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Title

Animal Cellulases with a Focus on Aquatic Invertebrates

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Abstract

Cellulose is utilized as a nutritional source by various organisms. It had been long believed that only protozoa, bacteria and fungi, in addition to plants and photo-synthetic bacteria, are able to synthesize cellulases encoded by their own genes. However, the wide spread distribution of cellulases throughout the animal kingdom has been recently recognized. Conventionally, animals digest cellulose by utilizing cellulases derived from symbiotic bacteria in the digestive organs. However, recent molecular biological studies have shown that some cellulase genes are actually encoded on animal chromosomes. In addition, the homologous primary structure of cellulases obtained from various phyla of invertebrates indicates the possible vertical transfer of the cellulase gene from ancient organisms that are now extinct. Studies on cellulase with unique enzymatic properties are expected to be applied to bioethanol production and aquaculture. In the present review, we describe cellulases, with a primary focus on aquatic invertebrates in which both endogenous and exogenous cellulases are involved in the breakdown of cellulose in the digestive organs.

Keywords

Breakdown · Cellulase · Cellulose · Endo-β-1,4-glucanase · Endogenous · GHF9 · Invertebrate · Symbiosis

Introduction

Cellulose synthesized by plants and phototrophic bacteria is the most abundant organic substance on the earth. Cellulose is chemically stable, and thereby plays an important role as a major component in the cell wall of plants and bacteria by providing physical strength [1]. This physical strength is attributed to the primary structure of cellulose, which consists of monomeric chains of D-glucopyranose bound by β-1,4-glycoside linkages that form cellulose microfibrils interconnected with hydrogen bonds (Fig. 1) [2]. In addition to cellulose, the cell wall contains lignin and various hemicelluloses, including mannan, xylan, and laminarin. The contents of the hemicelluloses differ across plant species [3].

Enzymes that degrade cellulose are collectively called cellulases, and are classified according to a range of characteristics. First, cellulases are classified according to the cleavage site on cellulose; comprising (1) β-1,4-endoglucanase (EC 3.2.1.4.), which cleaves cellulose at random sites, and (2) β-1,4-exocellobiohydrolase (EC 3.2.1.91.), which cleaves off glucose dimers from the terminal end of cellulose. Subsequently, β-glucosidase (EC 3.2.1.21.) cleaves glucose from the breakdown products formed by β-1,4-endoglucanase and β-1,4-exocellobiohydrolase (Fig. 2).

Second, cellulases are classified by the presence of the carbohydrate-binding module (CBM) within their molecules. Some carbohydrate degrading enzymes have a CBM that is independent of the catalytic site, which binds to substrates and stabilizes the enzymatic reaction. For example, cellulases that have a cellulose-binding domain (CBD) on a CBM constantly bind to cellulose, with the enzyme molecules

continuously moving to a subsequent cleavage site after each reaction. In comparison, cellulases that do not have a CBD detach from the cellulose after every cleaving reaction, and search for the cleaving site of the next hydrolytic reaction [2]. Cellulases that have a CBD are assumed to hydrolyze cellulose more efficiently than those that do not have (Fig. 3).

Third, cellulases are classified according to their primary structure. For instance, glycoside hydrolases are classified into the glycoside hydrolase family (GHF) by Henristatt et al., according to the amino acid sequence [4, CazyWeb: http://www.cazy.org/ "Accessed 6 May 2012".]. At present, 130 families are registered, with cellulases being classified into families 1, 3, 5, 6, 7, 8, 9, 10, 12, 19, 26, 30, 44, 45, 48, 51, 61, 74, 116, and 124 (Table 1).

Cellulose is utilized as a nutritional source by various organisms. It has long been believed that only protozoa, bacteria, and fungi, in addition to plants and photo-synthetic bacteria, are able to synthesize cellulases encoded by their own genes [1, 5-7]. Before 1998, it was assumed that metazoans degraded cellulose using cellulases derived from symbiotic protozoa and bacteria in their digestive organs [1]. However, a novel gene encoding cellulase (GHF9; β-1,4-endoglucanses) was identified in *Reticulitermes speratus* (Arthropoda, Insecta) in 1998 [7]. Subsequently, the presence of endogenous β-1,4-endoglucanses, belonging to various GHF families, has been reported in various insects, crustaceans, mollusks, echinoderms, and nematodes [8-16].

The origin of cellulase in metazoans may be explained by two alternative hypotheses [15, 16]. The

first hypothesis is the horizontal transfer of cellulase genes from symbiotic protozoa. The second hypothesis is the vertical transfer of cellulase genes from ancient organisms that are now extinct. In this second hypothesis, cellulase genes have been inherited for a long period of time from the ancestor to the offspring. According to the reports on GHF9 [15, 16], which is the most intensively studied cellulases, vertical transfer is considered more likely. The amino acid sequence of GHF9 is very similar in several organisms, indicating the presence of a shared common cellulase ancestor, from which the GHF9 gene has been inherited for a hundred million years (Fig. 4). Unfortunately, evolutionary evidence of other GHFs is not available, and information about their primary structures remains fragmentary [15].

Table 2 classifies cellulases according to origin (endogenous or exogenous) and habitat (terrestrial or aquatic). Endogenous origin means that the cellulase gene is encoded on the chromosomes of the organisms, whereas an exogenous origin means that the cellulase gene is encoded on the chromosomes of symbiotic microorganisms. As shown in Table 2, enzymes from archaea, eubacteria, fungi, and plants are all classified as endogenous, whereas cellulases of both endogenous and exogenous origin are found in invertebrates. The exogenous origin of cellulases in invertebrates is assumed to compensate for cellulases of endogenous origin. In contrast, cellulases of endogenous origin have yet to be reported in vertebrates.

In the present review, we describe cellulases, with a primary focus on aquatic invertebrates in which both endogenous and exogenous cellulases contribute to the breakdown of cellulose in the digestive organs.

