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ORIGINAL PAPER

Endoglucanase activity in *Neoteredo reynei* (Bivalvia, Teredinidae) digestive organs and its content

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Abstract
Cellulolytic enzymes have been studied in several organisms, such as insects, molluscs and other organisms, which can have enzymes endogenously produced or by symbiotic microorganisms. These enzymes are responsible for breaking down the cellulosic material upon which these organisms feed, probably with the aim of assimilating the sugars and nutrients. As *Teredinidae bivalves* grown in mangrove trees, this study aimed to measure endo-β-1,4-glucanase activity in different organs and its content. Endo-β-1,4-glucanase activity was detected in different organs of the *Teredinidae bivalves*, including gills and digestive organs tissues and its content. Moreover, organisms such as teredinids grow up inside wood and this process could perhaps be related to creating growth space. All the endoglucanase extracts, from organs tissues and contents, showed maximum activity at 40 °C. The maximum activity was observed at pH 5.5 for all the extracts, except for intestine tissue, which maximum was at pH 6. Moreover, some of the extracts showed a different profile of the activity as a pH influence, suggesting different distribution of enzymes over the digestive system of the teredinids. The results suggested that the endo-β-1,4-glucanase from Teredinidae could be applied in process that requires low temperature, such as, simultaneous saccharification and fermentation, since it presents lower optimum temperature in comparison to enzymes from terrestrial microorganisms.

Keywords Teredinidae · Cellulases · Wood digestion · Endo-β-1,4-glucanase · Optimum activity

Introduction

The Teredinidae family is a specialized bivalve, which bore and digest the wood to live inside trunks. Most of them are marine tropical molluscs found around the world, mainly in shallow environments. The digestion of wood in the shipworms, as they are commonly known, begins when the shell scrapes the wood, generating small particles that go into the mouth, pass through the esophagus and reach the stomach. In some teredinid species, there is only one part of the stomach, which is circular (Lopes et al. 2000). In others, the stomach is divided into three parts, one circular and other two elongated (Lopes and Narchi 1998). In the stomach, wood particles receive the enzymes from the digestive glands, whose ducts deflate into these organs. From the stomach, the material (wood particles) goes to the appendix, and it can stay there for a long time. From the appendix, it returns to the stomach and is conducted to the intestine. The material passes throughout the intestine length and flows into the anal canal. In some species, such as the *Neoteredo reynei*, the anal canal is closed by a valve and the wood particles remain in this organ for a long time while they go through the whole length of the organ. Finally, the material is ejected as feces, through an exhalant siphon, into the environment. A detailed illustrated anatomy of the *N. reynei* has been published elsewhere (Lopes et al. 2000). The material would be digested during its journey across the digestive system, however the digestion of wood in the appendix and anal canal was not found in some organisms, such as *N. reynei* (Lopes et al. 2000).

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Differently of termites, shipworms have few microorganisms in their digestive systems. Despite being in the gills, there are registers of some populations of a cellulolytic and nitrogen-fixing bacteria found in several species of shipworms (Betcher et al. 2012). It is the *Teredinibacter turnerae*, a kind of gamma proteobacterium that makes it possible for these molluscs to use nitrogen compounds that do not exist in wood and also to digest wood particles through the secretion of enzymes that break the cellulose chains (Waterbury et al. 1983; Iman et al. 1993; Distel et al. 2002; Distel 2003; Xu and Distel 2004; Yang et al. 2009; O'Connor et al. 2014).

Some studies indicated that molluscs can produce enzymes for wood decay. However, other studies point to a symbiotic relation with cellulolytic microorganisms in some spcies, such as *Mytilus, Corbicula*, and *Haliotis* (Xu et al. 2001; Suzuki et al. 2003). Some cellulase and protease enzymes produced by these endosymbiotic bacteria were identified and characterized (Greene et al. 1988; Iman et al. 1993; Xu and Distel 2004), but there is no previous register of cellulase enzymes produced endogenously or by other symbionts in the digestive system of teredinids.

