Reappraisal of cellulase activities in mangrove wetlands resulting from preliminary investigations in East Java, Indonesia

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インドネシア・ジャワ島東部のマングローブ湿地帯での
予備的調査結果に基づくセルラーゼ活性の再評価

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抄録：本研究ではインドネシア東ジャワのパンパン湾周辺のマングローブ地域において，以前の研究でセルラーゼ活性を有することが報告されていなかった軟体動物、節足動物、および腔腸動物に属する無脊椎動物にセルラーゼ活性が広く分布することを明らかにした。さらに、セルラーゼ活性は、パンパン湾マングローブ干潟の落ち葉や堆積物、また湾内の海底堆積物にも検出され、バクテリアやメイオベントスなどの関与が示唆された。また、これまで堆積物中の有機物はタンパク質やデンプンなどの易分解性とセルロースやリグニンなどの難分解性に区分されてきたが，セルロースはヘミセルロースなどと共に生物分解を受けることから，食物連鎖の視点では準難分解性有機物として取り扱うことが適切であると考えた．更に，湿地に生息する無脊椎動物が保持しているセルラーゼが食物連鎖で果たす役割を理解するために、既報告の研究成果をレビューし，水生無脊椎動物のセルラーゼに関する情報を更新した.

Abstract: Saline wetlands are empirically known to play an important role in the decomposition of terrestrial organic matter that is difficult to degrade, such as cellulose. The true nature of this role has been underestimated for a long time, because only microorganisms inhabiting saline wetland sediments, or those symbiotically living in aquatic invertebrates, were considered responsible until very recently. Although it is still unknown whether the majority of planktonic/benthic invertebrates possess endogenous cellulase, it is necessary to comprehensively investigate the distribution of cellulase activities in saline wetland invertebrates before any further studies. In the present study, we found that cellulase activities were widely distributed in invertebrates inhabiting a mangrove area of East Java, Indonesia, including Mollusca, Arthropoda and Cnidaria species that were not reported to have cellulase activity in previous studies. Moreover, cellulase activities were also detected in fallen leaves and sediments along the coastline, or below the water surface in Pang-Pang Bay of East Java, suggesting the involvement of small organisms such as meiobenthic invertebrates. To date, organic matter in sediments has been empirically classified into easy-to-decompose organic matter（EDOM）such as protein and starch, and non-degradable organic matter（NDOM）such as cellulose and lignin. However, from the viewpoint of food chain-related research, cellulose undergoes biodegradation together with hemicellulose. We thus propose to classify cellulose as quasi-hard-to-degrade organic matter（qHDOM）. To understand the role played by invertebrates in the decomposition of qHDOM in wetlands, we therefore updated the information on aquatic invertebrates' cellulase through a review of previous studies.

Key words: CMCase plate assay; benthic invertebrates; cellulose; cellulase; Pang-Pang Bay; Indonesia

Introduction

Saline wetlands are carbon sinks that have higher carbon density compared to other ecosystems. Large amounts of organic substances and inorganic nutrients, which are known as allochthonous inputs, are transported from terrestrial regions to saline wetlands by rivers（Pant et al., 2003）. In addition to allochthonous inputs, considerable amounts of organic substances and nutrients could also originate from adjacent salt marsh plant communities and forests, and are known as autochthonous inputs（Scholz, 2011; Sudip et al., 2005）. Allochthonous organic carbons accumulate and decompose in saline wetlands and then enter shallow seawaters. This process contributes to the reproduction of organisms in saline wetlands, and it is also the reason why saline wetlands, particularly mangrove wetlands, can maintain high biodiversity and bio-productivity（e.g., Barbier et al., 2011）.

Among these terrestrial organic carbon substances, cellulose constitutes the majority. Cellulose is characterized by a difficult-to-degrade feature that is caused by its chemically stable 1,4-glucoside linkage between glucose units. It was widely mistakenly thought that cellulose could be consumed by only microorganisms（i.e., bacteria, fungi and protists）until the discovery of the gene for an endogenous cellulase（enzymes that hydrolyzes cellulose）encoded in the genome of a terrestrial invertebrate, namely, the Japanese termite, *Reticulitermes speratus*（Kolbe, 1885）（Watanabe et al., 1998）. Before that study, the ability of invertebrates to consume cellulose was ascribed to symbiotic microorganisms（such as protists）in their intestines.

To date, various invertebrates inhabiting saline wetlands have been reported to possess cellulase activities, for example, polychaetes（Kanaya et al., 2018; 2019）, large decapods（Bui and Lee, 2015; Gray et al., 2018; Kawaida et al., 2013; Kanaya et al., 2019）, planktonic copepods（Liu et al., 2015）, and bivalves（Niiyama et al., 2012a; Kanaya et al., 2019）. However, except for studies on *Corbicula japonica* Prime, 1884 inhabiting temperate saline wetlands（Niiyama and Toyohara, 2011; Sakamoto et al., 2007; Liu et al., 2019）, most of these previous studies focused only on investigating the presence of cellulase activity, but never discussed the possibility that cellulose originating from terrestrial forests or local mangrove plants could contribute to the food chain system through energy source for these invertebrates.

In the present study, we firstly investigated the distribution of cellulase activities by checking all collected large-sized benthic invertebrates found in a typical tropical mangrove wetland around Pang-Pang Bay located on East Java Island, Indonesia. In addition, fallen leaves and sediments collected from the tidal flat and bottom of Pang-Pang Bay were also investigated because previous studies revealed that environmental cellulase activity（cellulase secreted, excreted or released from organisms）could exist on those components（Liu et al., 2019）. We discuss the classification of organic

Fig. 1. Sampling locations at Pang-Pang Bay and the surrounding mangrove wetland, East Java.