Cellulases derived from symbiotic microorganisms in terrestrial organisms

It had long been doubted as to whether herbivorous terrestrial animals actually digest cellulose and utilize it as a nutritional source. For instance, there have been substantial efforts to validate whether herbivorous mammals utilize cellulose during digestion [1]. Studies on symbiotic microorganisms in the ruminant stomach of herbivorous animals, which are involved in cellulose breakdown, are documented in Table 3. In 1942, Hungate reported the ability of the genera *Diplodinium* and *Entodinium* to breakdown protozoan cellulose, and the possible implication of these microorganisms in the breakdown of cellulose in the lumen of herbivorous mammals [17]. Various microorganisms in the digestive organs of sheep have also been investigated. For example, in 1982, Wood et al. isolated an anaerobic symbiotic microorganism *Ruminococcus albus* from the sheep lumen that was able to degrade cellulose, and successfully purified the cellulase from the extracts [18]. In 1986, Coleman et al. recorded the cellulose-degrading ability of the protozoa *Entodinium caudatum* in the sheep lumen [19]. Subsequently, in 1992, Bernaler et al. reported the cellulose-degrading ability of the anaerobic fungus *Neocallinastix frontalis* in the sheep lumen [20]. In addition to sheep, Varel et al. reported the cellulase degrading ability of the gram-positive bacillus *Bacteroides succinogenes* and the gram-negative coccus *Ruminococcus flavefaciens* in the porcine colon [21].

In addition to herbivorous mammals, there have been extensive studies of symbiotic bacteria

implicated in the breakdown of cellulose in herbivorous insects. The cellulose-degrading ability of symbiotic bacteria in the termite *Reticulitermes flavipes* was first reported in 1924 [22]. Subsequently, in 1932, Trager et al. reported the cellulase activity of flagellates in the digestive organs of the wood roach [23]. Recently, Delalibera et al. found symbiotic cellulose-degrading bacteria and fungi in the digestive organs of the wood borer and bark beetle [24]. The presence of symbiotic microorganisms has been extensively studied in termites. For example, Wenzel et al. successfully isolated symbiotic aerobic and anaerobic bacteria from damp wood termites (*Zootermopsis angusticollis*; Arthropoda, Insecta) in 2002 [25]. In 2006, Watanabe et al. isolated archaebacteria exhibiting cellulase activity from the digestive tract of the giant northern termite (*Mastotermes darwiniensis*; Arthropoda, Insecta) [26]. Interestingly, Martin et al. detected cellulase activity in the midgut extract of the fungus-growing termite, *Macrotermes natalensis* (Arthropoda, Insecta) in 1978 [27]. This termite species cultures cellulose-degrading bacteria in its hive, and obtains cellulase from these bacteria. A similar example was reported for another termite, *Macrotermes mulleri* (Arthropoda, Insecta) [28]. Hence, there are a large number of terrestrial animals that are associated with symbiotic microorganisms, allowing them to effectively utilize cellulose.

Cellulases derived from symbiotic microorganisms in aquatic organisms

The shipworm (Bivalvia, Teredinidae) is a major pest that bores holes in the hulls of wooden ships and other wooden marine structures, occasionally resulting in the sinking of ships. Symbiotic bacteria

were first found in granules of “the Gland of Deshayes” from one shipworm species *Bankia australis* (Mollusca, Bivalvia) in 1973 [29]. In 1983, Waterbury et al. found some species of bacteria that had both cellulose-degrading activity and nitrogen-fixing ability in shipworms, indicating that this species of shipworm utilizes cellulose and fixes nitrogen as carbon and nitrogen sources, respectively [30]. In 1991, the authors also reported the presence of a symbiotic bacteria belonging to Proteobacteria in various species of shipworm [31]. This was the first report demonstrating the widespread distribution of symbiotic bacteria belonging to the same phylum being present in a variety of shipworm species. After 2002, *Teredinibacter turnerae* classified into Proteobacterium, was found to be distributed in 24 species belonging to 9 distinct genera out of the total 14 genera of shipworm [32]. This observation indicates the ubiquitous distribution of specific bacteria among various species of shipworm.

Studies on symbiotic bacteria with cellulase activity have extended to the deep sea ecology of animals inhabiting wood that has fallen to the ocean floor at the sea bottom. In 1997, bacteria morphologically resembling those found in shipworms were discovered in the digestive organs of a bivalve species *Xylophaga washingtona* (Mollusca, Bivalvia), which belongs to the same class as the shipworm [33]. New symbiotic bacteria were also found in the gills of a sunken wood-associated mussel (Mytilidae) in 2008 using the fluorescence in situ hybridization (FISH) technique, which employs specific molecular probes to identify different species of bacteria [34]. In 2010, another species of symbiotic bacteria was found in the gills and digestive organs of *Pectinodonta* sp., belonging to the

Gastropoda [35]. On the basis of these findings, the mechanism of cellulose breakdown in the wood ecological system of the deep sea is gradually being revealed.

In comparison, Cary et al. reported the vertical transfer of symbiotic bacteria from "mother to baby" in related species of *Calyptogena* (cold-seep clams) and *Solemya reidi* [36]. In a later study of *Bankia setacea*, a type of shipworm, Sipe et al. reported the vertical transfer of symbiotic bacteria from "mother to baby" *via* the eggs [37]. Since the cellulose-degrading activity of these bacteria has yet to be demonstrated, further studies on the occurrence of cellulase activities in these bacterial species are required.

The gribble worm is an isopod that bores holes in wooden ships, similar to shipworm. The presence of symbiotic bacteria in the gribble worm has long been disputed [38]; however, in 2010, one gribble worm species, *Limnoria quadripunctata* (Arthropoda, Malacostracea), was confirmed to have endogenous cellulases, the genes of which are encoded on the chromosomes of the species [39].