Cellulase enzymes are a group of at least three enzymes: endo-β-1,4-glucanase (EC 3.2.1.4), exo-β-1,4-glucanase (EC 3.2.1.91), and β-glucosidases (EC 3.2.1.21). The endo-β-1,4-glucanase randomly attacks the internal and amorphous chain, the glycosidic bonds of cellulose, releasing oligomers, which are substrates for exo-β-1,4-glucanase to act on the ends of the chain and to release cellobiose; the β-glycosidases act specifically on the cellobiose (glucose disaccharide), producing glucose (Lynd et al. 2002). Cellulases have been increasingly receiving research attention and interest because of their potential in converting the biomass into bioethanol and also in processes that use fermentable sugars. There is a growing interest in finding new enzymes sources with improved catalytic activity. Furthermore, information about endoglucanase activities could bring useful insights when it comes to the Teredinidae as a biotechnological system of cellulosic material degradation. Moreover, enzymes with specific characteristics that can be find in different environmental could benefit biotechnology process such as biomass conversion. Therefore, the main objective of this work was to investigate the endo-β-1,4-glucanase profile over the digestive system of the *N. reynei*, a common shipworm found in the mangroves of Brazil.

Materials and methods

Collection of the *N. reynei* specimens

The animals were collected in the mangrove of Barra de Guaratiba, Rio de Janeiro city, State of Rio de Janeiro, in November, 2012 (coordinates 22°59′S, 43°36′W). The logs/trunks were taken to the laboratory at the National Institute of Metrology, Quality and Technology (Inmetro), and the specimens were carefully removed from the wood. *Neoteredo reynei* specimens were rinsed with sterile seawater before the dissection step.

Dissection and preparation of extracts

Three *N. reynei* specimens were dissected under a stereoscope microscope (Labomed Luxeo 4D) with sterile seawater, and the organs and their contents were separated. It was established that organ is the entire tissue (content free); and content is the liquid and particles inside of the organ. Considering this the organs where the wood particles could be digested, through enzymatic action, it has been chosen to perform the study with raw extracts of: appendix content; appendix tissue; anal canal content; anal canal tissue; intestine content and tissue; digestive diverticula; gills; stomach content; and stomach tissue. All the samples (organs or content) were collected in a 10 mL tube and the volume was completed to 4 mL with sodium citrate buffer pH 5.4, with exception of the anal canal content that volume was completed to 20 mL with buffer due to higher content compared to other organs content. The organs tissue was smashed with a rod until complete defragmentation. The samples were centrifuged (Beckman Coulter™ Microfuge®22R) for solids separation at 10,000×*g* for 5 min, volume corrected to 4 mL with the same buffer, and stocked at − 20 °C for further studies.

Endo-β-1,4-glucanase assays

The activity of endo-1,4-β-D-glucanase (endoglucanase) was determined by reducing the sugar content released by carboxymethylcellulose (CMC) solutions incubated with enzyme preparations at 50 °C, pH 5.4 (50 mM citrate-phosphate buffer). The reducing sugars were measured by the dinitrosalicylic acid (DNS), method standardized with glucose (Miller 1959). For this assay, 1 IU of endoglucanase activity is defined as the amount of enzyme catalyzing the release of 1 µmol/min of glucose. The activity was shone also based on the amount of total protein in solution as IU/mg of protein. The assay was performed with 20 µL of raw extracts and 180 µL of 0.44% (4.4 g/L) CMC solution in microtubes and incubated at 50 °C for 60 min, using a ThermoShaker (Benchmark Scientific, Model H 5000-H). The reaction was interrupted by the addition of 460 µL of DNS. The tubes were incubated at 100 °C for 5 min and, after cooling, analyzed in a spectrophotometer (Bioteck Synergy 2) at 540 nm. The absorbance was converted to glucose concentration by using an analytical curve (1–5 µmol/mL). The total protein amount was determined by using the Coomassie

blue method described by Bradford, using bovine serum albumin as a standard (Bradford 1976).

Temperature and pH influence on the activity of endo-β-1,4-glucanase

Endoglucanase activity was determined by using an extract of anal canal content to verify the effect of the temperature and pH. Anal canal content was used due the higher volume of enzymatic extract. The previously described endoglucanase assay was performed at temperatures of 20, 30, 40, 50, and 60 °C, and pH 5.4, in 50 mM citrate-phosphate buffer. The endoglucanase thermostability was determined at 50 °C because of the usual condition of the enzymatic hydrolysis. The residual enzyme activity was measured by incubating the enzymatic extract (1.5 mL in a sealed tube) up to 72 h, in the absence of substrate. Each of the sample at different periods, performed in triplicate, was done in individual tube, and the residual activity of the endoglucanase was assayed again under standard conditions.

The effect of the pH was determined at pH 4–7 in a 50 mM citrate-phosphate buffer, and at pH 7–9 in the sodium-phosphate buffer. The assays were performed at standard conditions.

