

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1 **Title**

2 **Animal Cellulases with a Focus on Aquatic Invertebrates**

3

4 **Aya Tanimura¹ · Wen Liu¹ · Kyohei Yamada¹ · Takushi Kishida² · Haruhiko Toyohara^{1*}**

5

6 ¹Division of Applied Biosciences, Graduate School of Agriculture, Kyoto University,

7 Kyoto 606-8502, Japan

8 ²Primate Research Institute, Kyoto University, Inuyama, Aichi 484-8506, Japan

9

10 **Corresponding Author**

11 Haruhiko Toyohara

12 Tel/Fax: 81-75-753-6446

13 **Email Address**

14 Haruhiko Toyohara: toyohara@kais.kyoto-u.ac.jp

15 Aya Tanimura: ayatani@kais.kyoto-u.ac.jp

16 Wen Liu: liuwen@kais.kyoto-u.ac.jp

17 Kyohei Yamada: kyohei1216@gmail.com

18 Takushi Kishida: takushi@zoo.zool.kyoto-u.ac.jp

19

1
2
3 **20 Abstract**
4
5

6 **21** Cellulose is utilized as a nutritional source by various organisms. It had been long believed that only
7
8
9 **22** protozoa, bacteria and fungi, in addition to plants and photo-synthetic bacteria, are able to synthesize
10
11
12 **23** cellulases encoded by their own genes. However, the wide spread distribution of cellulases throughout the
13
14
15
16 **24** animal kingdom has been recently recognized. Conventionally, animals digest cellulose by utilizing
17
18
19 **25** cellulases derived from symbiotic bacteria in the digestive organs. However, recent molecular biological
20
21
22 **26** studies have shown that some cellulase genes are actually encoded on animal chromosomes. In addition,
23
24
25 **27** the homologous primary structure of cellulases obtained from various phyla of invertebrates indicates the
26
27
28 **28** possible vertical transfer of the cellulase gene from ancient organisms that are now extinct. Studies on
29
30
31 **29** cellulase with unique enzymatic properties are expected to be applied to bioethanol production and
32
33
34 **30** aquaculture. In the present review, we describe cellulases, with a primary focus on aquatic invertebrates
35
36
37
38 **31** in which both endogenous and exogenous cellulases are involved in the breakdown of cellulose in the
39
40
41 **32** digestive organs.
42
43

44 **33**

45
46
47 **34 Keywords**
48
49

50
51 **35** Breakdown • Cellulase • Cellulose • Endo- β -1,4-glucanase • Endogenous • GHF9 • Invertebrate • Symbiosis
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1
2
3 **36 Introduction**
4
5

6 **37** Cellulose synthesized by plants and phototrophic bacteria is the most abundant organic substance on
7
8
9 **38** the earth. Cellulose is chemically stable, and thereby plays an important role as a major component in the
10
11
12 **39** cell wall of plants and bacteria by providing physical strength [1]. This physical strength is attributed to
13
14
15
16 **40** the primary structure of cellulose, which consists of monomeric chains of D-glucopyranose bound by
17
18
19 **41** β -1,4-glycoside linkages that form cellulose microfibrils interconnected with hydrogen bonds (Fig. 1) [2].
20
21
22 **42** In addition to cellulose, the cell wall contains lignin and various hemicelluloses, including mannan, xylan,
23
24
25 **43** and laminarin. The contents of the hemicelluloses differ across plant species [3].
26

27
28
29 **44** Enzymes that degrade cellulose are collectively called cellulases, and are classified according to a
30
31
32 **45** range of characteristics. First, cellulases are classified according to the cleavage site on cellulose;
33
34
35 **46** comprising (1) β -1,4-endoglucanase (EC 3.2.1.4.), which cleaves cellulose at random sites, and (2)
36
37
38 **47** β -1,4-exocellobiohydrolase (EC 3.2.1.91.), which cleaves off glucose dimers from the terminal end of
39
40
41 **48** cellulose. Subsequently, β -glucosidase (EC 3.2.1.21.) cleaves glucose from the breakdown products
42
43
44
45 **49** formed by β -1,4-endoglucanase and β -1,4-exocellobiohydrolase (Fig. 2).
46

47
48 **50** Second, cellulases are classified by the presence of the carbohydrate-binding module (CBM) within
49
50
51 **51** their molecules. Some carbohydrate degrading enzymes have a CBM that is independent of the catalytic
52
53
54 **52** site, which binds to substrates and stabilizes the enzymatic reaction. For example, cellulases that have a
55
56
57 **53** cellulose-binding domain (CBD) on a CBM constantly bind to cellulose, with the enzyme molecules
58
59
60

1
2
3 54 continuously moving to a subsequent cleavage site after each reaction. In comparison, cellulases that do
4
5
6 55 not have a CBD detach from the cellulose after every cleaving reaction, and search for the cleaving site of
7
8
9 56 the next hydrolytic reaction [2]. Cellulases that have a CBD are assumed to hydrolyze cellulose more
10
11
12
13 57 efficiently than those that do not have (Fig. 3).
14
15

16 58 Third, cellulases are classified according to their primary structure. For instance, glycoside
17
18
19 59 hydrolases are classified into the glycoside hydrolase family (GHF) by Henrissat et al., according to the
20
21
22 60 amino acid sequence [4, CazyWeb: <http://www.cazy.org/> “Accessed 6 May 2012”]. At present, 130
23
24
25 61 families are registered, with cellulases being classified into families 1, 3, 5, 6, 7, 8, 9, 10, 12, 19, 26, 30,
26
27
28
29 62 44, 45, 48, 51, 61, 74, 116, and 124 (Table 1).
30
31

32 63 Cellulose is utilized as a nutritional source by various organisms. It has long been believed that only
33
34
35 64 protozoa, bacteria, and fungi, in addition to plants and photo-synthetic bacteria, are able to synthesize
36
37
38 65 cellulases encoded by their own genes [1, 5-7]. Before 1998, it was assumed that metazoans degraded
39
40
41 66 cellulose using cellulases derived from symbiotic protozoa and bacteria in their digestive organs [1].
42
43
44 67 However, a novel gene encoding cellulase (GHF9; β -1,4-endoglucanase) was identified in *Reticulitermes*
45
46
47 68 *speratus* (Arthropoda, Insecta) in 1998 [7]. Subsequently, the presence of endogenous
48
49
50
51 69 β -1,4-endoglucanases, belonging to various GHF families, has been reported in various insects,
52
53
54 70 crustaceans, mollusks, echinoderms, and nematodes [8-16].
55
56

57 71 The origin of cellulase in metazoans may be explained by two alternative hypotheses [15, 16]. The
58
59
60
61
62
63
64
65

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

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

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

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

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

90
91
92
93
94
95
96
97
98
99
100
101
102
103
104
105
106
107

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

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1108 implicated in the breakdown of cellulose in herbivorous insects. The cellulose-degrading ability of
1109 symbiotic bacteria in the termite *Reticulitermes flavipes* was first reported in 1924 [22]. Subsequently, in
1110 1932, Trager et al. reported the cellulase activity of flagellates in the digestive organs of the wood roach
1111 [23]. Recently, Delalibera et al. found symbiotic cellulose-degrading bacteria and fungi in the digestive
1112 organs of the wood borer and bark beetle [24]. The presence of symbiotic microorganisms has been
1113 extensively studied in termites. For example, Wenzel et al. successfully isolated symbiotic aerobic and
1114 anaerobic bacteria from damp wood termites (*Zootermopsis angusticollis*; Arthropoda, Insecta) in 2002
1115 [25]. In 2006, Watanabe et al. isolated archaeobacteria exhibiting cellulase activity from the digestive tract
1116 of the giant northern termite (*Mastotermes darwiniensis*; Arthropoda, Insecta) [26]. Interestingly, Martin
1117 et al. detected cellulase activity in the midgut extract of the fungus-growing termite, *Macrotermes*
1118 *natalensis* (Arthropoda, Insecta) in 1978 [27]. This termite species cultures cellulose-degrading bacteria
1119 in its hive, and obtains cellulase from these bacteria. A similar example was reported for another termite,
1120 *Macrotermes mulleri* (Arthropoda, Insecta) [28]. Hence, there are a large number of terrestrial animals
1121 that are associated with symbiotic microorganisms, allowing them to effectively utilize cellulose.