Endogenous cellulases in terrestrial and aquatic animals

Table 3 summarizes the reported endogenous β-1,4-endoglucanses belonging to GHF9 from terrestrial and aquatic invertebrates. Most species containing endogenous cellulases belong to the Arthropoda and Nematoda. The first species reported to have endogenous cellulase was the termite *Reticulitermes speratus*, which is widely distributed across Japan, and has been studied intensively as a

harmful insect that decomposes wooden houses [7]. In 1998, Watanabe et al. succeeded in cloning the cDNA of β-1,4-endoglucanse from *R. speratus*, and the enzyme was classified as GHF9, according to the deduced amino acid sequence. The authors confirmed its endogenous origin by using PCR and Southern blot analysis of DNA extracted from termites. These procedures revealed the presence of an intron in the gene of β-1,4-endoglucanse. Later, the cDNA of another cellulase was cloned from *Coptotermes formosanus* (Arthropoda, Insecta), a termite species related to *R. speratus*, with it being classified as an endogenous GHF9 cellulase [40]. This cellulase was expressed in the foregut and midgut, with a symbiotic flagellate expressing a GHF7 cellulase in the hindgut [40]. These findings indicate that *C. formosanus* first degrades cellulose in the foregut and midgut *via* endogenous cellulases, and then the degraded products of cellulose are further digested by cellulases from the symbiotic flagellate in the hindgut, facilitating the effective digestion of cellulose. The presence of endogenous cellulases was subsequently reported for herbivorous arthropods, including the cockroach, which inhabits forests [41], and the well-known flour beetle *Tribolium castaneum* (Arthropoda, Insecta), which has a worldwide distribution [42].

In addition to arthropods, cellulases from Nematoda have been studied intensively. In 1998, the cDNA of cellulase was cloned from the plant-pathogenic cyst nematodes *Globodera rostochiensis* and *Heterodera glycines* (Nematoda, Tylenchida), and was classified as an endogenous GHF5 family enzyme [11]. In addition to cellulase, *Heterodera glycines* has endogenous genes encoding chitinase [43].

Cellulase genes were assumed to be horizontally transferred from bacteria or fungi to the ancestors of these two nematodes [44]. These nematodes infest the roots of host plants through an aperture that is formed by using their cellulases to degrade the plant cell walls [45]. In Japan, the gene of endogenous β-1,4-endoglucanase belonging to GHF45 was found in a major pine wood nematode *Bursaphelenchus xylophilus* (Nematoda, Tylenchida) which is parasitic to pine trees, causing pine tree death. These nematodes are assumed to bore into pine trees by degrading the cell wall in a similar way to the other two described nematode species. The β-1,4-endoglucanase gene of this nematode was demonstrated to be horizontally transferred from fungi. Thus, this species may have acquired the ability to degrade cellulose independently from other plant parasitic nematodes [46].

A small number of aquatic invertebrate species with endogenous cellulases have been reported. However, the phylum of the species found to have cellulases is diverse: Arthropoda, Mollusca, Annelida, Echinodermata, and Chordata. In aquatic animals, endogenous cellulase (β-1,4 endoglucanase) was first identified in *Cherax quadricarinatus* (common crayfish: Arthropoda, Crustacea). The primary structure of their cellulases shows homology with those of the termite, a terrestrial arthropod [47]. Although this finding indicates that cellulases distribute widely in arthropod, further studies on the distribution of cellulases among the other subphylums as Cheliceriformes, Myriopoda, or Trilobitomorpha is needed to confirm the widespread distribution of cellulases in arthropods.

The presence of cellulases has been most intensively studied in mollusks. At present, GHF9

cellulases have, for example, been reported in *Halliotis discus hannai* (Mollusca, Gastropoda) [48], *Halliotis discus discus* (Mollusca, Gastropoda) [49], *Corbicula japonica* (Mollusca, Bivalvia) [12], *Ampullaria crossean* (Mollusca, Gastropoda) [50], and *Mizuhopecten yessoensis* (Mollusca, Bivalvia) [CazyWeb: http://www.cazy.org/ "Accessed 6 May 2012".]. In addition, GHF45 cellulase has been reported in *Mytilus edulis* (Mollusca, Bivalvia) [51], *Corbicula japonica* [52], and *Ampullaria crossean* [53]. There are species differences in the feeding habits of these mollusks. For instance, *H. discus hannai and H. discus discus* feed on diatoms in the larval stage and macroalgae in the adult stage. *M. edulis* is an epifaunal suspension feeder in coastal marine areas. *C. japonica* preferentially feeds on terrestrial particulate organic matter over phytoplankton in brackish waters [49, 54–58]. To the best of our knowledge, the diet of *A. crossean* has not been reported. Nevertheless, *Pomacea canaliculata*, which belongs to the same family as *A. crossean*, is known to digest fresh leaves. In 2011, Qiu et al. reported that *P. canaliculata* (Mollusca, Gastropoda) feeds on both fresh and decayed leaves of a variety of macrophytes [59]. Qiu et al. also mentioned that several species of Ampullariidae (Pomacea) eat leaves of macrophytes in wetlands [59].

The widespread distribution of cellulases among various mollusks with different feeding habits may also indicate the importance of this enzyme in biochemical cellulose breakdown, particularly for *C. japonica*, which is thought to feed on detritus, including decaying plants. The detritus that accumulates on the substrate of brackish areas includes a large amount of plant fragments, mainly composed of cellulose,

and is considered an important food resource for estuarine benthos [60, 61]. A recent study revealed that *C. japonica* contains endogenous β-glucosidase, which degrades the digested products of cellulose formed by β-1,4-glucanase into free glucose [62]. In addition, immunological analysis using an antibody to GHF9 endo-β-1,4-glucanase from *C. japonica* confirmed the production of GHF9 endo-β-1,4-glucanase in the digestive gland [63]. Because of the synergistic action of β-1,4-glucanases [12, 52] and β-glucosidase, *C. japonica* is assumed to utilize cellulose efficiently as a nutritional resource. Furthermore, *C. japonica* is also able to degrade hemicellulose. Sakamoto et al. revealed that *C. japonica* contains xylanase, which degrades xylan, one of the hemicellulases [64]. A comparative study of cellulase and hemicellulase activities in bivalves confirmed that *C. japonica* exhibits significantly higher mannanase activity than other bivalves [65]. Hence, *C. japonica* appears to be well adapted to brackish environments rich in plant-derived detritus.

Furthermore, recent studies have confirmed that other mollusks also utilize multiple enzymes. For example, *H. discus hannai* contains mannanase, β-1,3-glucanase, and alginate lyase [66-68], and *M. edulis* contains mannanase [69]. More recently, Kumagai and Zahura found that *Aplysia kurodai* (Mollusca, Gastropoda), which is a sea hare that eats seaweed, contains β-1,3-glucanases and mannanase [70, 71]. The presence of endogenous cellulose-degrading enzymes, including β-1,4-endoglucanase, in these studied species indicates that these enzymes are widespread among mollusks.