SDS-gel electrophoresis

The molecular weight of studied proteins was determined by SDS-gel electrophoresis on a 12% polyacrylamide gel. A high molecular weight range was used for calibration Kaleidoscope Prestained (BioRad—161-0324). The gels were stained with Coomassie brilliant blue R-250 (0.2% in 40% methanol and 10% acetic acid). For the preparation of SDS–PAGE, 50 µg of protein was applied to the digestive diverticula, anal canal tissue, appendix tissue and stomach contents. The content of the anal canal was applied at a concentration of 22.5 µg of protein.

Zymogram gel was prepared by the addition of 0.01% of 4-methylumbelliferyl-β-D-glucopyranoside or 4-methylumbelliferyl-β-D-cellobioside to the polyacrylamide gel. The gels were incubated at 37 °C for 30 min, and the zones of clearance were visualized with ultravilolet (Cheng and Chang 2011).

Enzymatic hydrolysis

Enzymatic hydrolysis was performed with 2% carboximethilcellulose (CMC), by incubating 0.1 g of CMC in 5 mL of 0.05 M citrate buffer (pH 5), in 15 mL flasks, at 50 °C, and with constant agitation (in an orbital shaker, at 170 rpm). Reactions mixtures contained 0.15 IU/g CMC (Celluclast 1.5 L, Novozymes), and/or 0.15 IU/g cellobiase (β-glucosidase, Novozym 188—Novozymes), and or 0.15 IU/g anal canal content extract. The reaction was performed with commercial enzyme Celluclast and canal anal content extract separated, and also combined with cellobiase. The enzymatic digestibility of CMC was calculated from the glucose yield (measured by HPLC) after different reaction times (0, 24, 48 and 72 h). Enzymatic hydrolysis assays were performed in experimental triplicates, and averaged results were reported. The conversion yield was calculated based on the glucose and cellobiose released.

Results

Among the organs, the digestive diverticula showed the highest protein concentration: 1.23 mg/mL (Table 1). The digestive diverticula stocks enzymes and unload them into the stomach, probably decreasing the enzyme concentration by mixing them with particles of wood. The protein concentration increased in the appendix and intestine, probably due to microorganisms that grow in these organs. The anal canal, as a long tube, could cause the protein to dilute, decreasing its concentration. The enzyme concentration could not be

Table 1 Endoglucanase activity in the extracts from *N. reynei* organ tissues and contents

Sample	Endoglucanase (IU/mg)	Endoglucanase (IU/mL)	Protein (mg/mL)
Gill	2.75 ± 0.04	0.74 ± 0.01	0.27 ± 0.02
Normal digestive diverticula	0.36 ± 0.01	0.44 ± 0.03	1.23 ± 0.06
Specialized digestive diverticula	1.14 ± 0.15	0.47 ± 0.06	0.41 ± 0.02
Stomach tissue	1.65 ± 0.10	0.51 ± 0.03	0.31 ± 0.03
Stomach content	1.15 ± 0.01	0.49 ± 0.03	0.43 ± 0.04
Appendix tissue	1.26 ± 0.05	0.50 ± 0.02	0.40 ± 0.01
Appendix content	1.28 ± 0.22	0.38 ± 0.06	0.30 ± 0.02
Intestine	3.65 ± 0.15	0.58 ± 0.02	0.16 ± 0.05
Anal canal tissue	0.33 ± 0.01	0.07 ± 0.01	0.21 ± 0.03
Anal canal content	0.02 ± 0.01	0.01 ± 0.01	0.48 ± 0.05

distinguished from the total protein amount that was determined. However, it is expected to find a higher concentration of enzymes than of other proteins, considering the role they play in the material degradation. Interestingly, the gill, which is not an organ related to digestion, showed the highest endoglucanase activity, probably because its enzymes can be translocated to gut (O'Connor et al. 2014).

Enzymatic activities in the Teredinidae digestive system

To understand the enzyme presence, several of the *N. reynei* digestive organs were separated into content and tissue and the enzymatic activity of endoglucanase was determined. The endoglucanase activity was found in tissues of several organs, such as stomach tissue, appendix tissue and anal canal tissue (Table 1). The highest endoglucanase activity was found in the gill tissue, with 2.75 IU/mg. Among the digestive organs, the intestine showed higher endoglucanase activity: 3.65 IU/mg, and also per volume, showing the activity of 0.58 IU/mL (Table 1).