Table 1. Longitude and latitude of the sediment sampling sites in Pang-Pang Bay and mangrove wetlands.

	Latitude	Longitude
St 1	8°28'52" S	114°23'13" E
St 2	8°31'08" S	114°22'05" E
St 3	8°28'11" S	114°22'11" E
St 4	8°28'11" S	114°22'05" E
St 5	8°30'46" S	114°21'23" E
St A	8°31'52" S	114°21'55" E
St B	8°31'08" S	114°22'06" E
St C	8°31'00" S	114°22'09" E
St D	8°29'16" S	114°22'29" E
St E	8°27'49" S	114°22'26" E
St F	8°28'34" S	114°21'55" E
St G	8°28'48" S	114°21'53" E
St H	8°28'52" S	114°21'26" E

substances from the viewpoint of food-chain and propose a new category, "quasi-hard-to-degrade organic matter", for cellulose and other terrestrial organic matter that could be decomposed by wetland invertebrates. Besides those data, we also summarize and update the knowledge about aquatic invertebrates that possess cellulase activities.

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Material & Methods

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1. Environment of Pang-Pang Bay, East Java, Indonesia

Pang-Pang Bay is an oval-shaped bay that is located east of Muncar, East Java, and extends approximately 10 km from north to south and 2.5 km from east to west. The center of Pang-Pang Bay is located at 8°30'04" S, 114°22'23" E. The water depth in the center is approximately 5 m at high tide（Fig. 1）. The entrance of the bay is approximately 3.5 km wide facing the north. Along the east coast of the bay, a wide mangrove forest area is preserved as Alas Purwo National Park. From the western to the southern coast, however, most of the area is used for cultured fisheries and farming, except for some afforested mangroves. Along the western coast, up to 1 km of the muddy flat can be exposed in the offshore direction at low tide. On the eastern coast, the sediments are also muddy but contain cobbles and gravel.

2. Cellulase activity assay on macrobenthos, sediments, and fallen leaves distributed in mangrove wetlands around Pang-Pang Bay, East Java, Indonesia

Larger benthic invertebrates inhabiting the Pang-Pang Bay mangrove wetland were collected for the CMC

Table 2. Cellulase activity of large invertebrates, sediments, and leaves collected from Pang-Pang Bay and mangrove wetland, East Java, Indonesia. Species names are followed by WoRMS.

Subject		number of specimen	CMC Plate Assay	Remarks
Macro Benthos				
Cnidaria (Phylum)				
Anthozoa	*Zoanthus* aff. *kuroshio*	two samples from one specimen	detected	Fig. 4-5
Mollusca (Phylum)				
Bivalvia	*Tegillarca granosa*	two specimens	detected	Fig. 6-2,3
	Tegillarca nodifera	two specimens	detected	Fig. 5-5,6
	Gafrarium pectinatum	two specimens	detected	Fig. 4-4
	Paratapes undulata	two specimens	detected	Fig. 6-4,5
	Saccostrea malabonensis	two specimens	detected	Fig. 4-2
Gastropoda	*Cerithium coralium*	two specimens	detected	Fig. 4-1
	Murex trapa	one specimen	detected	Fig. 5-2
	Pirenella nipponica	one specimen	detected	Fig. 4-6
	Supplanaxis niger	one specimen	detected	Fig. 5-4
	Telescopium telescopium	one specimen	detected	Fig. 5-3
Arthropoda (Phylum)				
Decapoda	*Uca chlorophthalmus*	one specimen	detected	Fig. 5-1
Sediment				
	St. 1		not detected	Fig. 2-1
	St. 2		not detected	Fig. 2-2
	St. 3		not detected	Fig. 2-4
	St. 4		detected	Fig. 3-1
	St. 5		not detected	Fig. 3-2
	St. A		not detected	Fig. 3-3
	St. B		not detected	Fig. 3-4
	St. C		not detected	Fig. 3-5
	St. D		not detected	Fig. 3-6
	St. E		detected	Fig. 3-7
	St. F		not detected	
	St. G		not detected	
	St. H		not detected	
Leaf				
	Fresh (*Bruguiera gymnorhiza*)	one specimen	not detected	Fig. 2-5
	floating (orange yellow in color)	one specimen	detected	Fig. 2-6
	floating (brown in color)	one specimen	not detected	Fig. 2-7

（carboxymethyl cellulose）assay and photographed for species identification. Intestinal glands were removed from gastropods and bivalves and subjected to the cellulase activity assay described below. For cnidarians, parts of each individual were subjected to the assay. For small decapods, the whole body of each individual was cut with scissors and homogenized with proper quantity of Tris-HCl Buffer（pH=7.5）immersed the sample, and a part of the homogenized sample was used for the assay. Leaves that had dropped onto the surface of the mangrove wetland or floated on seawater were collected and cut into small pieces for the assay. In addition, surface sediments（approximately 1 cm sediment thickness）were collected from five sites in the mangrove wetland at low tide. Moreover, submarine sediments approximately 2 cm deep were collected by a TFO（**T**okyo University, **F**isheries **O**ceanography Laboratory）corer from five sites inside Pang-Pang Bay（Fig. 1, Table 1）.

Samples of the benthic invertebrates, leaves and sediments were then investigated using the CMC Assay. Briefly, these samples were placed in holes created on agar plates（0.1% CMC and 1% agarose in Tris-HCl Buffer, pH=7.5）. After incubation for 24 hours at room temperature（20～25℃）, 0.1%（w/v）Congo Red dissolved in 0.1 M Tris-HCl Buffer（pH=7.5）was added to cover the surface of the plates and the plates were shaken for 30 min. After the Congo Red was discarded, the plates were washed with 1 M sodium chloride（NaCl）overnight. A transparent halo would be observed around the sample if the CMC had been decomposed, as shown in Table 2. Specialists were consulted for the identification of each taxon.