1122
1123 **Cellulases derived from symbiotic microorganisms in aquatic organisms**

1124 The shipworm (Bivalvia, Teredinidae) is a major pest that bores holes in the hulls of wooden ships
1125 and other wooden marine structures, occasionally resulting in the sinking of ships. Symbiotic bacteria

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

126 were first found in granules of “the Gland of Deshayes” from one shipworm species *Bankia australis*
127 (Mollusca, Bivalvia) in 1973 [29]. In 1983, Waterbury et al. found some species of bacteria that had both
128 cellulose-degrading activity and nitrogen-fixing ability in shipworms, indicating that this species of
129 shipworm utilizes cellulose and fixes nitrogen as carbon and nitrogen sources, respectively [30]. In 1991,
130 the authors also reported the presence of a symbiotic bacteria belonging to Proteobacteria in various
131 species of shipworm [31]. This was the first report demonstrating the widespread distribution of
132 symbiotic bacteria belonging to the same phylum being present in a variety of shipworm species. After
133 2002, *Teredinibacter turnerae* classified into Proteobacterium, was found to be distributed in 24 species
134 belonging to 9 distinct genera out of the total 14 genera of shipworm [32]. This observation indicates the
135 ubiquitous distribution of specific bacteria among various species of shipworm.

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

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

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

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

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

156

157 **Endogenous cellulases in terrestrial and aquatic animals**

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

1
2
3 162 harmful insect that decomposes wooden houses [7]. In 1998, Watanabe et al. succeeded in cloning the
4
5
6 163 cDNA of β -1,4-endoglucanase from *R. speratus*, and the enzyme was classified as GHF9, according to the
7
8
9 164 deduced amino acid sequence. The authors confirmed its endogenous origin by using PCR and Southern
10
11
12 165 blot analysis of DNA extracted from termites. These procedures revealed the presence of an intron in the
13
14
15
16 166 gene of β -1,4-endoglucanase. Later, the cDNA of another cellulase was cloned from *Coptotermes*
17
18
19 167 *formosanus* (Arthropoda, Insecta), a termite species related to *R. speratus*, with it being classified as an
20
21
22 168 endogenous GHF9 cellulase [40]. This cellulase was expressed in the foregut and midgut, with a
23
24
25 169 symbiotic flagellate expressing a GHF7 cellulase in the hindgut [40]. These findings indicate that *C.*
26
27
28 170 *formosanus* first degrades cellulose in the foregut and midgut *via* endogenous cellulases, and then the
29
30
31
32 171 degraded products of cellulose are further digested by cellulases from the symbiotic flagellate in the
33
34
35 172 hindgut, facilitating the effective digestion of cellulose. The presence of endogenous cellulases was
36
37
38 173 subsequently reported for herbivorous arthropods, including the cockroach, which inhabits forests [41],
39
40
41 174 and the well-known flour beetle *Tribolium castaneum* (Arthropoda, Insecta), which has a worldwide
42
43
44 175 distribution [42].

46
47
48 176 In addition to arthropods, cellulases from Nematoda have been studied intensively. In 1998, the
49
50
51 177 cDNA of cellulase was cloned from the plant-pathogenic cyst nematodes *Globodera rostochiensis* and
52
53
54 178 *Heterodera glycines* (Nematoda, Tylenchida), and was classified as an endogenous GHF5 family enzyme
55
56
57 179 [11]. In addition to cellulase, *Heterodera glycines* has endogenous genes encoding chitinase [43].
58
59
60
61
62
63
64
65

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

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

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

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

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

198 cellulases have, for example, been reported in *Halliotis discus hannai* (Mollusca, Gastropoda) [48],
199 *Halliotis discus discus* (Mollusca, Gastropoda) [49], *Corbicula japonica* (Mollusca, Bivalvia) [12],
200 *Ampullaria crossean* (Mollusca, Gastropoda) [50], and *Mizuhopecten yessoensis* (Mollusca, Bivalvia)
201 [CazyWeb: <http://www.cazy.org/> “Accessed 6 May 2012”]. In addition, GHF45 cellulase has been
202 reported in *Mytilus edulis* (Mollusca, Bivalvia) [51], *Corbicula japonica* [52], and *Ampullaria crossean*
203 [53]. There are species differences in the feeding habits of these mollusks. For instance, *H. discus hannai*
204 and *H. discus discus* feed on diatoms in the larval stage and macroalgae in the adult stage. *M. edulis* is an
205 epifaunal suspension feeder in coastal marine areas. *C. japonica* preferentially feeds on terrestrial
206 particulate organic matter over phytoplankton in brackish waters [49, 54–58]. To the best of our
207 knowledge, the diet of *A. crossean* has not been reported. Nevertheless, *Pomacea canaliculata*, which
208 belongs to the same family as *A. crossean*, is known to digest fresh leaves. In 2011, Qiu et al. reported
209 that *P. canaliculata* (Mollusca, Gastropoda) feeds on both fresh and decayed leaves of a variety of
210 macrophytes [59]. Qiu et al. also mentioned that several species of Ampullariidae (Pomacea) eat leaves of
211 macrophytes in wetlands [59].

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

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

216 and is considered an important food resource for estuarine benthos [60, 61]. A recent study revealed that
217 *C. japonica* contains endogenous β -glucosidase, which degrades the digested products of cellulose
218 formed by β -1,4-glucanase into free glucose [62]. In addition, immunological analysis using an antibody
219 to GHF9 endo- β -1,4-glucanase from *C. japonica* confirmed the production of GHF9
220 endo- β -1,4-glucanase in the digestive gland [63]. Because of the synergistic action of β -1,4-glucanases
221 [12, 52] and β -glucosidase, *C. japonica* is assumed to utilize cellulose efficiently as a nutritional resource.
222 Furthermore, *C. japonica* is also able to degrade hemicellulose. Sakamoto et al. revealed that *C. japonica*
223 contains xylanase, which degrades xylan, one of the hemicellulases [64]. A comparative study of cellulase
224 and hemicellulase activities in bivalves confirmed that *C. japonica* exhibits significantly higher
225 mannanase activity than other bivalves [65]. Hence, *C. japonica* appears to be well adapted to brackish
226 environments rich in plant-derived detritus.

227 Furthermore, recent studies have confirmed that other mollusks also utilize multiple enzymes. For
228 example, *H. discus hannai* contains mannanase, β -1,3-glucanase, and alginate lyase [66-68], and *M.*
229 *edulis* contains mannanase [69]. More recently, Kumagai and Zahura found that *Aplysia kurodai*
230 (Mollusca, Gastropoda), which is a sea hare that eats seaweed, contains β -1,3-glucanases and mannanase
231 [70, 71]. The presence of endogenous cellulose-degrading enzymes, including β -1,4-endoglucanase, in
232 these studied species indicates that these enzymes are widespread among mollusks.