The lower tissue and organ content activity found in the anal canal were 0.33 and 0.02 IU/mg, respectively. The enzymatic activity followed an increasing path until the intestine, whereas it decreased in the anal canal. The specific activity of endoglucanase was higher for the intestine (3.65 IU/mg), gill (2.75 IU/mg), stomach tissue (1.65 IU/mg), appendix tissue (1.26 IU/mg) and also appendix content (1.28 IU/mg) (Table 1).

Temperature and pH effect on the endo-β-1,4-glucanase activity

The effects of the temperature on the endoglucanase activity of raw extracts from different digestive organs of Teredinidae were measured. All the enzymatic extracts of the organs have shown a similar optimum temperature: 40 °C (Fig. 1). The endoglucanase activity has been influenced by the temperature. Enzymatic extracts from the intestine content were more sensible to temperature, showing lower activities for assays at 30 and 60 °C (lower than 30% of total activity) (Fig. 1). The test at 50 °C showed 70% percent of the total activity. Enzymatic extracts from anal canal content, digestive diverticula, and stomach tissue showed a similar profile of endoglucanase activity according the effects of the temperature. With slight differences, appendix content showed more affected endoglucanase activity if compared to the described group.

In spite of the optimum temperature being around 40 °C, the thermal stability was determined at 50 °C due to be compared to fungi endoglucanase. Also, this is the most used temperature for studies that aim to determine the enzymatic activity and the enzymatic hydrolysis (saccharification) (Shimizu et al. 2018; Wallace et al. 2016; Brienzo et al. 2008). The enzymatic activity of the endoglucanase continuously decreased for 20 h, thereafter it stabilized at around 20% of the total activity (Fig. 2). The half-life of the endoglucanase at 50 °C were determined as 11 h.

The effects of the pH on the endoglucanase activities of raw extracts from different digestive organs of Teredinidae were measured. The optimum pH was between 5.5 and 6 for endoglucanase in extracts from anal canal tissue, digestive diverticula, stomach content, and canal anal content. Differently, the endoglucanase from intestine content showed

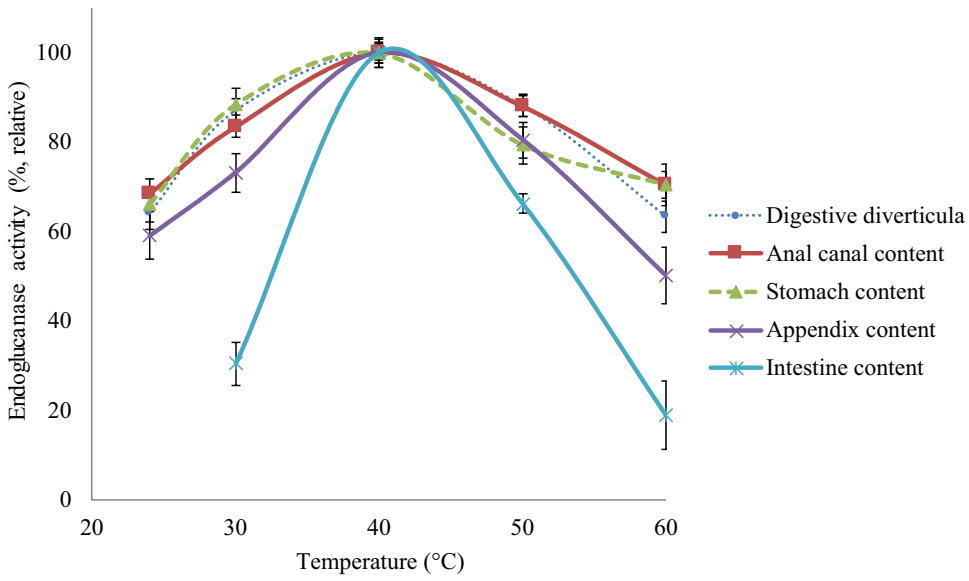

Fig. 1 Temperature effect on the endoglucanase activity in extracts from *N. reynei* organs

