to observe the cellulolytic activities of the strains under extreme salinity conditions. The strains were also incubated at different temperatures for 48 hours using the same Bairagi method (adapted) described previously (15°C representing the upwelling temperature, 25°C room temperature, and 33°C as the optimal growth temperature for *T. turnerae* according to Greene & Freer (1986)). All tests were performed in triplicate.

Analyzes of wood degradation

The total amount of collector wood degraded by Teredinidae was calculated from the differences between the dry weights of the collector sheets before immersion and after removal. The results were expressed as mean values of wood destruction.

RESULTS

Water temperature

A total of 1,412 temperature records were obtained at AC, and 2,435 records at IGB.

At AC, 40% of the records were of water temperatures below 20.0°C, with the minimum temperature (12°C) recorded in February and the maximum (29.5°C) in January; all of the recorded months, however, showed values below 20.0°C, characterizing the upwelling phenomenon

(especially in February, with 86.6% of the temperature measurements below that value).

At IGB, sea surface temperature ranged from 21.5°C (February and May) to 31°C (February) with 99.6% of the records below 30°C. We did not record any signal of upwelling at that site.

Teredinidae abundance and diversity

Only two Teredinidae species were recorded during the surveys: *Bankia gouldi* (Bartsch 1908) and *Lyrodus floridanus* (Bartsch 1922). The former had a higher population density at AC, with an average of 31.8 individuals per collector, while *L. floridanus* dominated at IGB, with an average of 20.8 individuals per collector (Table I).

Wood degradation rates

Collectors installed at AC showed greater wood consumption than those at Ilha Grande. The average degradation of collectors at AC (70.7% ± 2.0) was much higher than that recorded at Ilha Grande (7% ± 1.3). One of the collectors at AC showed 80.5% destruction, while 12% was the maximum destruction percentage at Ilha Grande.

Cellulose degradation tests

Fifty-eight morphologically distinct (14 at AC and 44 at Ilha Grande) bacterial strains were isolated; 39 (67.2%) showed some cellulose degradation activity, with enzymatic indices ranging from 1.1 to 3.3. Three strains isolated from each site (two from *B. gouldi* and one from *L. floridanus*) were considered as potential enzyme producing strains (EI ≥ 2.0) with the highest values recorded for *B. gouldi* from Ilha Grande (3.3) and AC (3.0).

All strains showing cellulolytic activity (EI ≥ 2.0) functioned at different temperatures and salinities. Strains 2BgAC, 12LfAC, and 69LfIGB all exhibited high EI indices when exposed to 170 ppt of salt, with 12LfAC and 69LfIGB demonstrating high EIs for cellulose degradation at both 25°C and 33°C. The highest EI (3.5) was observed with strain 12LfAC at 25°C and 170 ppt salt (Table II). All of the strains tested at 15°C and at salinities of 170 ppt and 350 ppt, at 25°C and 350 ppt, and at 33°C and 350 ppt, exhibited EI = 0 (absence of halo), and those results are not presented in table II.

Table I. Total number of individuals, mean number of Teredinidae individuals and (standard deviation between bracket) found at Arraial do Cabo and Ilha Grande Bay.

Site/ Species	*L. floridanus*	*B. gouldi*	Total in all collectors
Praia Grande (N=5)	35 - 7(4.0)	159 - 31.8 (13.8)	194
Ponta Leste (N=4)	83 - 20.8 (11.4)	5 - 1.3 (1.2)	88
Total of specimens	118	164	282

N = Number of collectors retrieved from water.

Table II. Cellulolytic activity (EI) presented by the strains isolated from *Bankia gouldi* (Bg) and *Lyrodus floridanus* (Lf) collected in Praia Grande - Arraial do Cabo (AC) and Ponta Leste - Ilha Grande Bay (IGB) at different temperatures (15, 25 and 33°C) and salinities (0, 17, 35 and 170 ppt of salt).

Temperature-Salinity/ Strains	15°C 0ppt	15°C 17ppt	15°C 35ppt	25°C 0ppt	25°C 17ppt	25°C 35ppt	25°C 170ppt	33°C 0ppt	33°C 17ppt	33°C 35ppt	33°C 170ppt
2 Bg AC	3.33	2	2	2.14	2	2	0	3	3	2.33	2.5
6 Bg AC	0	2.5	0	2	2.5	0	0	2	2.5	0	0
12 Lf AC	0	1.5	0	0	3	2.25	3.5	2.5	2	2	3
60 Bg IGB	2.5	1.25	0	2.25	2.25	0	0	3	2	3	0
61 Bg IGB	2.25	2.5	2.5	0	3	0	0	0	2.33	0	0
69 Lf IGB	0	2	2	0	2.33	2.5	2.5	0	2	2.5	2.66

Bg: *Bankia gouldi*; Lf: *Lyrodus floridanus*; AC: Praia Grande (Arraial do Cabo); IGB: Ponta Leste (Ilha Grande Bay); ppt: parts per thousand; 0 = absence of halo.