3. Updates of invertebrates possessing endogenous cellulase

Niiyama and Toyohara（2011）and Tanimura et al.（2013）summarized the aquatic invertebrates that possess cellulase activities. In addition to the data of these previous studies, we also reviewed the Carbohydrate-Active enZYmes（CAZy）database（www.cazy.org）as well as the GenBank database, and updated their works by adding new species processing endogenous cellulase gene or cellulase enzymic activity.

Results

1. Cellulase activities in leaves and sediments

Newly dropped（fresh）and decayed（brown colored）leaves of *Bruguiera gymnorhize*（Linnaeus, 1758）showed no cellulase activity, while cellulase activity was detected on orange-yellow leaves of the same species. Regarding various sediments, cellulase activity was detected in surface sediment of 1 site in the mangrove wetland, and in submarine sediment of 2 sites in Pang-Pang Bay. Results are shown in Figs. 2-6 and Table 2.
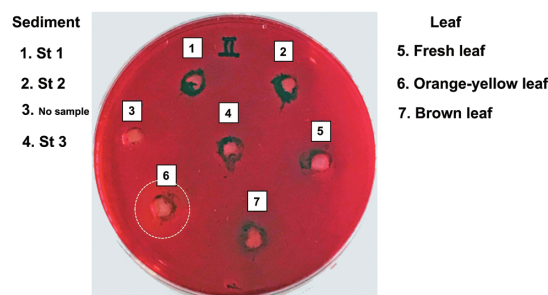

Fig. 2. Result of the CMC-plate assay for sediments and leaves collected from mangrove wetlands and Pang-Pang Bay, East Java.
1. Sediment St-1, 2. Sediment St-2, 3. No sample, 4. Sediment St-3, 5. Fresh leaf, 6. Orange-yellow leaf, 7. Brown leaf. Halo indicated by dotted circle shows cellulase activity.
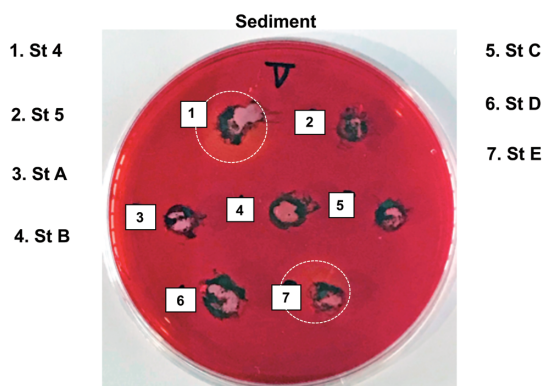

Fig. 3. Result of the CMC-plate assay for sediments collected from mangrove wetlands and Pang-Pang Bay, East Java.
1. St-4, 2. St-5, 3. St-A, 4. St-B, 5, St-C, 6. St-D, 7. St-E. Halos indicated by dotted circle show cellulase activity.

2. Cellulase activities of larger benthic invertebrates

Most of the large invertebrates collected around the Pang-Pang Bay mangrove wetlands clearly showed cellulase activities, namely *Zoanthus* aff. *kuroshio* Reimer & Ono in Reimer, Ono, Iwata, Takishita, Tsukahara & Maruyama, 2006
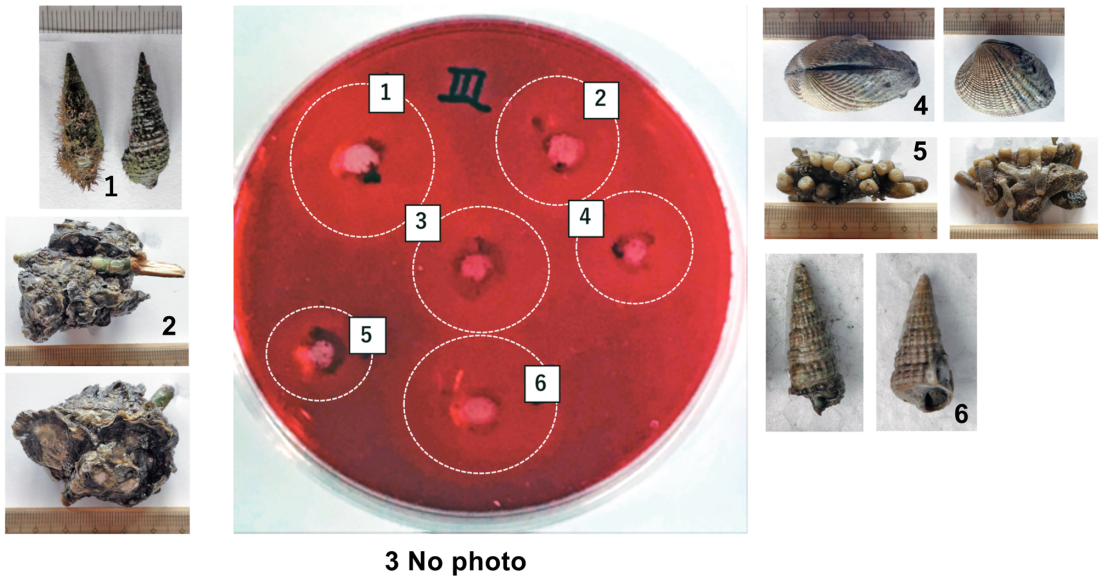

3 No photo

Fig. 4. Results of the CMC-plate assay for large invertebrates collected from mangrove wetlands around Pang-Pang Bay, East Java.
1. *Cerithium coralium*（Gastropoda）, 2. *Saccostrea malabonensis*（Bivalvia）, 3. Unknown（no photo）, 4. *Gafrarium tumidum*（Bivalvia）,
5. *Zoanthus* aff. *kuroshio*（Cnidaria）, 6. *Pirenella nipponica*（Gastropoda）. Halos indicated by dotted circle show cellulase activity.