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

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

234 and crabs inhabiting a wide range of environments. Niiyama recorded high cellulase and hemicellulase
235 activities in a variety of temperate region macrobenthos [72]. Toyohara et al. suggested that either
236 meiobenthos (small annelids or nematodes) or sediments in temperate areas exhibit cellulase activity [73].
237 Furthermore, Yamada and Toyohara confirmed the presence of cellulase activity in the meiobenthos and
238 sediments of the subantarctic region, which was dependent on climatic and sediment features [74]. A
239 recent study by Liu and Toyohara proposed that sediment complexes harbor enzymes including cellulase
240 [75], and it is suggested that plant-degrading enzymes are widely distributed in sediments and the
241 environment.

242 β -1,4-Endoglucanases of GHF9 have been reported in the echinoderm *Storonylocentrotus nudus*
243 (Echinodermata, Echinoidea), which typically feeds on macroalgae [10], and *Ciona intertinalis* (Chordata,
244 Ascidiacea) [76], for which the genome structure has been intensively studied. Although *C. intertinalis*
245 belongs to the Chordata, which also includes humans, it is known to synthesize cellulose to protect its
246 body. Genomic analyses have shown that the genome of this species harbors endogenous enzymes that
247 synthesize cellulose, which were horizontally transferred from bacteria 530 million years ago. This
248 species has evolved a specific biochemical system to synthesize cellulose that is distinct from that of
249 plants [77]. While the β -1,4-endoglucanases of this species share a homologous structure with other
250 GHF9 cellulases that have been reported in various aquatic invertebrates, they have a distinct function
251 [76]. *C. intertinalis* contains cellulases to degrade cellulose in the tunic, not to degrade cellulose ingested

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

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

255

256 **Utilization of animal cellulases**

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

264 Recently, cellulases have attracted attention as potential energy sources, such as bioethanol.
265 Bioethanol is synthesized from glucose derived from plant cellulose and hemicellulose. Bioethanol differs
266 from conventional fossil fuels, such as petroleum and coal, in that it is a carbon-neutral fuel, which does
267 not increase the amount of CO₂ in the air. This is because the amount of CO₂ released into the air when
268 consuming bioethanol is equal to that fixed by plants used for bioethanol production [80]. An important
269 aspect of bioethanol production is to reduce energy expenditure when degrading cellulose. At present,

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

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

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

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

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

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

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

302
303 **Acknowledgements**

304 This study was partly supported by a Grant-in-Aid for Scientific Research from the Ministry of
305 Education, Culture, Sports, Science and Technology of Japan (No. 21380131).

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

306

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

307 **References**

308 1 Watanabe H and Tokuda G (2001) Animal cellulases. *Cell Mol Life Sci* 58: 1167-1178

309 2 Watanabe H and Tokuda G (2010) Cellulolytic systems in insects. *Annu Rev Entomol Annu*
310 *Rev* 55: 609-632

311 3 Cosgrove DJ (2005) Growth of the plant cell wall. *Nat Rev Mol Cell Bio* 6: 850-861

312 4 Henrissat B, Claeysens M, Tomme P, Lemesle L and Mornon JP (1989) Cellulase families
313 revealed by hydrophobic cluster-analysis. *Gene* 81: 83-95

314 5 Karrer P, Schubert P and Wehrli W (1925) Polysaccharides XXXIII on the enzymatic
315 breakdown of artificial silk and native cellulose. *Helv Chim Acta* 8: 797-810

316 6 Lenski RE (2003) Life's solution: Inevitable humans in a lonely universe. *Nature* 425: 767-768

317 7 Watanabe H, Noda H, Tokuda G and Lo N (1998) A cellulase gene of termite origin. *Nature*
318 1998; 394: 330-331

319 8 Martin MM (1991) The evolution of cellulose digestion in insects. *Philos Trans Roy Soc B*
320 333: 281-288

321 9 Sugimura M, Watanabe H, Lo N and Saito H (2003) Purification, characterization, cDNA
322 cloning and nucleotide sequencing of a cellulase from the yellow-spotted longicorn beetle, *Psacotha*
323 *hilaris*. *Eur J Biochem* 270: 3455-3460

324 10 Nishida Y, Suzuki K, Kumagai Y, Tanaka H, Inoue A and Ojima T (2007) Isolation and primary

1
2
3 325 structure of a cellulase from the Japanese sea urchin *Strongylocentrotus nudus*. *Biochimie* 89: 1002-1011
4
5
6 326 11 Smant G, Stokkermans J, Yan YT, de Boer JM, Baum TJ, Wang XH, Hussey RS, Gommers FJ,
7
8
9 327 Henrissat B, Davis EL, Helder J, Schots A and Bakker J (1998) Endogenous cellulases in animals:
10
11
12 328 Isolation of beta-1,4-endoglucanase genes from two species of plant-parasitic cyst nematodes. *Proc Natl*
13
14
15 329 *Acad Sci USA* 95: 4906-4911
16
17
18
19 330 12 Sakamoto K, Touhata K, Yamashita M, Kasai A and Toyohara H (2007) Cellulose digestion by
20
21
22 331 common Japanese freshwater clam *Corbicula japonica*. *Fish Sci* 2007; 73: 675-683
23
24
25 332 13 Imjongjirak C, Amparyup P and Sittipraneed S (2008) Cloning, genomic organization and
26
27
28 333 expression of two glycosyl hydrolase family 10 (GHF10) genes from golden apple snail (*Pomacea*
29
30
31 334 *canaliculata*). *DNA Sequence* 19: 224-236
32
33
34
35 335 14 Kostanjsek R, Milatovic M and Srus J (2010) Endogenous origin of endo-beta-1,4-glucanase in
36
37
38 336 common woodlouse *Porcellio scaber* (Crustacea, Isopoda). *J Comp Physiol B* 180: 1143-1153
39
40
41 337 15 Lo N, Watanabe H and Sugimura M (2003) Evidence for the presence of a cellulase gene in the
42
43
44 338 last common ancestor of bilaterian animals. *Proc Roy Soc Lond B Bio* 270: S69-S72
45
46
47
48 339 16 Davison A and Blaxter M (2005) Ancient origin of glycosyl hydrolase family 9 cellulase genes.
49
50
51 340 *Mol Biol Evol* 22: 1273-1284
52
53
54 341 17 Hungate RE (1946) The cellulose-decomposing bacteria in the rumen of cattle. *J Bacteriol* 51:
55
56
57 342 28-28
58
59
60
61
62
63
64
65

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

343 18 Wood TM, Wilson CA and Stewart CS (1982) Preparation of the cellulase from the cellulolytic
anaerobic rumen bacterium *Ruminococcus-albus* and its release from the bacterial-cell wall. *Biochem J*
205: 129-137

344
345

346 19 Coleman GS (1986) The distribution of carboxymethylcellulase between fractions taken from
the rumens of sheep containing no protozoa or one of 5 different protozoal populations. *J Agr Sci* 106:
121-127

347
348

349 20 Bernalier A, Fonty G, Bonnemoy F and Gouet P (1992) Degradation and fermentation of
cellulose by the rumen anaerobic fungi in axenic cultures or in association with cellulolytic bacteria. *Curr*
Microbiol 25: 143-148

350
351

352 21 Varel VH, Fryda SJ and Robinson IM (1984) Cellulolytic bacteria from pig large-intestine. *Appl*
Environ Microb 47: 219-221

353

354 22 Cleveland LR (1924) The physiological and symbiotic relationships between the intestinal
protozoa of termites and their host, with special reference to *Reticulitermes flavipes* Kollar. *Biol Bull-US*
46: 178-201