Cellulase activity has also been well documented in other aquatic invertebrates, such as polychaetes

and crabs inhabiting a wide range of environments. Niiyama recorded high cellulase and hemicellulase activities in a variety of temperate region macrobenthos [72]. Toyohara et al. suggested that either meiobenthos (small annelids or nematodes) or sediments in temperate areas exhibit cellulase activity [73]. Furthermore, Yamada and Toyohara confirmed the presence of cellulase activity in the meiobenthos and sediments of the subantarctic region, which was dependent on climatic and sediment features [74]. A recent study by Liu and Toyohara proposed that sediment complexes harbor enzymes including cellulase [75], and it is suggested that plant-degrading enzymes are widely distributed in sediments and the environment.

β-1,4-Endoglucanases of GHF9 have been reported in the echinoderm *Storongylocentrotus nudus* (Echinodermata, Echinoidea), which typically feeds on macroalgae [10], and *Ciona intertinalis* (Chordata, Ascidiacea) [76], for which the genome structure has been intensively studied. Although *C. intertinalis* belongs to the Chordata, which also includes humans, it is known to synthesize cellulose to protect its body. Genomic analyses have shown that the genome of this species harbors endogenous enzymes that synthesize cellulose, which were horizontally transferred from bacteria 530 million years ago. This species has evolved a specific biochemical system to synthesize cellulose that is distinct from that of plants [77]. While the β-1,4-endoglucanases of this species share a homologous structure with other GHF9 cellulases that have been reported in various aquatic invertebrates, they have a distinct function [76]. *C. intertinalis* contains cellulases to degrade cellulose in the tunic, not to degrade cellulose ingested

into the digestive organs. Hence, *C. intertinalis* may serve as a good model to study the horizontal gene transfer mechanism from prokaryotes to eukaryotes, although supplementary genetic information must be collected for other sea squirts.

Utilization of animal cellulases

Cellulose is the most abundant organic material on the earth. A variety of animals that have flourished on this planet are dependent on this widespread resource. Cellulose is even essential for humans as an industrial material for generating paper and clothes, as well as nutritional food fiber materials. The degradation and reconstitution of cellulose has been extensively studied with the aim of producing desirable industrial materials or food, in addition to synthesizing various cellulose derivatives. The modification of cellulose has been studied intensively, and cellulase is probably one of the most industrially utilized enzymes [2, 78, 79].

Recently, cellulases have attracted attention as potential energy sources, such as bioethanol. Bioethanol is synthesized from glucose derived from plant cellulose and hemicellulose. Bioethanol differs from conventional fossil fuels, such as petroleum and coal, in that it is a carbon-neutral fuel, which does not increase the amount of CO_2 in the air. This is because the amount of CO_2 released into the air when consuming bioethanol is equal to that fixed by plants used for bioethanol production [80]. An important aspect of bioethanol production is to reduce energy expenditure when degrading cellulose. At present,

microbial cellulases with heat-stable and acid-stable properties are available for the breakdown of cellulose. To date, anaerobic bacteria have been intensively studied for this purpose, as described in a review by Demain, in which the co-culture of anaerobic bacteria was shown to be effective for producing bioethanol under moderate conditions, and at low cost by using crude substrates [81]. Animal cellulases are potential candidates for bioethanol production, because they are expected to be equipped with specific enzymatic properties that are different to microbial cellulases. In 2011, Xu et al. reported that endogenous β-1,4 endoglucanase from a gastropod *Ampullaria crossean* showed acid and heat stability [82]. Yanagisawa et al. reported that drips containing cellulase and amylase from the mid-gut gland of scallops sacchalified sea lettuce and suggested that these enzymes could be used to produce ethanol when combined with yeast [83].

The digestive efficiency of termite enzymes is reported to be as high as 99 % for cellulose and 87 % for hemicelluloses, driving researchers to investigate potential industrial applications. It has been suggested that the ability of chewing and biting plants led to the high efficiency of cellulose breakdown by termites [2, 84]. Aquatic invertebrates, such as gastropods, also scrape plants using radula. Hence, further studies on both insect and aquatic animal cellulases might contribute toward improving the efficiency of bioethanol production.

Information about aquatic animal cellulases might also be beneficial for aquaculture. The feeding habits of algae eaters that are important to fisheries, such as *Haliotis discus hannai*, *Mytilus*

edulis, and *Mizuhopecten yessoensis*, have been intensively studied [54–56, 85]. For example, abalone is known to feed on diatoms in the larval stage, and feeds on macroalgae in the adult stage. It has also been reported that climatic events, such as tidal streams, may affect ecological circumstances by changing levels of competition or predation stress [54, 55]. Hence detailed information about the feeding habits of species important to fisheries may help in the development of efficient aquaculture systems. Other useful studies in associated fields, including protoplast preparation [86] and improving fruit yields [87], are also advancing.

Recent studies on cellulases have revealed that these enzymes are present in many organisms, reflecting the widespread distribution of cellulose on the earth. Organisms, including bacteria, fungi, and invertebrates, have developed a system to digest cellulose, which may originally have evolved in the bacteria or plants for cell wall construction [2]. Compared to studies of cellulases in microorganisms, such as bacteria and fungi, limited information is available about aquatic invertebrates. In conclusion, further studies on the cellulases of aquatic invertebrates are anticipated; these will contribute toward improving the efficient use of plants by human beings.

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Figure legends

Fig.1 Three types of cellulose hydrolysis enzymes collectively named cellulase (Exocellubiohydrolase, Endo-β-1,4-glucanase, and β-glucosidase) are involved in the cellulose degradation process

Exocellubiohydrolases (exocellulase) cut cellulose from reducing (R) or nonreducing (NR) termini to release cellubiose (dotted circle). Endo-β-1,4-glucanases cut the cellulose randomly from the internal β-1,4 linkages (broken circle). β-Glucosidases hydrolyze cellubiose or cellu-oligomers from the reducing temini to produce glucoses (double circle). Scissors represents the cutting site of each enzyme.