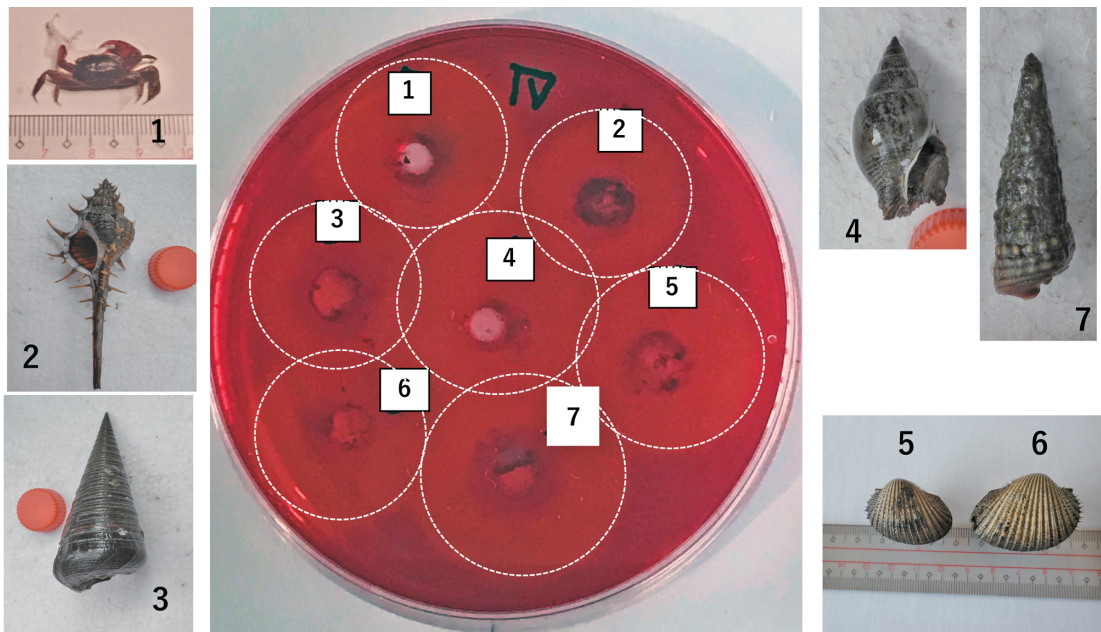Fig. 5. Results of the CMC-plate assay for large invertebrates collected from mangrove wetlands around Pang-Pang Bay, East Java.
1. *Uca chlorophthalmus*（Decapoda）, 2. *Murex trapa*（Gastropoda）, 3. *Telescopium telescopium*（Gastropoda）, 4. *Supplanaxis niger*（Gastropoda）, 5 & 6. *Tegillarca nodifera*（Bivalvia）, 7. *Pirenella nipponica*（Gastropoda）. Halos indicated by dotted circles show cellulase activity.
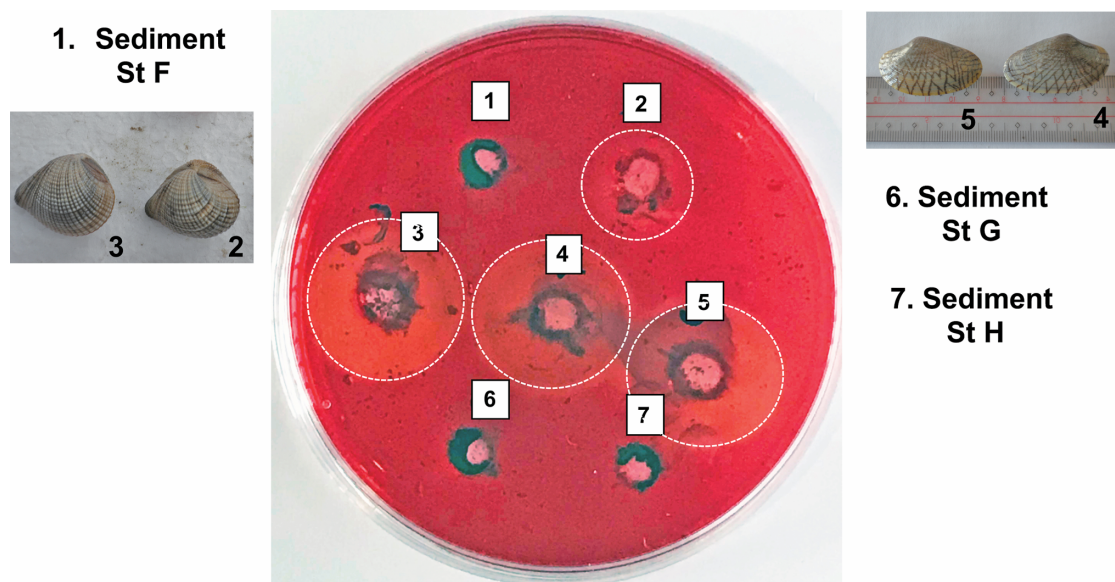
Fig. 6. Results of the CMC-plate assay for large invertebrates collected from mangrove wetlands around Pang-Pang Bay, East Java. 1. Sediment St. F, 2 & 3. *Tegillarca granosa*（Bivalvia）, 4 & 5. *Paratapes undulata*（Bivalvia）, 6. Sediment St-G, 7. Sediment St-H. Halos indicated by dotted circle show cellulase activity.