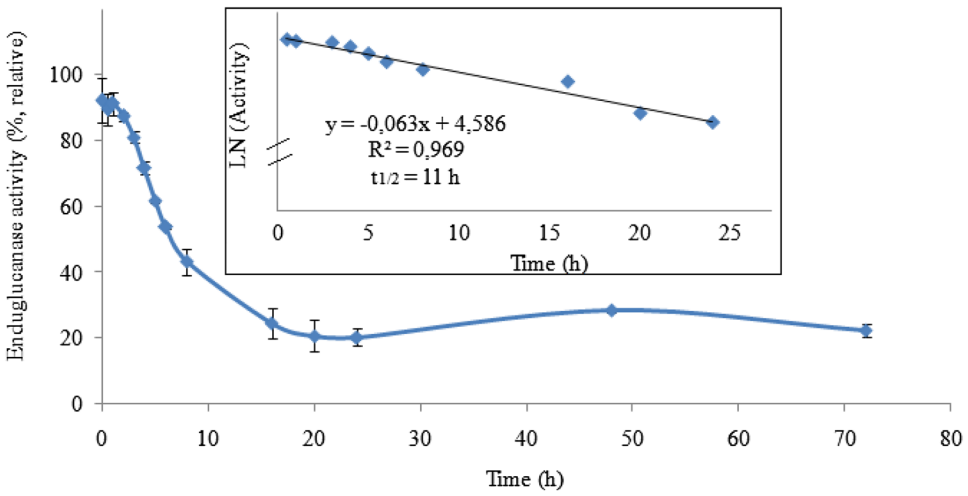

Fig. 2 Thermal stability of endoglucanase activity in extracts from *N. reynei* canal anal content

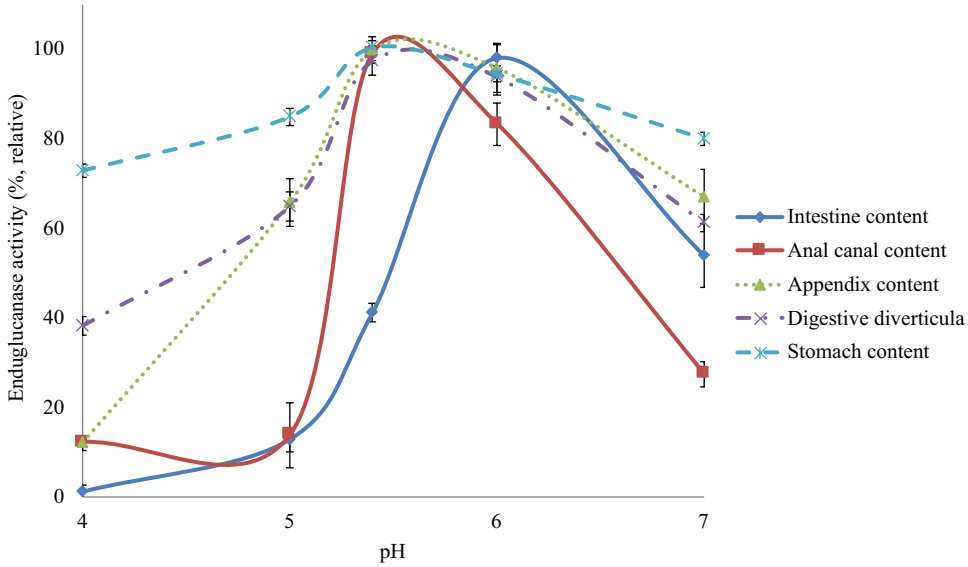

Fig. 3 Effect of pH on the endoglucanase activity in extracts from *N. reynei* organs

optimum pH at 6 (Fig. 3). It was possible to observe that all the endoglucanase activities were more stable at alkaline than at acidic pH. Extracts from anal canal content and stomach content showed an optimum region between 6.5 and 6, with a slow decrease in the activity for pH higher than 6.

Electrophoresis study of enzymes

The crude enzymatic extracts were analyzed by electrophoretic techniques. SDS–PAGE analysis revealed a similar number of proteins with a similar molecular weight in all samples (Fig. 4). The result indicates that there is a more intense band, of molecular weight of approximately 17.4 kDa in all samples. Other lower intense bands of molecular weights ranging from 32.1 to 48.2 kDa can also be seen in all the samples. However, it is more intense in the appendix tissue and less in the anal canal content. Probably,