Fig.2 Schematic view of the crystalline structure of cellulose chains

Glucose units are joined by β-1,4 linkages. Different cellulose chains are linked by hydrogen bonds (marked by dotted lines) to form cellulose crystals.

Fig.3 Two different types of cellulase act on the cellulose binding domain (CBD); multi-domain cellulases and single-domain cellulases

Multi-domain cellulases comprise a catalytic domain that is linked to a CBD by a peptide strand, termed a linker. In this instance, CBDs are assumed to bind to cellulose, which improves the efficiency of the hydrolytic process of multi-domain cellulases by continuing catalytic action on the cellulose surface, while following CBD movement. In contrast, single-domain cellulases leave the cellulose surface after

catalytic action is complete, and subsequently approach a new linkage to participate in another catalytic action.

Fig.4 Phylogenetic tree of GHF9 endo-β-1,4-glucanase based on a nucleotide sequence (CazyWeb: http://www.cazy.org/ "Accessed 6 May 2012".)

This tree was prepared according to the method of Davidson et al. (2005) [16]. Please note that this tree was prepared according to the nucleotide sequence of part of the endogenous endo-β-1,4-glucanases reported to date.

和文要旨

動物セルラーゼ ― 水生無脊椎由来酵素に注目して

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本総説では，動物セルラーゼに関する研究について 1900 年代半ばから最新のものに至る論文を渉猟し，陸生と水生，共生と内源性の観点から分類した。特に自身の染色体 DNA 上にコードされる内源性セルラーゼについては詳述した。これらの酵素について，糖加水分解酵素ファミリーにおける分類や一次構造上の類似性についても述べた。なかでも，近年著しく研究が進んだ軟体動物，節足動物，棘皮動物などの水生生物については，それらの食性や生態との関連について概説するとともに，水生生物由来のセルラーゼの今後の応用の可能性についても論じた。