（Cnidaria）, *Tegillacera granosa*（Linnaeus,1758）, *T. nodifera*（Maetens, 1860）, *Saccostrea malabonensis*（Faustino, 1932）, *Gafrarium pectinatum*（Linnaeus, 1758）, *Paratapes undulata*（Born, 1778）（Mollusca, Bivalvia）, *Cerithium coralium* Kiener, 1841, *Murex trapa* Rörding, 1798, *Supplanaxis niger* Quoy & Gaimard, 1833, *Telescopium telescopium* Linnaeus,1758（Mollusca, Gastropoda）, and *Uca chlorophthalmus*（H. Milne Edwards, 1837）（Crustacea, Decapoda）. Results are shown in Figs. 4 and 5 and Table 2.

3. Updating of marine invertebrates that have been reported to possess endogenous cellulase

Animals that have been reported to possess cellulase were distributed in various phyla, namely Porifera, Cnidaria, Platyhelminthes, Annelida, Crustacea, Mollusca, Rotifera, Echinodermata, Chordata, and Vertebrata. Among these phyla, Annelida, Crustacea and Mollusca have larger numbers of members（Table 3）.

Discussion

1. Cellulase activities in leaves

Large amounts of leaves that originate from mangrove trees float in Pang-Pang Bay and its upstream rivers. According to Liu et al.（2019）, cellulase activities are positively correlated with the organic matter contents of the sediment in saline wetlands, and in many wetlands, leaves constitute the largest proportion of the organic matter. We therefore investigated the cellulase activities associated with the fallen leaves in Pang-Pang Bay. As shown in Fig. 2 and Table 2, newly dropped（fresh）and decayed（brown colored）leaves of *B. gymnorrhiza* showed no cellulase activity, while cellulase activity was detected on orange-yellow leaves of the same species. These facts suggest that the cellulase activity might not be related to the condition（color）of the leaves. Matsunaga et al.（1999）reported that the proteins contained in mangrove fallen leaves were decomposed by bacteria, as indicated by the change in the C/N ratio. Cellulase activity detected in the leaves might be from some bacteria that were attached to the fresh leaves.

2. Cellulase activities of sediments

Sediments have recently been reported to play an important role in cellulose decomposition in saline wetlands by

acting as a scaffold for secreted cellulase from large size（macro-）invertebrates, or from other sources of independent cellulase such as excreted or escaped cellulase from various organisms that inhabit wetlands（Liu and Toyohara, 2012; Liu et al., 2019）. In the present study, we also investigated the cellulase activity in sediments, and confirmed that the surface sediments from two sites in the saline wetland, as well as the bottom sediment from two sites of Pang-Pang Bay, exhibited cellulase activity. The bottom sediments in the other two sites of the bay had halos in the CMC-plate assay （Figs. 2, 3）. There were two possibilities about why the sediment showed cellulase activity: 1）the activity could be ascribed to meio- and/or micro-benthos that live in the sediment. For example, cellulase activity in lugworms has been reported by Niiyama and Toyohara（2011）. However, cellulase activities in meiobenthic organisms, which could be separated using microscopes, have rarely been investigated and reported（e.g., Kanaya et al., 2019）. This community could be investigated in the future to better understand the mechanism of cellulose decomposition in saline wetlands. 2） The activity could also be derived from macrobenthos. Liu et al.（2019）reported that a brackish bivalve, *C. japonica*, secretes its own cellulase on the saline wetland sediment, suggesting a complex composition of cellulase activities in saline wetland sediment. However, since the present study investigated sediment samples without any separation or treatments to separate meiobenthos, the cellulase activity detected in the sediments of Pang-Pang Bay could be from both origins described above.

3. Cellulase activities of larger benthic invertebrates

The cellulase activities in larger benthic invertebrates, mainly the phyla Arthropoda and Mollusca, were also confirmed by the present study, as shown in Figs. 4-6, and Table 2. Among these invertebrates, the cellulase activity of *Zoanthus* aff. *kuroshio* was the first report on the phylum Cnidaria.

The method of detecting cellulase activities in this study could not differentiate endogenous cellulase, because detected cellulase activity could also originate from symbiotic bacteria/protists. This fact also applies to the result of the fallen leaves and sediments. However, many bivalve species such as *T. granosa, C. japonica*, and *Crassostrea gigas* （Thunberg, 1793）have a crystalline style, which contains endogenous carbohydrate hydrolases, including cellulase （Ogino et al., 2018）. In *T. telescopium* taken from mangrove wetland of Queensland, Australia, Alexander et al.（1979） also observed a crystalline style and showed its cellulase activity. This fact suggests that the cellulose activities detected from the 2 molluscs（*T. telescopium* and *T. granosa*（=*Anadara granosa*（Linnaeus, 1758））in the present study might also be ascribed to their endogenous cellulase. Except for *T. telescopium* and *T. granosa*, the other large invertebrates listed in Table 2 were reported here for the first time to have cellulase activities. Further investigation, however, is needed to clarify whether these cellulase activities are endogenous or not.