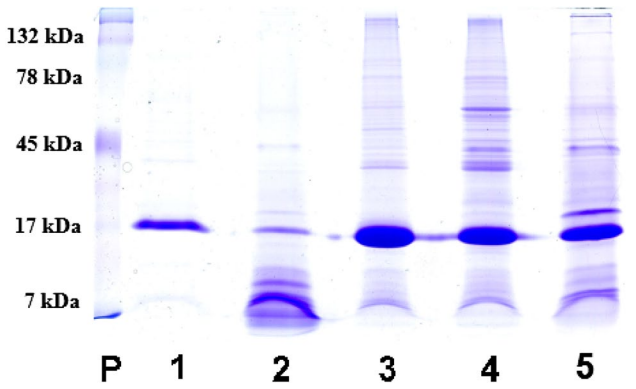

Fig. 4 SDS-gel electrophoresis of the crude enzymatic extract in 12% polyacrylamide gel extracts. Sample applied contained 50 µg of protein, except for sample 1, where 22.5 µg of protein was applied. Sample: 1—anal canal content; 2—digestive diverticula; 3—anal canal tissue; 4—appendix tissue; 5—stomach content

the contents of the anal canal presented the same pattern of protein distribution, as it is with lower concentration of protein compared to the other samples.

The zymogram of enzymatic activity of β-glycosidase indicated the presence of an enzyme with molecular weight of 129.3 kDa (Fig. 5a). The extract corresponding to the anal canal content was concentrated, and all samples had an equal amount of protein applied into the gel. Only the samples corresponding to the digestive diverticula and to the anal canal tissue had lower intense bands in relation to the other samples.

The enzyme activity zymogram of exoglucanase indicated the presence of an enzyme with a molecular weight of 20 kDa (Fig. 5b). All the organs analyzed presented the band corresponding to this enzyme, however only one band was detected in each crude extract. The bands corresponding to the contents and tissue of the anal canal proved to be lower intense when compared to the others, indicating a lower activity of this enzyme in this organ.

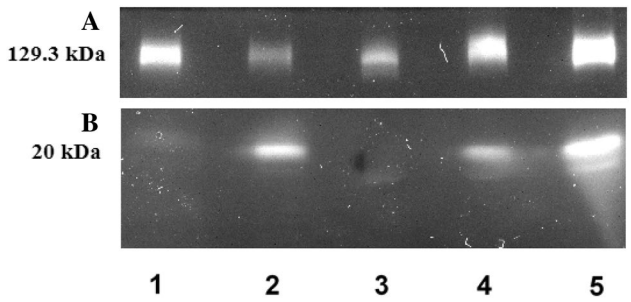

Fig. 5 Zymogram of crude enzymatic extract for β-glucosidase (**a**) and exoglucanase (**b**) activity in 8% polyacrylamide gel. Assay with 40 µg of protein per sample. Sample: 1—anal canal content; 2—digestive diverticula; 3—anal canal tissue; 4—appendix tissue; 5—stomach content

Enzymatic hydrolysis

The anal canal content was applied in the enzymatic hydrolysis of CMC comparatively to commercial enzymatic extracts. The enzymatic hydrolysis was performed with a soluble cellulose derivative, using low amount of enzyme activity (0.15 IU/g, for all the enzymatic extracts). The conversion of CMC into cellobiose was similar to anal canal content extract and commercial enzyme (Celluclast) (Fig. 6). The glucose release was three times higher to the commercial extract compared to the anal canal content extract. The enzymatic hydrolysis was performed also adding commercial β-glucosidase extract (Novozym—Novozymes). The glucose release adding β-glucosidase provided different results comparing commercial and anal canal content extract. The anal canal content supplemented with β-glucosidase resulted in higher glucose yield compared to commercial extract supplemented.

Discussion

The protein concentration increased in the appendix and intestine, probably due to microorganisms that grow in these organs. The anal canal, as a long tube, could cause the protein to dilute, decreasing its concentration. The enzyme concentration could not be distinguished from the total protein amount that was determined. However, it is expected to find a higher concentration of enzymes than of other proteins, considering the role they play in the material degradation. Teredinids feeding is based on wood particles, but also on organic material in suspension in the surrounding water (Turner 1996). Paalvast and Velde (2013) suggested, specifically for *Teredo navalis*, that the main source of carbon, instead of wood, as it would be expected, comes from filter