355
356

357 23 Trager W (1932) A cellulase from the symbiotic intestinal flagellates of termites and of the
roach, *Cryptocercus punctulatus*. *Biochem J* 26: 1762-1771

358

359 24 Delalibera I, Handelsman J and Raffa KF (2005) Contrasts in cellulolytic activities of gut
microorganisms between the wood borer, *Saperda vestita* (Coleoptera: Cerambycidae), and the bark

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

361 beetles, *Ips pini* and *Dendroctonus frontalis* (Coleoptera: Curculionidae). *Environ Entomol* 34: 541-547

362 25 Wenzel M, Schonig I, Berchtold M, Kampfer P and Konig H (2002) Aerobic and facultatively
363 anaerobic cellulolytic bacteria from the gut of the termite *Zootermopsis angusticollis*. *J Appl Microbiol*
364 92: 32-40

365 26 Watanabe H, Takase A, Tokuda G, Yamada A and Lo N (2006) Symbiotic "archaezoa" of the
366 primitive termite *Mastotermes darwiniensis* still play a role in cellulase production. *Eukaryot Cell* 5:
367 1571-1576

368 27 Martin MM and Martin JS (1978) Cellulose digestion in midgut of fungus-growing termite
369 *Macrotermes natalensis* - Role of acquired digestive enzymes. *Science* 199: 1453-1455

370 28 Rouland C, Civas A, Renoux J and Petek F (1988) Purification and properties of cellulases
371 from the termite *Macrotermes mulleri* (Termitidae, Macrotermitinae) and its symbiotic fungus
372 *Termitomyces* sp. *Comp Biochem Phys B* 91: 449-458

373 29 Popham JD and Dickson MR (1973) Bacterial associations in the teredo *Bankia australis*
374 (Lamellibranchia: Mollusca). *Mar Biol* 19: 338-340

375 30 Waterbury JB, Calloway CB and Turner RD (1983) A cellulolytic nitrogen-fixing bacterium
376 cultured from the Gland of Deshayes in shipworms (Bivalvia: Teredinidae). *Science* 221: 1401-1403

377 31 Distel DL, DeLong EF and Waterbury JB (1991) Phylogenetic characterization and in situ
378 localization of the bacterial symbiont of shipworms (Teredinidae: Bivalvia) by using 16S rRNA sequence

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

379 analysis and oligodeoxynucleotide probe hybridization. *AEM* 57: 2376-2382

380 32 Distel DL, Morrill W, Maclaren-Toussaint N, Franks D and Waterbury J (2002) *Teredinibacter*

381 *turnerae* gen. nov., sp. nov., a dinitrogen-fixing, cellulolytic, endosymbiotic γ -proteobacterium isolated

382 from the gills of wood-boring molluscs (Bivalvia: Teredinidae). *IJSEM* 52: 2261-2269

383 33 Distel DL and Roberts SJ (1997) Bacterial endosymbionts in the gills of the deep-sea

384 wood-boring bivalves *Xylophaga atlantica* and *Xylophaga washingtona*. *Biol Bull* 192: 253-261

385 34 Duperron S, Laurent MCZ, Gaill F and Gros O (2008) Sulphur-oxidizing extracellular bacteria

386 in the gills of Mytilidae associated with wood falls. *FEMS Microbiol Ecol* 63: 338-349

387 35 Zbinden M, Pailleret M, Ravaux J, Gaudron SM, Hoyoux C, Lambourdiere J, Waren A, Lorion

388 J, Halary S and Duperron S (2010) Bacterial communities associated with the wood-feeding gastropod

389 *Pectinodonta* sp. (Patellogastropoda, Mollusca). *FEMS Microbiol Ecol* 74: 450-463

390 36 Cary SC (1994) Vertical transmission of a chemoautotrophic symbiont in the protobranch

391 bivalve, *Solemya reidi*. *Mol Mar Biol Biotech* 3: 121-130

392 37 Sipe AR, Wilbur AE and Cary SC (2000) Bacterial symbiont transmission in the wood-boring

393 shipworm *Bankia setacea* (Bivalvia: Teredinidae). *Environ Microbiol* 66: 1685-1691

394 38 Boyle J. P (1978) Absence of Microorganism in Crustacean Digestive Tracts. *Science* 200:

395 1157-1159

396 39 King AJ (2010) Molecular insight into lignocelluloses digestion by a marine isopod in the

1
2
3 397 absence of gut microbes. Proc Natl Acad Sci USA 107: 5345-5350
4
5
6 398 40 Nakashima K, Watanabe H, Saitoh H, Tokuda G and Azuma JI (2000) Dual cellulose-digesting
7
8
9 399 system of the wood-feeding termite, *Coptotermes formosanus* Shiraki. Insect Biochem Molec 32:
10
11
12 400 777-784
13
14
15
16 401 41 Lo N, Tokuda G, Watanabe H, Rose H, Slaytor M, Maekawa K, Bandi C and Noda H (2000)
17
18
19 402 Evidence from multiple gene sequences indicates that termites evolved from wood-feeding cockroaches.
20
21
22 403 Curr Biol 10: 801-804
23
24
25 404 42 Willis JD, Oppert B, Oppert C, Klingeman WE and Jurat-Fuentes JL (2011) Identification,
26
27
28 405 cloning, and expression of a GHF9 cellulase from *Tribolium castaneum* (Coleoptera: Tenebrionidae). J
29
30
31 406 Insect Physiol 57: 300-306
32
33
34
35 407 43 Gao BL, Allen R, Maier T, McDermott JP, Davis EL, Baum TJ and Hussey RS (2002)
36
37
38 408 Characterisation and developmental expression of a chitinase gene in *Heterodera glycines*. Int J Parasitol
39
40
41 409 32: 1293-1300
42
43
44 410 44 Yan YT, Smant G, Stokkermans J, Qin L, Helder J, Baum T, Schots A and Davis E (1998)
45
46
47 411 Genomic organization of four beta-1,4-endoglucanase genes in plant-parasitic cyst nematodes and its
48
49
50 412 evolutionary implications. Gene 220: 61-70
51
52
53
54 413 45 Jones JT, Furlanetto C and Kikuchi T (2005) Horizontal gene transfer from bacteria and fungi
55
56
57 414 as a driving force in the evolution of plant parasitism in nematodes. Nematology 7: 641-646
58
59
60
61
62
63
64
65

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

415 46 Kikuchi T, Jones JT, Aikawa T, Kosaka H and Ogura N (2004) A family of glycosyl hydrolase
416 family 45 cellulases from the pine wood nematode *Bursaphelenchus xylophilus*. *FEBS Lett* 572: 201-205
417 47 Byrne KA, Lehnert SA, Johnson SE and Moore SS (1999) Isolation of a cDNA encoding a
418 putative cellulase in the red claw crayfish *Cherax quadricarinatus*. *Gene* 239: 317-324
419 48 Suzuki K, Ojima T and Nishita K (2003) Purification and cDNA cloning of a cellulase from
420 abalone *Haliotis discus hannai*. *Eur J Biochem* 270: 771-778
421 49 Nikapitiya C, Oh C, De Zoysa M, Whang I, Kang DH, Lee SR, Kim SJ and Lee J (2010)
422 Characterization of beta-1,4-endoglucanase as a polysaccharide-degrading digestive enzyme from disk
423 abalone, *Haliotis discus discus*. *Aquacult Int* 18: 1061-1078
424 50 Li YH, Yin QY, Ding M and Zhao FK (2009) Purification, characterization and molecular
425 cloning of a novel endo-beta-1,4-glucanase AC-EG65 from the mollusc *Ampullaria crossean*. *Comp*
426 *Biochem Phys B* 153: 149-156
427 51 Xu BZ, Janson JC and Sellos D (2001) Cloning and sequencing of a molluscan
428 endo-beta-1,4-glucanase gene from the blue mussel, *Mytilus edulis*. *Eur J Biochem* 268: 3718-3727
429 52 Sakamoto K and Toyohara H (2009) Molecular cloning of glycoside hydrolase family 45
430 cellulase genes from brackish water clam *Corbicula japonica*. *Comp Biochem Phys B* 152: 390-396
431 53 Guo R, Ding M, Zhang SL, Xu GJ and Zhao FK (2008) Molecular cloning and characterization
432 of two novel cellulase genes from the mollusc *Ampullaria crossean*. *J Comp Physiol B* 178: 209-215