4. Update of marine invertebrates that have been reported to possess cellulase

Saline wetlands and mangrove forests prosper on coastlines, particularly in subtropical to tropical regions. They are well known for their high bio-diversities and bio-productivities（e.g., Dittmann, 1995; 2000; Vargas, 1987）. These features make these environments ones that should be preserved globally（e.g., Spalding et al., 2010）. With their ability to decompose terrestrial organic matter, aquatic invertebrates might strongly affect these features and be worth investigating. As shown in Table 3, in addition to the list of endogenous cellulase-possessing aquatic invertebrates that were previously summarized by Tanimura et al.（2013）, more large invertebrates inhabiting saline wetlands have been reported to have endogenous cellulase or detected together with cellulase activities. Among these newly found cases, most decapods（crabs）showed strong cellulase activity, suggesting that they are both carnivores and deposit（detritus） feeders. Integration of these results mainly based on previous and present studies reveals that more than 85 species have cellulase activities, suggesting that the activity could be ubiquitous in aquatic invertebrates, including members of Cnidaria, Platyhelminthes, Annelida, Arthropoda, Mollusca, Rotifera, Echinodermata, and Chordata. Among those phyla, Annelida, Crustacea and Mollusca have the largest numbers of members in saline wetlands.

Table 3. List of animals showing cellulose activities reported in previous studies and the present study. UP: unpublished data, DS: Direct submission, GB: Carbohydrate-Active enZYmes（CAZy）database, Asterisk（*）: Since *Labyrinthula zosterae* D. Porter & Muehlstein can consume cells of eel grass（Muehlstein and Porter, 1991）, this species probably possesses cellulase activity. Since species names are followed by WoRMS, some names used in references are changed in this table.

Organisms (Taxa in Alpahbet)	Cellulase A:activity detected E: endogenous N: No activity detected EM: endogenous molecular	Habitat F: freshwater P: planktonic N: nectonic B: benthic	Feeding Habit C: carnivorous H: harbivorous O: omnivorous L: limnivorous D: detrivorous	Tropical-Subtropical Habitatl	Reference	Accession Number
Labyrinthulea (Class)						
Labyrinthula zosterae	A*	P	H		Muehlstein & Porter (1991)	
Cnidaria (Phylum)						
Zoanthus aff. *Kuroshio*	A	B	H?	◎	present study	
Platyhelminthes (Phylum)						
Turbellarea(Class)	A	B	C		Yamada et.al (2013) Toyohara et.al (2012)	
Annelida (Phylum)						
Oligochaete (Class)						
Tubificidae sp.	N	B	L		Niiyama & Toyohara (2011)	
Polychaeta (Class)						
Hediste atoka	A	B	O?		Kanaya et.al (2019)	
Hediste diadroma	A	B	O?		Kanaya et.al (2019)	
Heteromastus sp.	A	B	D		Kanaya et.al (2019)	
Notomastus sp.	A	B	D		Kanaya et.al (2019)	
Perinereis aibuhitensis	A	B	D?		Niiyama & Toyohara (2011)	GB ANR02619
Perinereis nuntia	A	B	L		Niiyama & Toyohara (2011)	
Perinereis nuntia brevicirris	E	B	D			GB BAK20401
Tylorrhynchus osawai	A	B	D		Kanaya et.al (2019)	
Crustacea (Phylum)						
Copepod (Subclass)						
Acartia spinicauda	A	P	H	◎	Liu et.al (2015)	
Acartia sp.	A	P	H	◎	Liu et.al (2015)	
Pseudodiaptomus annandalei	A	P	H	◎	Liu et.al (2015)	
Pseudodiaptomus trihamatus	A	P	H	◎	Liu et.al (2015)	
Maxillopoda (Class)	A				Yamada et.al (2013)	
Amphipod (Order)						
Gammaridae gen. sp.	A	P	B/L		Niiyama & Toyohara (2011)	
Hirondella gigas	E	B	D		Kobayashi et al (2012)	
Decapoda (Order)						
Acetes indicus	A	N	O	◎	Liu et.al (2015)	
Acetes japonicus	A	N	O	◎	Liu et.al (2015)	
Gammarus pulex	EM	F, B	C/H/D		Sambles et al. (UP)	EH272443, 273125, 275051, 269547 269941; GHF 9
Caridina multidentata	A	N	O		Niiyama & Toyohara (2011)	
Exopalaemon styliferus	A	N	unknown	◎	Liu et al. (2015)	
Fenneropenaeus merguiensi	A	N	O	◎	Liu et al. (2015)	
Ilyoplax pusilla	A	B	D		Kanaya et al. (2019)	
Macrophthalmus japonicus	A	B	D		Kanaya et al. (2019)	
Metapenaeus ensis	A	N	O	◎	Liu et al. (2015)	
Metapenaeus lysianassa	A	N	O	◎	Liu et al. (2015)	
Mierspenaeopsis sculptilis	A	N	unknown	◎	Liu et al. (2015)	
Neocaridina denticulate	A	B	O		Niiyama & Toyohara (2011)	
Palaemon semmelinkii	N	N	unknown	◎	Liu et al. (2015)	
Penaeus monodon	A	N	O	◎	Liu et al. (2015)	
Scopimera globosa	A	B	D		Kanaya et al. (2019)	
Cherax destructor	EM	F, B	C		Crawford et al. (DS)	DQ359104, 359105; GHF 9
Cherax tenuimanus	EM	F, B	C		Crawford et al. (up)	DQ359106, 359107, 359108, 359109; GHF 9
Cherax quadricarinatus	EM	F, B	C		Crawford et al. (DS)	DQ359114; GHF 9
Cherax robustus	EM	F, B	C		Crawford et al. (DS)	DQ359110, 359111; GHF 9
Euastacus balanensis	EM	F, B	C		Crawford et al. (up)	DQ359115, 359116; GHF 9
Euastacus robertsi	EM	F, B	C		Crawford et al. (up)	DQ359115, 359117, 359118; GHF 9
Procambarus clarkii	EM	F, B	C		Osuna-Jimenez et al. 2014	JZ135474, 135475, 135380, 135553; GHF 9
Homarus americanus	EM	B	C		Towle & Smith (2006)	CN951583, 853218, 85436 ; GHF 9
Uca chlorophthalmus	A	B	D	◎	present study	
Mictyris platychele	EM	B	C		Gray & Linton (DS)	KX158846, 158847; GHF 9
Gecarcoidea natalis	EM	B	C/H/D		Gray & Linton (DS)	KX158844; GHF 9
Coenobita brevimanus	EM	F, B	C/H/D		Gray & Linton (DS)	KX158848; GHF 9
Coenobita rugosu	EM	F, B	C/H/D		Gray & Linton (DS)	KX158849; GHF 9
Macrobrachium lar	EM	F, B	C		Crawford et al. (up)	DQ359103; GHF 9
Diplostraca						
Cladocera (Order)						
Daphnia pulex	EM*	F, P	H		Colbourne et al. (2011)	GL732637, 732546; GHF 9
Harpacticoida (Order)	A	P	H		Toyohara et al. (2012)	
Hexanauplia (Order)	A	B/P			Toyohara et al. (2012)	
Mysid (Order)						
Acathomysis thailandica	E	N	unknown	◎	Niiyama et al. (2012a)	
Daphnia pulex	E	P	H		Colbourne et al. (2011)	
Euastacus sp. SL-2005	E (Fragmental)	B	H/C/D		Linton et al. (2006)	
Gecarcoidea natalis	E	B	H/C/D		Gray et al. (2018)	
Heloecius cordiformis	E	B	D	◎	Bui et al. (2014)	
Ilyograpsus daviei	E	B	C/H	◎	Bui et al. (2014)	
Limnoria quadripunctata	E	B	H		Kinga et.al (2010)	
Macrophthalmus setosus	E	B	C/H	◎	Bui et al. (2014)	
Mesopodopsis orientalis	A	N	C/H/D		Niiyama et al. (2012a)	
Mesopodopsis tenuipes	N	N	C/H/D		Niiyama et al. (2012a)	
Mictyris platycheles	E	B	C/H/D		Gray et al. (2018)	
Neomysis intermedia	E	N	C/H		GB BAL60587	
Neosarmatium trispinosum	E	B	C/H/D	◎	Bui et al. (2014)	
Notacanthomysis hodgarti	N	N	unknown	◎	Niiyama et al. (2012a)	
Parasesarma erythrodactyla	E	B	C/H/D		GB AIT11911	
Phopalophthalmus egregius	A	N	C/H	◎	Niiyama et al. (2012a)	
Phopalophthalmus orientalis	A	N	C/H	◎	Niiyama et al. (2012a)	
Pseudohelice subquadrata	E	F, B	C	◎	Bui et.al (2014)	