キーワード

エンド-β-1,4-グルカナーゼ，共生，セルラーゼ，セルロース，内源性，分解，無脊椎動物，GHF9

Fig 1

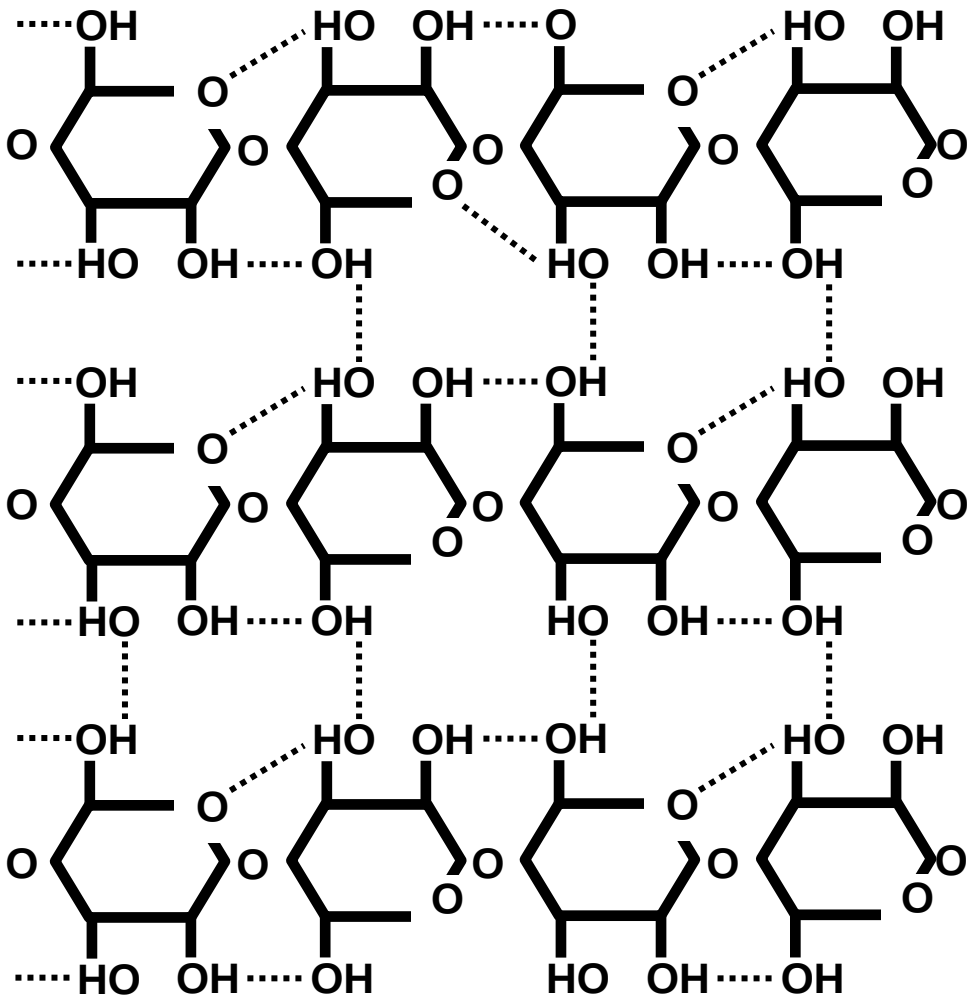


Fig 2

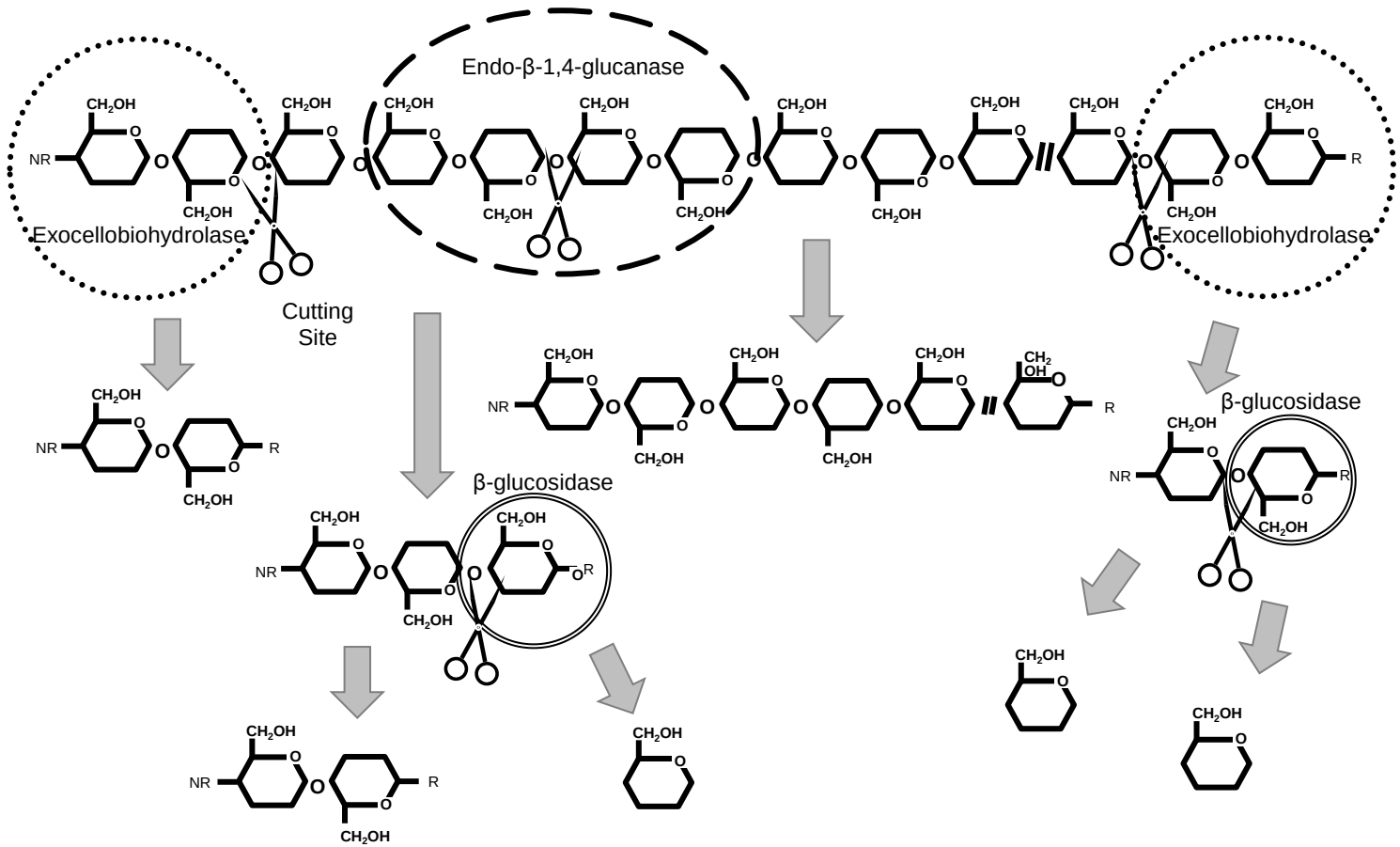

Endo-β-1,4-glucanase
Exocellobiohydrolase
Exocellobiohydrolase
Cutting Site
β-glucosidase
β-glucosidase

Fig 3

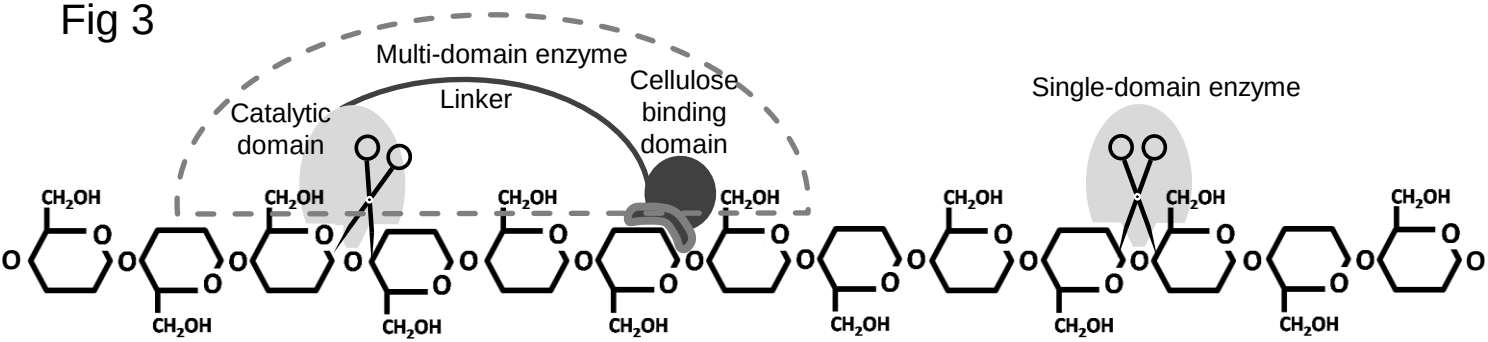
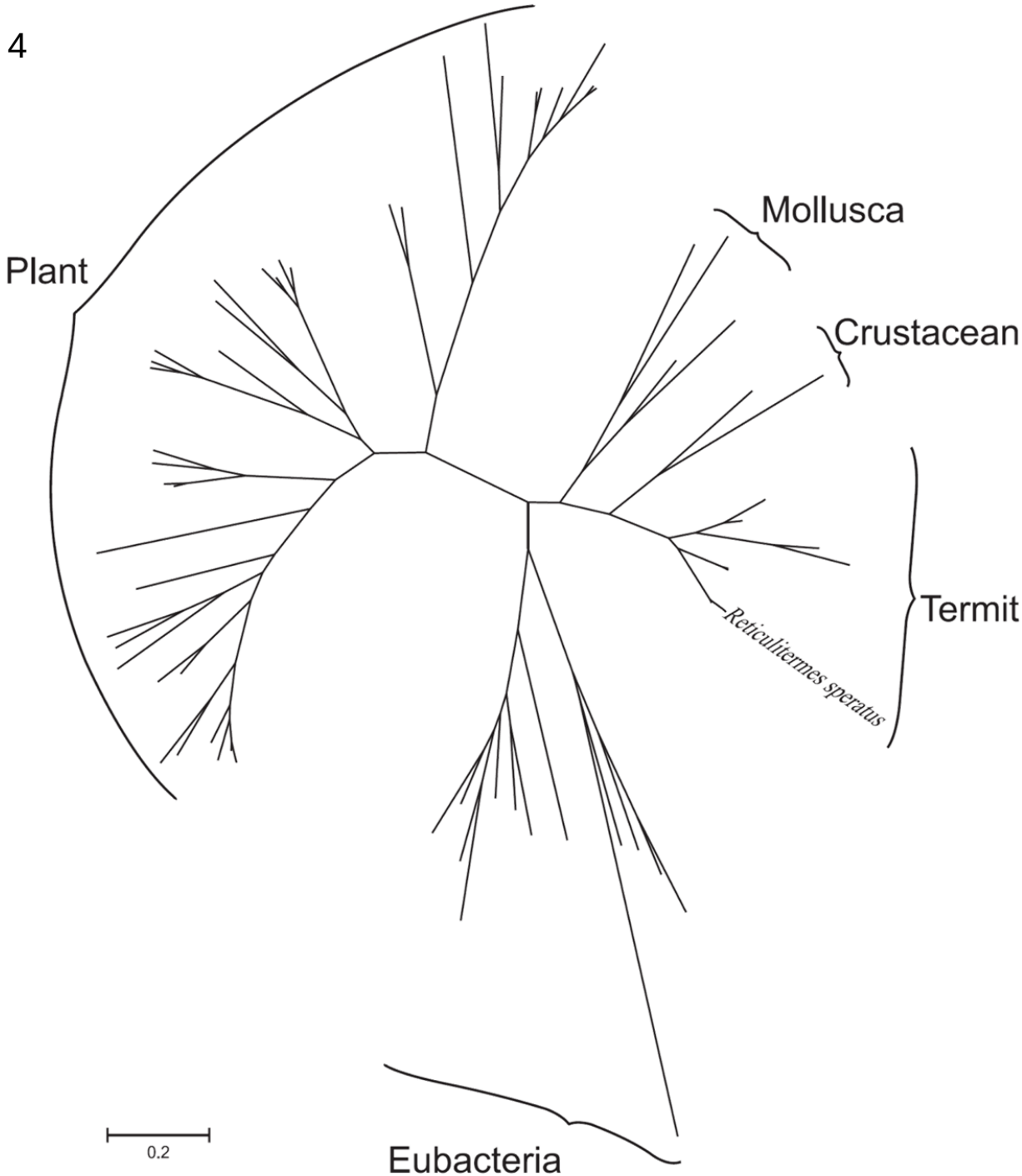