DISCUSSION

Understanding the roles of Teredinidae and their associated bacteria in marine environments is of great economic importance, both in terms of minimizing adverse effects caused by the degradation of wood structures and for searching for biotechnological alternatives for biofuel production. Despite that economic importance, and extent of the Brazilian coast, studies of Teredinidae in that country have been scarce.

Abundance and diversity of teredinidae species

The two species encountered in this study had already been recorded for the south-eastern coast of Brazil (Junqueira et al. 1989, Lopes & Narchi 1993, Muller & Lana 2004, Maldonado & Skinner 2016). The dominance of *L. floridanus* in IGB had likewise been previously recorded by other authors since the 1980's (Junqueira et al. 1989, Omena et al. 1990, Maldonado & Skinner 2016). That species was replaced by *B. gouldi* as dominant in only one study (Barreto et al. 1993).

According to Turner (1966), Teredinidae distribution is conditioned basically by temperature, salinity, and wood availability, with that substrate supplying the main food and spatial resource for those animals. One of the most significant sources of wood in the marine environment along the south-eastern coast of Brazil is the Atlantic Forest, which borders many bays and estuaries along the shore. That Atlantic Forest formation is present near the coast at IGB in Rio de Janeiro State, becoming scarce, however, in the coastal regions from Sepetiba Bay northwards (where AC is located), with the Serra do Mar mountain chain and its forests occupying more inland locations there (Maldonado & Skinner 2016).

In addition to the high availability of wood (food and habitat) resources at IGB, *L. floridanus* is larviparous, and its larvae are incubated by the adults during the initial (and therefore most) critical phases of their development. That incubation provides protection against both predators and unfavourable environmental conditions (such as low salinity during the rainy season) (Barreto et al. 2000), thus increasing their chances of survival. Those larvae are subsequently able to rapidly colonize wood substrates, as they do not become dispersed far from those sources. Due to this reproductive strategy, they are usually the most abundant species in the environments where they occur (Turner & Johnson 1971, Mann & Gallager 1985a, b). Similar results were reported by Borges et al. (2014) for *Lyrodus pedicellatus* (Quatrefages 1849) in the Mediterranean Sea.

Bankia gouldi was the dominant species at the upwelling site at AC. Muller & Lana (1986) consider that species as being typically marine, probably stenohaline, and only rarely occurring estuary environments. The characteristics of the AC environment therefore probably explain the abundance of that specie.

During previous studies in the region, Maldonado & Skinner (2016) observed that *L. floridanus* was the most abundant species at Porto do Forno, while *B. gouldi* was the dominant species in the Itajuru channel. Junqueira et al. (1989) reported the dominance of *Teredo furcifera* (Martens 1894) at Itajuru followed by *B. gouldi*. Barreto et al. (1993) found a different *Bankia* species as dominant close to Forno cove, although at a much lower density. Those variations in species dominance in the study region may be explained by the extreme variations in oceanographic features there, mainly related to sea water temperature, salinity, and water mass changes (Fernandes et al. 2017, Valentin 2001). Additionally, our data represents the first information concerning the Teredinidae family in an area under the direct influence of upwelling.

Wood degradation

Studies of wood degradation due to the activity of Teredinidae species have not been common in Brazil (or anywhere around the world). Some research has been developed in Europe (Eaton et al. 1989, Paalvast & van der Veld 2011, Borges 2014, Sivrikaya et al. 2016) – although under temperate climatic influences and thus under oceanographic conditions different from our study site. The study regions in the present work are under the influence of a tropical regime with an average SST of approximately 20.6°C, with a maximum of 29.5°C at AC and mean of 25.8°C, and a maximum of 31.0°C at Ilha Grande. Upwelling was observed several times at AC, decreasing water temperatures to 12.0°C.