Ostracoda (Order)	E	B/P	C/H/D		Yamada et.al (2013)	
Sergestidae (Family)						
Acetes sibogae	E	N	C/H/D	◎	Niiyama et al. (2012a)	
Acetes indicus	N	N	C/H/D	◎	Niiyama et al. (2012a)	
Acetes japonics	N	N	C/H/D	◎	Niiyama et al. (2012a)	
Chelura terebrans	E	B?	H(wood)		Kern et al. (2013)	
Macrobrachium nipponense	E	F, B	C		Jin et al. (2013)	
Mollasca (Phylum)						
Bivalvia (Class)						
Tegillarca granosa	A	B	H/D	◎	Niiyama et al. (2012b)	
Tegillarca nodifera	A	B	H/D	◎	present study	
Anodonta woodiana	A	F, B	H/D		Niiyama & Toyohara (2011)	
Corbicula japonica	E	F, B	H/D		Niiyama & Toyohara (2011) Sakamoto et al. (2007)	
Gafrarium pectinatum	A	B	H/D	◎	present study	
Laternula marilina	A	B	H/D		Kanaya et al. (2019)	
Macoma contabulata	A	B	H/D		Kanaya et al. (2019)	
Mizuhopecten yessoensis	E	B	H/D		GB BAH85844	
Mytilus edulis	E	B	D		Xu et al. (2001)	
Nuttallia japonica	A	B	H/D		Kanaya et al. (2019)	
Paratapes undulata	A	B	H/D	◎	present study	
Ruditapes philippinarum	A	B	H/D		Niiyama & Toyohara (2011)	
Saccostrea malabonensis	A	B	H	◎	present study	
Xylophaga rikuzenica	E	B	H(wood)		GB BBJ26607	
Gastropoda (Class)						
Ampullaria crossean	E	B	H/D		Li et al. (2009)	
Aplysia kurodai	E	B	H		Rahman et al. (2014)	
Bellamya sp.	E	B	H/D		Ueda et al. (2016)	
Biomphalaria glabrata	E	B	H/D		GB AAT76428	
Cerithium coralium	A	B	H/D	◎	present study	
Cipangopaludina sp. A	A	B	H/D		Niiyama & Toyohara (2011)	
Cipangopaludina sp. B	A	B	H/D		Niiyama & Toyohara (2011)	
Cipangopaludina sp. C	A	B	H/D		Niiyama & Toyohara (2011)	
Clithon retropictus	A	B	H/D (biofilm)		Niiyama & Toyohara (2011)	
Murex trapa	A	B	H/D	◎	present study	
Physa acuta	A	B	C/H/D (biofilm)		Niiyama & Toyohara (2011)	
Pirenella nipponica	A	B	H/D	◎	present study	
Semisulcospira libertina	A	B	H/D		Niiyama & Toyohara (2011)	
Semisulcospira nipponica	A	B	H/D		Niiyama & Toyohara (2011)	
Semisulcospira reiniana	A	B	H/D		Niiyama & Toyohara (2011)	
Supplanaxis niger	A	B	H/D	◎	present study	
Haliotis discus hannai	EM	B	H(algae)		Suzuki et al. (DS)	AB125892
Telescopium telescopium	A,E	B	H/D	◎	Alexander et al. (1979) present study	
Apylysia kurodai	EM	B	H(algae)		Rahman et al. (DS)	AB920344(GHF 45)
Terebralia palustris	A	B	H/D		Niiyama & Toyohara (2011) Yamada et al. (2013)	
Rotifera (Phylum)						
Adineta ricciae	E	F(also marine), P	H/C		Szydlowski et al. (2015)	
Echinodermata (Phylum)						
Apostichopus japonicus	E	B	D/C		Zhao et al. (2014)	
Mesocentrotus nudus	E	B	H/C		Nishida et al. (2007)	
Chordata (Phylum)						
Oikopleura dioica	E	P	H/D		Seo et al. (2004)	