Multi-domain enzyme
Linker
Catalytic domain
Cellulose binding domain
Single-domain enzyme

Fig 4


Plant
Mollusca
Crustacean
Termit
Reticulitermes speratus
0.2
Eubacteria

Table

Table 1 List of members of the Gglycoside Hhydrolase Ffamily (GHF) *

GHF	Cellulase Type	Archaea	Eukaryote			
			Eubacteria	Fungus	Plant	Animal(Invertebrate)
1	β-glucosidase	+	+	+	+	+
3	β-glucosidase	+	+	+	+	+
5	Endoglucanase, β-glucosidase	+	+	+	+	+
6	Endoglucanase, Cellobiohydrolase	-	+	+	-	-
7	Endoglucanase, Cellobiohydrolase	-	-	+	+	+
8	Endoglucanase	+	+	-	-	-
9	Endoglucanase, Cellobiohydrolase, β-glucosidase	+	+	+	+	+
10	Endoglucanase	-	+	-	-	-
12	Endoglucanase	+	+	+	+	-
19	Endoglucanase	+	+	-	-	-
26	Endoglucanase	-	+	-	-	-
30	β-glucosidase	+	+	+	+	+
44	Endoglucanase	-	+	-	-	-
45	Endoglucanase	-	+	+	-	+
48	Endoglucanase	-	+	+	-	-
51	Endoglucanase	+	+	+	+	-
61	Endoglucanase	-	-	+	+	-
74	Endoglucanase	-	+	+	-	-
116	β-glucosidase	+	+	+	+	+
124	Endoglucanase	-	+	-	-	-

* The GHF includes comprises all of glycoside hydrolases, including cellulases. Cellulases are further classified into 20 families [CazyWeb: http://www.cazy.org/ "Accessed 6 May 2012".], most of which belongs to endo-β-1, 4-glucanase. Only GHF5, 9, 10, and 45 are found in metazoans, while and GHF9 and 45 are exclusively found in aquatic invertebrates. β-Glycosidases have beenare reported infrom most animal phyla phylum of animals, because since this enzyme hydrolyzes various dimers of sugars besidein addition to cellubiose.

Table 2 Classification of cellulases based on habitatliving area and enzyme genetic origin*

Habitat type	Organism	Exogenous/Endogenous
Terrestrial	Archaea	Endo
	Eubacteria	Endo
	Fungus	Endo
	Plant	Endo
	Invertebrate Animal	Exo/Endo
	Vertebrate Animal	Exo
Aquatic	Archaea	Endo
	Eubacteria	Endo
	Fungus	Endo
	Plant	Endo
	Invertebrate Animal	Exo/Endo
	Vertebrate Animal	Exo

* Animals are classified asinto “aquatic” and” terrestrial.”. Then, cCellulases are classified into “endogenous” and “exogenous.”. Note,It should be noted that only exogenous cellulases have been so far reported forin vertebrates to date.