Borges (2014) studied 15 sites along the coast of Europe and recorded the presence of several species of Teredinidae and Limnoriidae, with the indices of wood destruction and species composition being influenced by site location. In northern Europe, from Sweden to the Netherlands, *Teredo navalis* (Linnaeus 1758) was predominant; from the British Channel to the Mediterranean Sea, *L. pedicellatus* was the dominant species. After one year, the destruction index in both regions reached the maximum value of 4 on the EN 275 (1992) ranking. Eaton et al. (1989) found differences in the species composition in Sweden, and lower destruction rates than reported by Borges et al. (2014). *Pinus sylvestris* panels were totally destroyed after only 36 months in Eaton's experiments, indicating that interannual variations related to differences in oceanographic characteristics are important to both species composition and wood destruction rates. Sivrikaya et al. (2016) performed chemical analyses of polysaccharides

(such as cellulose and holocellulose) before and after the exposure of wood panels in the Black Sea region, and observed a reduction in the proportion of black pine cellulose from 56% to 50%, and of holocellulose from 76% to 71% in heavily attacked test panels after 14-months of exposure.

In comparing our results with those of Borges et al (2014) and Eaton et al . (1989) in Europe, the rate of wood degradation was seen to be much higher in our study (even at Ilha Grande, which showed an average degradation of just 12%), even with only 3 months of collector immersion (unlike the 12 to 36 months observed in the above mentioned studies).

Several factors can influence the rates of wood degradation by teredinids, including wood availability and environmental variables such as temperature and salinity. In addition to those factors, the type of wood, the time of exposure, and the colonizing species also influence wood degradation rates (Turner 1966, Borges et al. 2014, Pati et al. 2014).

The upwelling phenomenon at AC appeared to have contributed to a greater abundance of teredinids in the collectors and greater wood destruction as compared to Ilha Grande and Europe. Although the classical influence of upwelling is to promote increases in primary productivity, as teredinids obtain their energy mainly from wood, a direct connection with greater destruction is not obvious. Fernandes et al. (2017) recently presented a new possibility for the effect of upwelling on region that views benthic invertebrates as being favoured due to high concentrations of larvae and phytoplankton close to the surface. Temperature variations such as those recorded at AC (alternating between cold and warm waters due to the shifts in upwelling strength) may also explain the high abundance of teredinids in those collectors and the dominance of the oviparous species

B. gouldi. Some marine invertebrates in the upwelling region of Cabo Frio have reproductive peaks during this season (e.g., barnacles, Skinner & Coutinho 2002, and echinoderms, Junqueira et al. 1997 and Ventura et al. 1997), and thermal shocks or high phytoplankton availability could influence gamete or larvae releases. Also, SST oscillations could affect the metabolic activities of some species, influencing their growth and behaviour (Skinner et al. 2007).

The dominance of teredinid species and the apparent absence of *Limnoria tripunctata* (Menzies 1951) at the survey sites is probably related to biogeographical patterns along the south-eastern Brazilian coast, as there are very few records of large abundances of limnoriids (Barreto et al. 2000 reported from 473 up to 1643 individuals per collector; M.J. Martins-Silva (unpublished data) encountered from 0 up to 83 individuals per collector). According to Lum (1981), *L. tripunctata* could survive in a wide range of temperature (2 up to 35°C) and salinity (19 to 50 ppt) under laboratory conditions, but reproduction only occurs between 15 - 30°C and 20 - 40 ppt salinity. The dominant species (*L. floridanus* and *B. gouldi*) are regularly described as the main species along the southwest Brazilian coast in the outer regions of estuaries or in coastal waters (Maldonado & Skinner 2016, Moraes et al. 2015, Junqueira et al. 1989). A similar pattern has been recorded, for example, in Europe (Roch 1932, Beckman & Menzies 1960, Eltringham 1961, Kristensen 1969, Eckelbarger & Reish 1972, Borges 2014, Borges et al. 2014).

The type of collector wood used may also help explain the higher degradation rates observed in our work. The artificial collectors used in our study were composed of *Pinus elliottii*, a soft, low-cost wood that is easily attacked by teredinids (Silva et al. 1988). Paalvast & van der Veld (2011) in their four-year study (2004-2008) in the region near the port of

Rotterdam in the Netherlands, use oak and fir panels and observed degradation values lower than those found in the present study and differences in the rates of degradation between the two types of wood. Those authors reported that the amount of oak wood consumed by each teredo was less than half that of the fir panels. Those results indicate that the harder the wood, the more energy the organisms must spend to perforate it.

Cellulolytic activity

Several methodologies have been published that can be used to evaluate the ability of microorganisms to produce extracellular enzymes in solid media. According to some authors, among the variables that will influence the choice of the most appropriate method of microbial screening is the direct relationship between the size of the halo and the degradative capacity of the microorganisms (Stamford et al. 1998, Molina et al. 2001). Lealem & Gashe (1994) suggested an enzymatic index ≥ 2.0 as the baseline for considering a microorganism as a potential producer of enzymes in solid medium.