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

433 54 Won NI, Kawamura T, Takami H, Hoshikawa H and Watanabe Y (2011) Comparison of
434 abalone (*Haliotis discus hannai*) catches in natural habitats affected by different current systems:
435 Implication of climate effects on abalone fishery. *Fish Res* 110: 84-91

436 55 Xing RL, Wang CH, Cao XB and Chang YQ (2008) Settlement, growth and survival of abalone,
437 *Haliotis discus hannai*, in response to eight monospecific benthic diatoms. *J Appl Phycol* 20: 47-53

438 56 Hawkins AJS, Navarro E and Iglesias JIP (1990) Comparative allometries of gut-passage time,
439 gut content and metabolic fecal loss in *Mytilus edulis* and *Cerastoderma edule*. *Mar Biol* 105: 197-204

440 57 Antonio ES, Kasai A, Ueno M, Kurikawa Y, Tsuchiya K, Toyohara H, Ishihi Y, Yokoyama H
441 and Yamashita Y (2010) Consumption of terrestrial organic matter by estuarine molluscs determined by
442 analysis of their stable isotopes and cellulase activity. *Estuar Coast Shelf S* 86: 401-407

443 58 Kasai A and Nakata A (2005) Utilization of terrestrial organic matter by the bivalve *Corbicula*
444 *japonica* estimated from stable isotope analysis. *Fish Sci* 71: 151-158

445 59 Qiu JW, Chan MT, Kwong KL and Sun J (2011) Consumption, Survival and growth in the
446 invasive freshwater snail *Pomacea Canaliculata*: Does food freshness matter? *J Mollus Stud* 77: 189-195

447 60 Kanaya G, Suzuki T and Kikuchi E (2011) Spatio-temporal variations in macrozoobenthic
448 assemblage structures in a river-affected lagoon (Idoura Lagoon, Sendai Bay, Japan): Influences of
449 freshwater inflow. *Estuar Coast Shelf S* 92: 169-179

450 61 Kanaya G, Takagi S and Kikuchi E (2008) Spatial dietary variations in *Laternula marilina*

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

451 (Bivalva) and Hediste spp. (Polychaeta) along environmental gradients in two brackish lagoons. Mar
452 Ecol-Prog Ser 359: 133-144

453 62 Sakamoto K, Uji S, Kurokawa T and Toyohara H (2009) Molecular cloning of endogenous
454 beta-glucosidase from common Japanese brackish water clam *Corbicula japonica*. Gene 435: 72-79

455 63 Sakamoto K, Uji S, Kurokawa T and Toyohara H (2008) Immunohistochemical, in situ
456 hybridization and biochemical studies on endogenous cellulase of *Corbicula japonica*. Comp Biochem
457 Phys B 150: 216-221

458 64 Sakamoto K and Toyohara H (2009) Putative endogenous xylanase from brackish-water clam
459 *Corbicula japonica*. Comp Biochem Phys B 154: 85-92

460 65 Sakamoto K and Toyohara H (2009) A comparative study of cellulase and hemicellulase
461 activities of brackish water clam *Corbicula japonica* with those of other marine Veneroida bivalves. J Exp
462 Biol 212: 2812-2818

463 66 Ootsuka S, Saga N, Suzuki K, Inoue A and Ojima T (2006) Isolation and cloning of an
464 endo-beta-1,4-mannanase from Pacific abalone *Haliotis discus hannai*. J Biotechnol 125: 269-280

465 67 Shimizu E, Ojima T and Nishita K (2003) cDNA cloning of an alginate lyase from abalone,
466 *Haliotis discus hannai*. Carbohydr Res 338: 2841-2852

467 68 Kumagai Y and Ojima T (2009) Enzymatic properties and the primary structure of a
468 beta-1,3-glucanase from the digestive fluid of the Pacific abalone *Haliotis discus hannai*. Comp Biochem

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

469 Phys B 154: 113-120

470 69 Xu BZ, Hagglund P, Stalbrand H and Janson JC (2002) endo-beta-1,4-mannanases from blue
471 mussel, *Mytilus edulis*: purification, characterization, and mode of action. *J Biotechnol* 92: 267-277

472 70 Zahura UA, Rahman MM, Inoue A, Tanaka H and Ojima T (2011) cDNA cloning and bacterial
473 expression of an endo-beta-1,4-mannanase, AkMan, from *Aplysia kurodai*. *Comp Biochem Phys B* 159:
474 227-235

475 71 Kumagai Y and Ojima T (2010) Isolation and characterization of two types of
476 beta-1,3-glucanases from the common sea hare *Aplysia kurodai*. *Comp Biochem Phys B* 155: 138-144

477 72 Niiyama T and Toyohara H (2011) Widespread distribution of cellulase and hemicellulase
478 activities among aquatic invertebrates. *Fish Sci* 77: 649-655

479 73 Toyohara H, Park Y, Tsuchiya K and Liu W (2012) Cellulase activity in meiobenthos in
480 wetlands. *Fish Sci* 78: 133-137

481 74 Yamada K and Toyohara H (2012) Functions of meiobenthos and microorganisms in cellulose
482 breakdown in sediments of wetlands with different origins in Hokkaido. *Fish Sci* 78: 699-706

483 75 Liu W and Toyohara H (2012) Sediment-complex-binding cellulose breakdown in wetlands of
484 rivers. *Fish Sci* 78: 661-665

485 76 Dehal P, Satou Y, Campbell RK, Chapman J et al (2002) The draft genome of *Ciona*
486 *intestinalis*: Insights into chordate and vertebrate origins. *Science* 298: 2157-2167

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

487 77 Nakashima K, Yamada L, Satou Y, Azuma J and Satoh N (2004) The evolutionary origin of
488 animal cellulose synthase. *Dev Genes Evol* 214: 81-88

489 78 Bhat MK (2000) Cellulases and related enzymes in biotechnology. *Biotechnol Adv* 18: 355-383

490 79 Murad HA and Azzaz HH (2010) Cellulase and dairy animal feeding. *Biotechnology* 9: 1-19

491 80 Demirbas A (2007) Progress and recent trends in biofuels. *Prog Energ Combust* 33: 1-18

492 81 Demain AL (2009) Biosolutions to the energy problem. *J Ind Microbiol Biol* 36: 319-332

493 82 Yin QY, Teng YG, Li YH, Ding M and Zhao FK (2011) Expression and characterization of
494 full-length *Ampullaria crosseana* endoglucanase EG65s and their two functional modules. *Biosci Biotech*
495 *Biochem* 75: 240-246

496 83 Yanagisawa M, Ojima T and Nakasaki K (2011) Bioethanol from sea lettuce with the use of
497 crude enzymes derived from waste. *J Mater Cycl Waste Manag* 13: 321-326