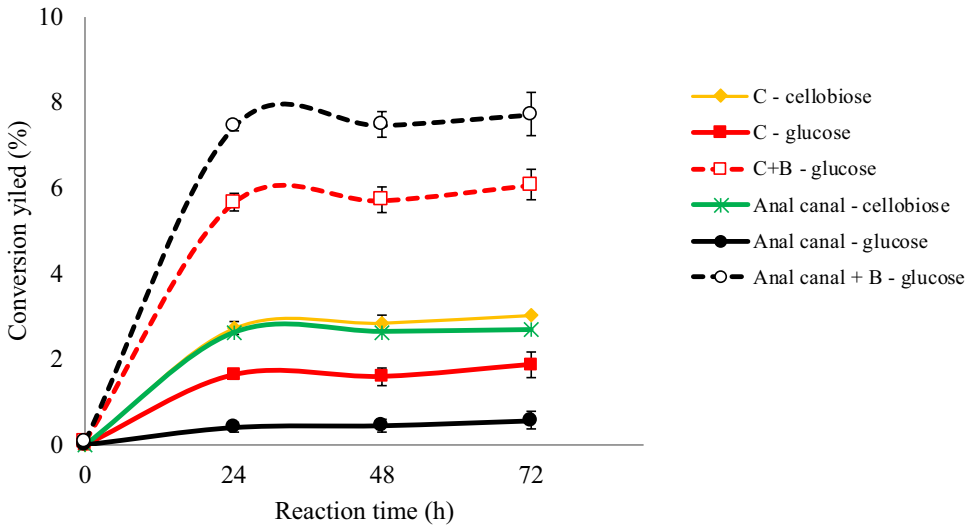

Fig. 6 Enzymatic hydrolysis using anal canal content extract from *Neoteredo reynei* and commercial enzymes (Cellulcast, supplemented with Novozym—Novozymes) 0.15 IU/g os substrate

feeding via siphons. However, the symbiotic system provides enzymatic tools of wood degradation (Betcher et al. 2012). At least, the presence of enzymes could not be ignored as a contributor to processing wood along the digestive system. To understand the enzyme presence, several of the *N. reynei* digestive organs were separated into content and tissue and the enzymatic activity of endoglucanase was determined.

The endoglucanase activities found in stomach tissue, appendix tissue and anal canal tissue (Table 1) suggest that the wood degradation could be more relevant than just being processed in order to secrete as feces. The wood degradation is enhanced with symbiotic association with microorganisms or groups of microorganisms. In fact, endoglucanase activity was found in all of the organs studied, including the gills (Table 1). Moreover, there is a study suggesting that enzymes are translocated from the gills to the stomach (O'Connor et al. 2014). Trindade-Silva et al. (2009) reported the presence of eubacteria in the intestine and in the gills of *N. reynei*, but there is no clear understanding of the host's biology.

The specific endoglucanase activity was higher in the tissue than in the content for the stomach and anal canal. The activity was similar for the appendix tissue and content (Table 1). Among the tissue and organ content the intestine was higher endoglucanase activity. The intestine higher endoglucanase activity is interesting since some authors have suggested that the appendix is the wood digestion site (Cragg et al. 2015; O'Connor et al. 2014; Elshahawi et al. 2013). In *N. reynei*, the wood particles stayed for a long time in the appendix (Lopes et al. 2000), allowing the enzymes to act, and the products of digestion to be absorbed. This was suggested by the microvilli epithelium found in the appendix (Lopes et al. 2000). Otherwise, a poor bacteria community has been reported in the appendix, but a very well-developed bacteria community was found in the intestine of five species of shipworms (Betcher et al. 2012). The endoglucanase activities found can suggest that it is possible to run a biochemical breakdown of wood cellulose and of the organic matter. Extracts from the bivalve *Corbicula japonica* have shown an endoglucanase activity of 0.80 IU/mg. Species that degrade organic matter from marine origin, such as *Ruditapes philippinarum* and *Mactra veneriformis*, have shown an endoglucanase activity of 0.30 IU/mg (Sakamoto and Toyohara 2009). Digestive tracts tissue homogenates of *Bankia gouldi*, a shipworm, have shown an endoglucanase activity of 0.023 IU/mg, with an enzymatic hydrolysis rate that is comparable to the ones found in *Trichoderma viride* and *Aspergillus niger* (Dean 1978).

The relative lower optimum temperature of 40 °C (Fig. 1), when compared to fungi endoglucanases, is probably related to the environmental characteristics of the teredinids. The molluscs were collected on the mangrove, where the common water temperature probably is not higher than 25 °C. The mangrove water temperature depends on several factors, and a range of 17–25 °C has been reported for a mangrove in some countries (Moraes et al. 2015). The difference on the temperature effects on the endoglucanase activity may suggest that this enzyme could be from a different microorganism, or from a different symbiotic system. The *Teredinibacter turnerae* isolated from *Lyrodus pedicellatus* gills was cultivated and its endoglucanase was characterized. The optimum temperature for this enzyme was 50 °C, with a strong decrease in the residual activity at temperatures lower than 40 °C and higher than 60 °C (Xu and Distel 2004). The half-life of the endoglucanase at 50 °C were 11 h what is a range common for microorganisms classified as mesophilic; however, a higher stability could be observed for enzymes from thermostable microorganisms (Wallace et al. 2016; Heidorne et al. 2006).