5. A new category, "quasi-hard-to-degrade organic matter (qHDOM)", which can be utilized by many more aquatic invertebrates than previously thought

Organic matter excreted or secreted from living organisms or that escapes from their dead bodies exists in natural sediments. Some of this matter is easily decomposed (easy-to-decompose organic matter, EDOM) into inorganic nitrogen and phosphates and finally recycled by photosynthetic organisms such as phytoplankton and macro-algae, while some of it is not so easily decomposed, such as insoluble organic matter and corrosion products. Although different kinds of insoluble organic matter and corrosion products share the same chemical feature of insolubility, other features such as their origins and their formulation vary due to the diversity of the chemical/microbiological reaction processes performed by different organisms (Ishiwatari, 2010). In newly produced sediments, except for lipids, proteins/amino acids and carbohydrates, the composition of 40 ~ 90% of the total organic matter is not fully understood (Ishiwatari, 2003). The origin of this unknown organic matter includes lignin, for example, which is the major component of higher plants. The so-called "non-degradable organic matter (NDOM)" accumulates in the sediments, becomes buried, and will finally become coal or petroleum though the geological process called diagenesis under high temperature and pressure in sediments (Hutton et al., 1994). Like lignin, cellulose and hemi-cellulose originate from terrestrial plants. They are also not easily decomposed in comparison with EDOM such as lipids and proteins (Reid et al., 2000; Pariatamby and Kee, 2016). However, in contrast to the highly complex chemical structures of lignin, the chemical stability of cellulose and hemi-cellulose comes from their relatively stable linkages (α-glucoside bonds) and the crystallization of their mono-polysaccharide chains. Since they can be decomposed by a kind of enzyme collectively called cellulase, or hemi-cellulase

in the case of hemi-cellulose（e.g., Yoshida, 2009）, we propose to call them quasi-hard-to-degrade organic matter（qHDOM）. In spite of regional differences, muddy sediments distributed around saline wetlands, including mangrove areas, all contain several percent organic matter（Dittmann, 1995; Wijensma et al., 1998; JICA, 1999）. This organic matter is originally derived from dead leaves or twigs of higher plants（Matsunaga et al., 1999）. Since cellulose constitutes a large fraction of the content of leaves of these plants, muddy sediments that contain many dead leaves and twigs should have a considerable amount of cellulose.

After the discovery of endogenous cellulase in land animals（Watanabe et al., 1998）, many aquatic animals were also found to possess endogenous cellulases, as shown in Table 3. Moreover, a brackish clam, *Corbicula japonica*, was demonstrated to not only decompose cellulose in the sediment but also absorb glucose released via cellulase activity（Liu et al., 2019）. Based on these facts, it became increasingly clear that cellulase is a useful tool to change qHDOM into low-molecular-weight EDOM（such as glucose in this case）, which allows animals that possess cellulase to be able to use qHDOM as alternative energy sources（foods）. Members of Polychaeta, Crustacea, Mollusca, Echinodermata, Platyhelminthes and Amphipoda are well known as benthic animals that inhabit saline wetlands, including mangrove areas. Polychaeta, Crustacea, and Mollusca are especially dominant in terms of species numbers and populations（Dittmann, 1995; Vargas, 1987; Wijesma et al., 1998）. These invertebrates are thought of as carnivorous or omnivorous; however, they also include deposit（detritus）and suspension feeders（Vargas, 1987）. Yurimoto et al.（2014）found plant fragments and amorphous organic flocks as well as frustules of diatoms in the digestive tubes of *T. granosa* collected from mangrove wetlands in Peninsular Malaysia. The photographs in Yurimoto et al. 2014（their Fig. 2）show that these plant fragments appear to be vascular bundles consisting of lignin and amorphous organic flocks. The results of our study indicate that *T. granosa* possessed cellulase activity. These two results suggest that *T. granosa* can digest both phytoplankton such as diatoms as well as cellulose included in amorphous flocks. Recently Kanaya et al.（2019）reported that several benthic invertebrates which have cellulase activities can digest qHDOM as alternative energy sources in temperate saline wetlands of northern Japan. In the present study, we also found many types of other macro-benthos inhabiting mangrove wetlands, including sediment and/or filter feeders, herbivores, and omnivores, that showed strong cellulase activities. These facts imply that these macrobenthic organisms can also use cellulose as an energy source, like *T. granosa*. The ability to use this alternative energy source from qHDOM could be widely distributed in many phyla of invertebrates; thus, further studies focusing on not only the enzyme activity but also the existence of the endogenous genes encoding the corresponding enzymes are needed. To understand the food source of these invertebrates, investigations using stable isotopes of δ^{13}C and δ^{15}N will also be required.

Sample collection

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Acknowledgments

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