498 84 Ohkuma M (2003) Termite symbiotic systems: efficient bio-recycling of lignocellulose.
499 *Appl.Microbiol Biot* 61: 1-9

500 85 Silina AV and Zhukova NV. Growth variability and feeding of scallop *Patinopecten yessoensis*
501 on different bottom sediments: Evidence from fatty acid analysis (2007) *J Exp Mar Biol Ecol* 348:
502 46-59

503 86 Inoue A, Mashino C, Kodama T and Ojima T (2011) Protoplast preparation from *Laminaria*
504 *japonica* with recombinant alginate lyase and cellulase. *Mar Biotechnol* 13: 256-263

1
2
3 505 87 Zhao FY, Lin JF, Zeng XL, Guo LQ, Wang YH and You LR (2010) Improvement in fruiting
4
5
6 506 body yield by introduction of the *Ampullaria crossean* multi-functional cellulase gene into *Volvariella*
7
8
9 507 *volvacea*. *Biores Technol* 101: 6482-6486
10
11
12 508 87 Zhao FY, Lin JF, Zeng XL, Guo LQ, Wang YH and You LR (2010) Improvement in fruiting
13
14
15 509 body yield by introduction of the *Ampullaria crossean* multi-functional cellulase gene into *Volvariella*
16
17
18 510 *volvacea*. *Biores Technol* 101: 6482-6486
19
20
21
22 511 88 Nozaki M, Miura C, Tozawa Y and Miura T (2009) The contribution of endogenous cellulase to
23
24
25 512 the cellulose digestion in the gut of earthworm (*Pheretima hilgendorfi*: Megascolecidae). *Soil Biol*
26
27
28 513 *Biochem* 41: 762-769
29
30
31
32 514 88 Shimada K and Maekawa K (2008) Correlation between social structure and nymphal
33
34
35 515 wood-digestion ability in the xylophagous cockroaches *Salgallea esakii* and *Panesthia angustipennis*
36
37
38 516 (Blaberidae: Panesthiinae). *Sociobiology* 52: 417-427
39
40
41 517 90 Tokuda G, Lo N, Watanabe H, Slaytor M, Matsumoto T, and Noda H (1999) Metazoan
42
43
44 518 cellulase genes from termites: intron/exon structures and site of expression. *BBA-Struct expr* 1447:
45
46
47 519 146-159
48
49
50
51 520 91 Tokuda G, Lo N, Watanabe H, Arakawa G, Matsumoto T and Noda H (2004) Major alternation
52
53
54 521 of the expression site of endogenous cellulases in members of an apical termite lineage. *Mol Ecol* 13:
55
56
57 522 3219-3228
58
59
60
61
62
63
64
65

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

523 92 Zhang DH, Lax AR, Bland JM and Allen AB (2011) characterization of a new endogenous
524 endo-beta-1,4-glucanase of Formosan subterranean termite (*Coptotermes formosanus*). Insect Biochem
525 Molec 41: 211-218

526 93 Zhang DH, Lax AR, Raina AK and Bland JM (2009) Differential cellulolytic activity of
527 native-form and C-terminal tagged-form cellulase derived from *Coptotermes formosanus* and expressed
528 in *E. coli*. Insect Biochem Molec 39: 516-522

529 94 Li L, Frohlich J, Pfeiffer P and Konig H (2003) Termite gut symbiotic archaezoa are becoming
530 living metabolic fossils. Eucaryot Cell 2: 1091-1098

531 95 Scharf ME, Wu-Scharf D, Zhou X, Pittendrigh BR and Bennett GW (2005) Gene expression
532 profiles among immature and adult reproductive castes of the termite *Reticulitermes flavipes*. Insect Mol
533 Biol 14: 31-44

534 96 Itakura S, Masuta T, Tanaka H and Enoki A (2006) Identification of two subterranean termite
535 species (Isoptera: Rhinotermitidae) using cellulase genes. J Econ Entomol 99: 123-128

536 97 Kim N, Choo YM, Lee KS, Hong SJ, Seol KY, Je YH, Sohn HD, Jin BR (2008) Molecular
537 cloning and characterization of a glycosyl hydrolase family 9 cellulase distributed throughout thr
538 digestive tract of the cricket *Teleogryllus emma*. Comp Biochem Phys B 150: 368-376

539 98 Ito K, Nozakli M, Ohta T, Miura C, Tozawa Y and Miura T (2011) Differences of two
540 po;ychaete specis reflected in enzyme activities. Mar Biol 158: 1211-1221

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

541 99 Davison A and Blaxter ML (2005) An expression sequence tag survey of gene expression in the
542 pond snail *Lymnaea stagnails*, an intermediate vector of *Fasciola hepatica*. *Parasitology* 130: 539-552

543 100 Clark NL, Gasper J, Sekino M, Springer SA, Aquadro CF and Swanson WJ (2009) Coevolution
544 of interacting Fertilization Proteins. *Plos Genet* 5: e1000570

545 101 Crawford AC, Kricker JA, Anderson AJ, Richardson NR and Mather PB (2004) Structure and
546 function of a cellulase gene in redclaw crayfish, *Cherax quadricarinatus*. *Gene* 340: 267-274

547 102 Zhu XD, Mahairas G, Illies M, Cameron RA, Davidson EH and Etensohn CA (2001) A
548 large-scale analysis of mRNAs expressed by primary mesenchyme cells of the sea urchin embryo.
549 *Development* 128: 2615-2627

550 103 Edvardsen RB, Seo HC, Jensen MF, Mialon A, Mikhaleva J, Bjordal M, Cartry J, Reinhardt R,
551 weissenbach J, Wincker P and Chourrout D (2005) Remodeling of the homeobox gene complement in the
552 tunicate *Oikopleura dioica*. *Curr Biol* 15: R12-R13

553 104 Seo HC, Edvardsen RB, Maelnd AD, Bjordal M, Jansen MF, Hansen A, Flaar M, Weissenbach
554 J, Lahrach H, Wincker P, Reinhardt R and Chourrout D (2004) Hox cluster disintegration with persistent
555 anteroposterior order of expression in *Oikopleura dioica*. *Nature* 431: 67-71

556 105 Schloss PD, Delalibera I, Handelsman J and Radda KF (2006) Bacteria associated with the guts
557 of two wood-boring beetles: *Anoplophora glabripennis* and *Saperda vestita* (Cerambycidae). *Environ*
558 *Entomol* 35: 625-629

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

559 **Figure legends**

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

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

566
567 **Fig.2** Schematic view of the crystalline structure of cellulose chains

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

570
571 **Fig.3** Two different types of cellulase act on the cellulose binding domain (CBD); multi-domain
572 cellulases and single-domain cellulases

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

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

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

579

580 **Fig.4** Phylogenetic tree of GHF9 endo- β -1,4-gluconase based on a nucleotide sequence (CazyWeb:
581 <http://www.cazy.org/> “Accessed 6 May 2012”.)