Endoglucanase from intestine content has shown optimum pH at 6, differently that found in extracts from anal canal content and tissue, digestive diverticula, and stomach content that showed 5.5. The endoglucanase from *Teredinibacter turnerae* isolated from *Lyrodus pedicellatus* gills has shown optimum pH between 6 and 7, with a higher sensibility for extreme pH (Xu and Distel 2004). The extracts from digestive diverticula and stomach content have shown higher endoglucanase activity at acidic pH than the other extracts. The different optimum pH between digestive diverticula and stomach and the other organs content showed that the endoglucanase is different, and possibly, there are from different microorganisms or groups of microorganisms populating the organs (Cragg et al. 2015; Betcher et al. 2012). The rumen degradation of cellulosic material is associated to microorganisms that are related to protozoa, fungi and bacteria. Among the several microorganisms are common *Ruminococcus flavefaciens, Prevotella bryantii* and *Pseudobutyrivibrio xylanivorans*. These microorganisms are specialized for exemplo on cellulosomes production, xylanases and cellulases (Zorec et al. 2014). Termites are adapted to cellulose degradation due own mechanical and enzymatic tools, associated with the gut symbiotic (Brune 2014). Among the microorganisms in symbiosis there are different pattern related to high and low termites (Peterson and Scharf 2016). A multi microorganisms community probably benefit with the diversity of the enzyme production.

A better activity at alkaline pH could be positive for enzyme application if the process is carried in a condition close to neutral. Also, the optimum temperature at 40 °C could be interesting for the enzymatic hydrolysis of lignocellulosic biomass that happens simultaneously to the fermentation process (SSF) (Shane et al. 2017; Berbowska et al. 2016). Performing the enzymatic hydrolysis and fermentation in a combined step is pointed out as an advantage for the process of producing bioethanol: it increases the hydrolysis rate due to the conversion of glucose, which

inhibits the enzyme activity; it lowers the enzyme loading and the requirements for sterile conditions because glucose is removed for fermentation (Balat et al. 2008). In order to complete the feasibility of the process, it is ideal that the enzymes act in conditions that are similar to the ones found in the fermentation microorganism. The endoglucanase activity at alkaline pH and optimum at a temperature of 40 °C (Figs. 2, 3) converges into the required conditions for fermentation.

The SDS-gel electrophoresis of the enzymatic extracts showed several proteins with different molecular weights (Fig. 5). These proteins appeared in a large number but with wide molecular weight distribution. The most intense band appeared with 17.4 kDa. The zymogram was applied to identify activities of other enzymes of the cellulolytic complex. Activities of the β-glucosidase and exoglucanase were observed with molecular weight of 129.3 and 20 kDa, respectively. Considering that the wood is degraded over the digestive system of the mollusc, activities of the β-glucosidase and exoglucanase were expected to be found.

Beside of the presence of the cellulolytic complex, the enzymatic hydrolysis using anal canal content extract resulted in high amount of cellobiose in the medium (Fig. 6). Probably, the enzymatic activity of the β-glucosidase was low and did not collaborate to a fast hydrolysis into glucose. In fact, the supplementation with commercial β-glucosidase resulted in improvement of the glucose release, with no cellobiose accumulation. The commercial enzyme showed better result to glucose release compared to anal canal content extract. However, the supplementation with β-glucosidase improve the anal canal content extract, reaching higher glucose yield compared to the commercial extract.

Conclusion

This study has shown endoglucanase activities in different organ tissues and in their contents. The presence of cellulase enzymes suggests the importance of processing the wood along with the Teredinidae digestive system. The enzymes present in all the organs tissues and contents points to an important role for the teredinids development collaborating to cellulosic material degrading. The enzyme function cold be related to being part of the digestive system, and also to decaying the wood, allowing the teredinids to grow inside the wood trunk and branches. The extracts from stomach contents and digestive diverticula showed more stable activity at a range of acid and alkaline pH. An important characteristic of the Teredinae enzymes is the optimum temperature at 40 °C, which is interesting for some industrial application of enzymes. Enzymatic hydrolysis showed similar results compared to commercial extracts, evidencing the potential application in cellulosic material sugars release.

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