Table 3 List of invertebrate cellulases*

Exogenous/ Endogenous	Terrestrial/ Aquatic	Organism					Reference
		Phylum	Class	Order	Species	Common Name	
Endogenous	Terrestrial	Platyhelminthe	Trematoda	Strigeidida	*Schistosoma mansoni*		[16]
		Annelida	Oligochaeta	Haplotaxida	*Eisenia andrei*		CazyWeb
					Eisenia andrei		CazyWeb
					Eisenia fetida	branding worm	CazyWeb
					Lumbricus rubellus	humus earthworm	[16]
					Pheretima hilgendorfi	earthworm	[88]
		Arthropoda	Insecta	Blattaria	*Panesthia angustipennis*	cockroach	[89]
					Panesthia cribrata	cockroach	[41]
					Salganea esakii	cockroach	[89]
				Coleoptera	*Timarcha balearica*	beetle	[16]
				Copelata	*Tribolium castaneum*	red flour beetle	[42]
				Hymenoptera	*Apis mellifera*	honeybee	[16]
				Isopoda	*Glyptotermes sp. Wyong*	termite	[90]
					Hodotermopsis sjoestdi	termite	[91]
					Sinocapritermes mushae	termite	[91]
				Isoptera	*Coptotermes acinaciformis*	termite	CazyWeb
					Coptotermes formosanus	Formosan subterranean termite	[92]
					Coptotermes formosanus	Formosan subterranean termite	[93]
					Coptotermes formosanus Shiraki	termite	[40]
					Mastotermes darwiniensis	termite	[94]
					Nasutitermes takasagonesis	termite	[91]
					Nasutitermes takasagonesis	termite	[90]
					Nasutitermes walkeri	termite	[90]
					Neotermes koshunensis	termite	[91]
					Odontotermes formosanus	termite	CazyWeb
					Odontotermes formosanus	termite	[91]
					Reticulitermes flavipes	eastern subterrenean termite	[95]
					Reticulitermes speratus	termite	[7]
					Reticulitermes speratus	termite	[90]
					Reticulitermes speratus	termite	[96]
				Orthoptera	*Teleogryllus emma*	emma field cricket	[97]
			Malacostraca	Mysida	*Neomysis intermedia*		CazyWeb

continued

Exogenous/ Endogenous	Terrestrial/ Aquatic	Organism					Reference
		Phylum	Class	Order	Species	Common Name	
Endogenous	Aquatic	Annelida	Polychaeta	Aciculata	*Perinereis nuntia brevicirris*		[98]
		Mollusca	Bivalvia	Ostreoidea	*Crassostrea virginica*	eastern oyster	[16]
				Pectinoida	*Argopecten irradians*	bay scallop	[16]
					Argopecten irradians	bay scallop	[16]
					Mizuhopecten yessoensis	ezo giant scallop	CazyWeb
				Veneroida	*Corbicula japonica*		[12]
			Gastropoda	Architaenioglossa	*Ampullaria crossean*		[50]
				Pulmonata	*Biomphalaria glabrata*	bloodfluke planorb	[16]
					Lymnaea stagnalis	great pond snail	[99]
				Vetigastropoda	*Haliotis corrugata*	pink abalone	[100]
					Haliotis cracherodii	black abalone	[100]
					Haliotis discus discus	disc abalone	[49]
					Haliotis discus hannai	ezo abalone	[48]
					Haliotis fulgens	green abalone	[100]
					Haliotis kamtschatkana		[100]
					Haliotis rufescens		[100]
					Haliotis sorenseni	white abalone	[100]
					Haliotis walallensis	flat abalone	[100]
		Arthropoda	Branchiopoda	Diplostraca	*Daphnia magna*	water flea	[16]
			Insecta	Isoptera	*Macrotermes barnevi*		CazyWeb
			Malacostraca	Amphipoda	*Gammarus pulex*	shrimp	[16]
				Decapoda	*Austrothelphusa transversa*		CazyWeb
					Callinectes sapidus	blue crab	[16]
					Cherax quadricarinatus	crayfish	[47]
					Cherax quadricarinatus	crayfish	[101]
					Euastacus sp. SL-2005		CazyWeb
					Homarus americanus	American lobster	[16]
				Isopoda	*Limnoria quadripunctata*		[39]
					Porcellio scaber		[14]
		Echinodermata	Echinoidea	Echinacea	*Strongylocentrotus nudus*	sea urchin	[10]
					Strongylocentrotus purpuratus	purple sea urchin	[102]

continued

Exogenous/ Endogenous	Terrestrial/ Aquatic	Organism					Reference
		Phylum	Class	Order	Species	Common Name	
Endogenous	Aquatic	Chordata	Appendiculari	Copelata	*Oikopleura dioica*	sea squirt	[103]
					Oikopleura dioica	sea squirt	[104]
			Ascidiacea	Enterogona	*Ciona intestinalis*	sea squirt	[76]
					Ciona savignyi	sea squirt	[16]
				Stolidobranchia	*Botryllus schlosseri*	sea squirt	[16]
					Halocynthia roretzi	sea squirt	[16]
					Molgula tectiformis	sea squirt	[16]
Exogenous	Terrestrial	Arthropoda	Insecta	Blattaria	*Cryptocercus punctulatus*	brown-hooted cockroach	[23]
				Coleoptera	*Dendroctonus frontalis*	southern pine beetle	[24]
					Ips pini	North American pine engraver	[24]
					Saperda vestita	Wood Borer	[105]
				Isoptera	*Macrotermes mulleri*	termite	[28]
					Macrotermes natalensis	termite	[27]
					Mastotermes darwiniensis	Giant Northern Termite	[26]
					Reticulitermes flavipes	eastern subterrenean termite	[22]
					Zootermopsis angusticollis	Pacific dampwood termite	[25]
		Chordata	Mammalia	Artiodactyla	*Sus sp.*	pig	[21]
				Catartiodactyla	*Bos primigenius*	aurochs	[17]
					Ovis aries	sheep	[19]
					Ovis aries	sheep	[18]
					Ovis aries	sheep	[20]
	Aquatic	Mollusca	Bivalvia	Teredinidae	*Bankia gouldi*	shipworm	[30]
					Lyrodus pedicellatus	shipworm	[32]
					Psiloteredo healdi	shipworm	[30]
					Teredo bartschi	shipworm	[30]
					Teredo furcifera	shipworm	[30]
					Teredo navalis	shipworm	[30]
			Gastropoda	Patellogastropoda	*Pectinodonta sp.*		[35]

* Invertebrate cellulases are classified according to the standard described in Table 2. Please note that endogenous cellulases include only GHF9, while exogenous cellulases include some of the cellulases reported to date. Animal classification and common names are presented according to the Species Dictionary: http://bm2.genes.nig.ac.jp/Integrated_BodyMap/species.php "Accessed 6 May 2012". Endogenous GHF9s are summarized according to the CazyWeb: http://www.cazy.org/ "Accessed 6 May 2012". and Davison et al (2005) [16].