67.2% of the strains isolated in the present study showed some cellulose degradation activity, with 10.3% displaying enzymatic indices ≥ 2.0 – and therefore considered potential strains for producing enzymes in solid medium and confirming the great biotechnological potential of the bacteria present in the Teredinidae as secretors of enzymes with potential industrial applicability.

Nogueira & Cavalcanti (1996) analyzed cellulase production in 48 strains of fungi isolated from industrialized oats, and observed that some of the colonies showing low growth had the highest enzymatic indexes. A similar result was reported by Ruegger & Tauk-Tornisielo (2004) using fungal strains isolated from the soil at the Juréia-Itatins Ecological Station, in São Paulo State, Brazil (such as *Penicillium Herquei* and *Trichoderma hematum*, which had colony diameters of 2.0 cm and enzyme indices of 6.0). Carmo et al. (2014) analyzed the microbial biodiversity in bromeliad tank water, and Araujo et al. (2017) analyzed the bacterial community of sponges on the coast of Rio de Janeiro, and both groups observed that some of the strains showing little growth in the cellulolytic tests showed the highest enzymatic indices. That same situation was observed in the present work, with the strains showing the highest enzymatic indices (3 and 3.33) having the slowest growth rates (2.0 cm).

All strains tested at different temperatures and salinities showed enzymatic indices ≥ 2.0 at 33°C and at salinity levels of 17 ppt, corroborating the work of Greene & Freer (1986), which determined those conditions as optimal for the growth of *T. turnerae*.

The largest number of strains with the highest enzymatic indices at all temperatures and different salinities were isolated from *B. gouldi*, indicating that it is probably more effective than *L. floridanus* at degrading wood, and suggesting it to be more efficient in nutrient cycling and therefore of greater ecological importance. *Bankia gouldi* is known to grow faster than other teredinid species (Nair & Saraswathy 1971, Mann & Gallager 1985) and those physiological responses could help explain that phenomenon. The higher growth rates and higher EI values seen with *B. gouldi* indicates that it may have a high biotechnological potential, especially in light of the fact that the bacteria isolated from it efficiently degraded cellulose at a wide variety of temperatures and salinities.

The highest enzyme index (3.5) found in our work was seen with a bacterial strain isolated from *L. floridanus* growing at AC (in the test at 25°C and 170 ppt of salt). That enzyme index was lower, however, than the maximum enzymatic

index obtained with fungi in other studies (e.g., Nogueira & Cavalcanti [1996] and Ruegger & Tauk-Tornisielo [2004]), which showed enzyme indices of 6.0 after 4 days of growth. Bacteria isolated by Carmo et al. (2014) from bromeliad tank waters also showed enzyme indices of 6.5 after 2 days of growth. Nonetheless, our results are very relevant as the cellulose degrading bacteria demonstrated excellent enzymatic indices even at very high salinity levels. It is also important to note that the EI values were obtained after 48 hours of growth. As the symbiont bacteria occur in the gills, the presence of high salinities in those structures is suggested.

Little is known about the mechanisms by which bacteria degrade cellulose as compared to fungal cellulases. Considering the importance of cellulases in the most diverse branches of biotechnology, it will be important to develop research focusing on bacterial cellulases, even though they currently show enzymatic activities lower than those observed in fungi – for bacterial enzymes may be better adapted to fermentation conditions and may exhibit hypercellulolytic mutation rates greater than those seen with fungi – allowing the easier purification of cellulases for large-scale use (Lima et al. 2005).

Differences were observed in the rates of wood degradation at the different study sites, revealing a greater breakdown of the wood at AC (possibly due to the effects of upwelling). The associated bacteria found in teredinids can represent potential producers of enzyme in solid medium with great biotechnological potentials in industrial applications and the production of biofuels. The greater degradation of wood by *Bankia gouldi* as compared to *Lyrodus floridanus* at AC suggests its greater ecological importance and its greater efficiency in recycling nutrients – indicating it as a reservoir for bio-prospecting bacteria with high biotechnological potential.

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Each author presented relevant contribution to elaboration of the present manuscript as follows: Maldonado GC idealized and developed this study, collected and identified the species, processed the data, performed the analysis, interpreting the results and worked on the manuscript. Silva MM conceived of the presented idea, contributed to sample preparation and microbiological analysis, interpreting the results. Skinner LF collected and identified the species, interpreting the results, supervised the research. Araujo FV contributed to sample preparation and microbiological analysis interpreting the results, supervised the research. All authors discussed the results and contributed to the final manuscript and revisions.