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

585

586

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

谷村 彩, 劉 文, 山田 京平 (京大院農), 岸田 拓士 (京大霊長研), 豊原 治彦 (京大院農)

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

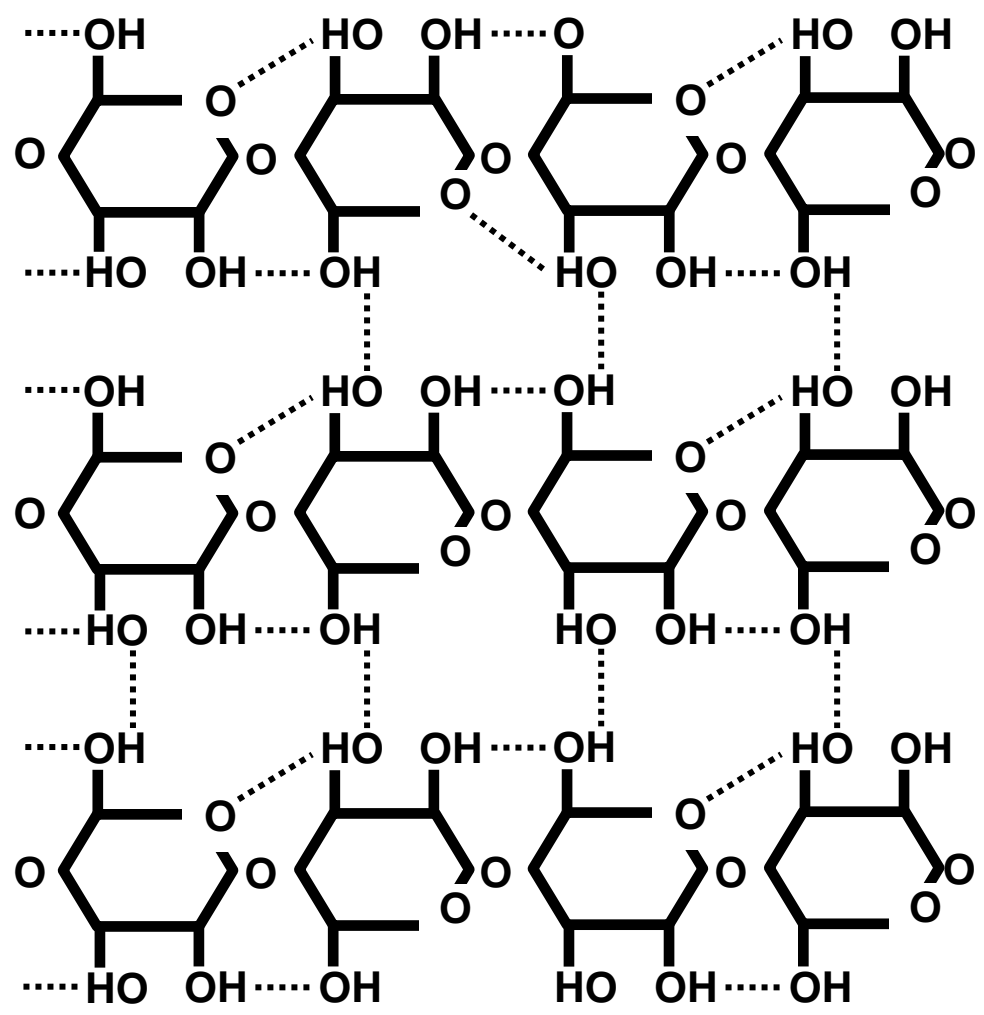


Fig 2

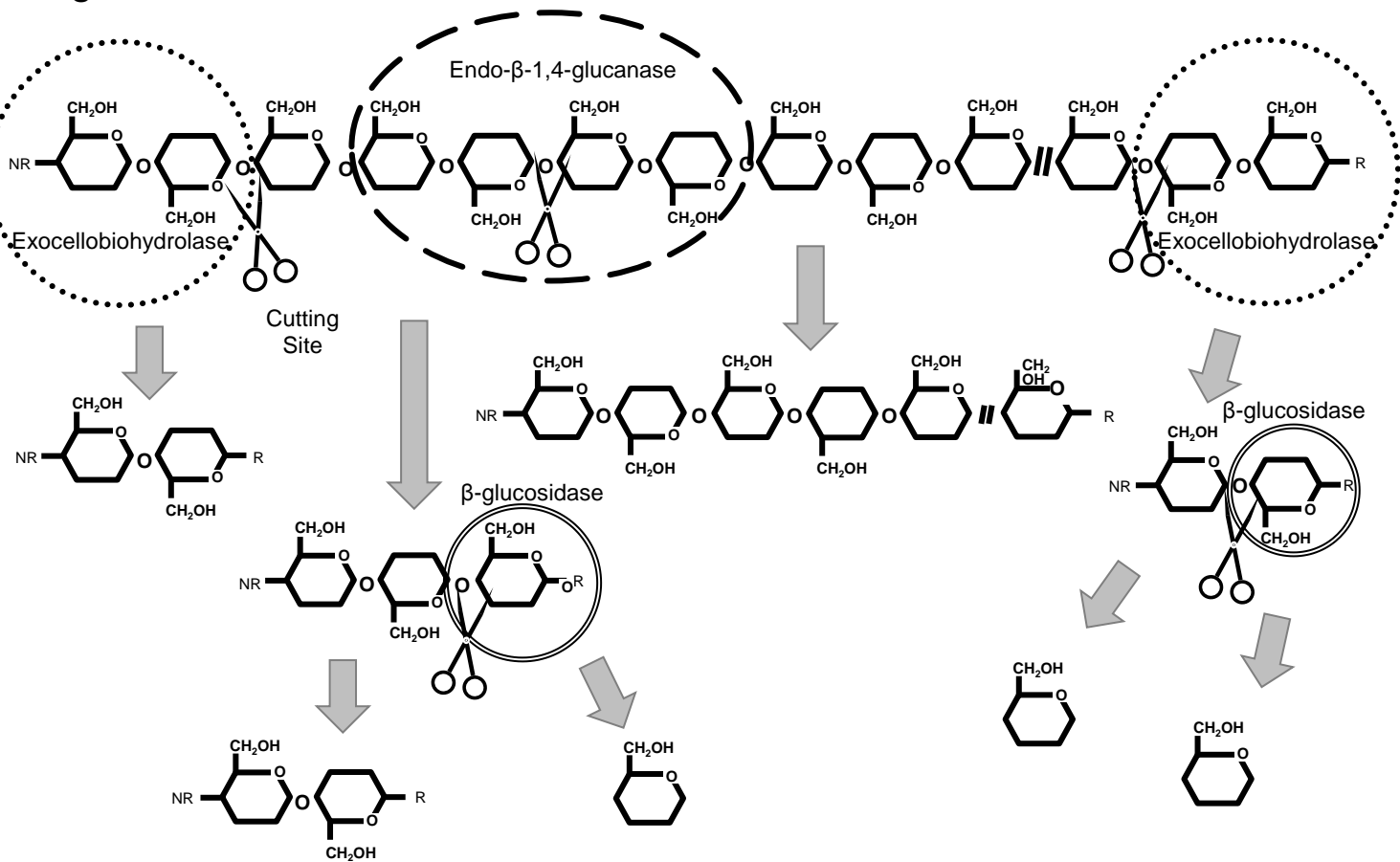


Fig 3

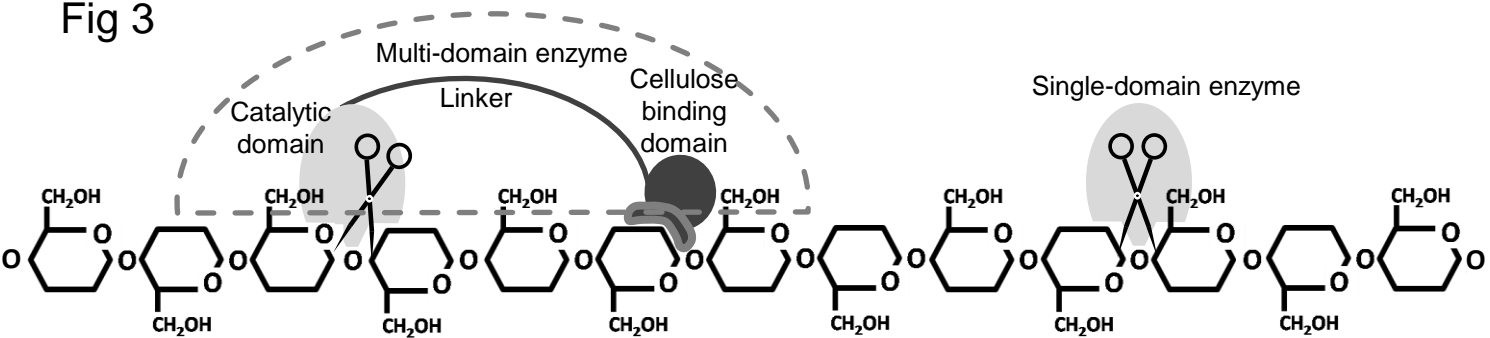


Fig 4

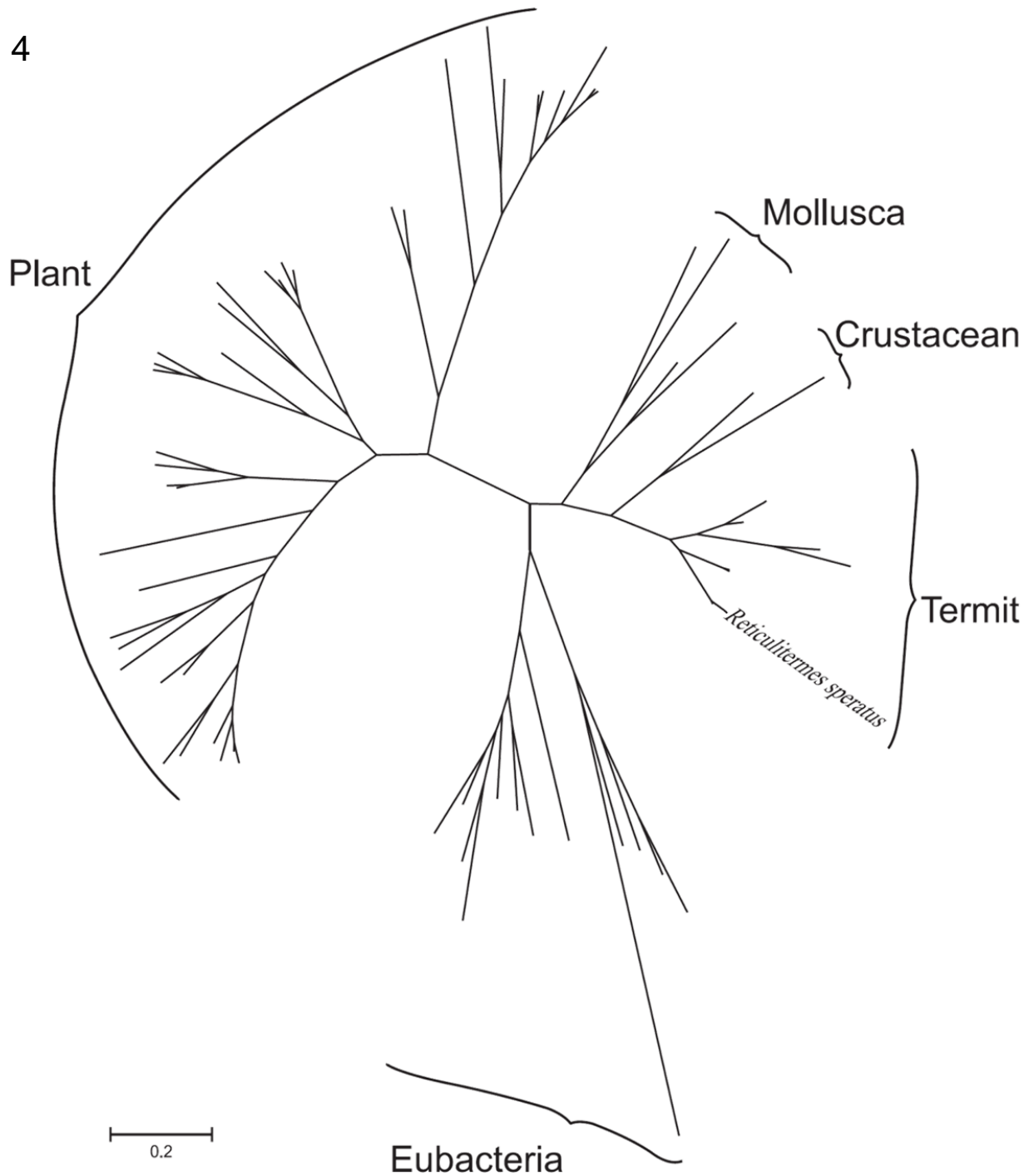


Table 1 List of members of the Gglycoside Hhydrolase Ffamily (GHF) *

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 cellulbiose.

Table 2 Classification of cellulases based on habitat living 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 as into “aquatic” and “terrestrial.”. Then, cellulases are classified into “endogenous” and “exogenous.”. Note, It should be noted that only exogenous cellulases have been so far reported for in 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	<i>Schistosoma mansoni</i>		[16]
		Annelida	Oligochaeta	Haplotaxida	<i>Eisenia andrei</i>		CazyWeb
					<i>Eisenia andrei</i>		CazyWeb
					<i>Eisenia fetida</i>	branding worm	CazyWeb
					<i>Lumbricus rubellus</i>	humus earthworm	[16]
					<i>Pheretima hilgendorfi</i>	earthworm	[88]
		Arthropoda	Insecta	Blattaria	<i>Panesthia angustipennis</i>	cockroach	[89]
					<i>Panesthia cribrata</i>	cockroach	[41]
					<i>Salganea esakii</i>	cockroach	[89]
				Coleoptera	<i>Timarcha balearica</i>	beetle	[16]
				Copelata	<i>Tribolium castaneum</i>	red flour beetle	[42]
				Hymenoptera	<i>Apis mellifera</i>	honeybee	[16]
				Isopoda	<i>Glyptotermes sp. Wyong</i>	termite	[90]
					<i>Hodotermopsis sjoestdi</i>	termite	[91]
					<i>Sinocapritermes mushae</i>	termite	[91]
				Isoptera	<i>Coptotermes acinaciformis</i>	termite	CazyWeb
					<i>Coptotermes formosanus</i>	Formosan subterranean termite	[92]
					<i>Coptotermes formosanus</i>	Formosan subterranean termite	[93]
					<i>Coptotermes formosanus Shiraki</i>	termite	[40]
					<i>Mastotermes darwiniensis</i>	termite	[94]
					<i>Nasutitermes takasagonesis</i>	termite	[91]
					<i>Nasutitermes takasagonesis</i>	termite	[90]
					<i>Nasutitermes walkeri</i>	termite	[90]
					<i>Neotermes koshunensis</i>	termite	[91]
					<i>Odontotermes formosanus</i>	termite	CazyWeb
					<i>Odontotermes formosanus</i>	termite	[91]
					<i>Reticulitermes flavipes</i>	eastern subterranean termite	[95]
			<i>Reticulitermes speratus</i>	termite	[7]		
			<i>Reticulitermes speratus</i>	termite	[90]		
			<i>Reticulitermes speratus</i>	termite	[96]		
		Orthoptera	<i>Teleogryllus emma</i>	emma field cricket	[97]		
		Malacostraca	Mysida	<i>Neomysis intermedia</i>		CazyWeb	

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

continued

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