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Guaiacol oxidation activity of herbivorous land crabs, *Chiromantes haematocheir* and *Chiromantes dehaani*

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The land crabs, *Chiromantes haematocheir* (Akate-gani) and *Chiromantes dehaani* (Kurobenkei-gani) inhabit seaside forests in Japan. The crabs mainly consume plant material and its detritus. Therefore, they are expected to possess the ability to degrade the major components of biomass, cellulose and lignin in order to digest plant materials. In this study, we analyzed biomass-degrading activities of the land crabs, especially guaiacol oxidation activity, which seems to be related to lignin degradation. Cellulase activity was detected from almost all gut samples including the stomach, midgut gland and intestine of all dissected crabs. Conversely, high guaiacol oxidation activity was detected in the midgut gland of all *C. dehaani* and several female *C. haematocheir* crabs. This is consistent with a previous study showing that female crabs were more herbivorous than male crabs were and observation that *C. dehaani* crabs are more herbivorous than *C. haematocheir*. Guaiacol oxidation activity might play an important role in the herbivorous behavior of land crabs.

[**Key words:** Land crab; *Chiromantes haematocheir*; *Chiromantes dehaani*; Lignin; Biomass; Guaiacol]

Land crabs influence ecosystems of tropical coastal forests (1). Several reports have suggested that matter cycle between forests and waters is essential for ecosystems (2,3). Material supplies from seaside forests are thought to be important for the preservation of aquatic resources of inshore waters. Several organisms, including land crabs in forests, may have important roles in the supply of materials. In seaside forests of Japanese provinces, several crustaceans, including land crabs and amphipods, survive by eating fallen leaves. Therefore, it is possible that these crustaceans play important roles in the cycle of matter between forests and coastal waters.

Chiromantes haematocheir, or Akategani (red-handed crab) is a land crab that lives in the maritime forests of Japan (Fig. 1A). This land crab is thought to play major roles in the matter cycle because of its mode of life; it lives in seawater during its larval stage and returns to forests after metamorphosing into small crabs. *Chiromantes dehaani*, or Kurobenkeigani is a closely related species of *C. haematocheir* and often observed in the vicinity of the habitat of *C. haematocheir* (Fig. 1B). Many land crabs are herbivorous or omnivorous and survive by eating plant material and its detritus (4). Akategani and Kurobenkeigani land crabs are also thought to be partly herbivorous since they eat fallen or green leaves and survive only on plant material in the laboratory. Therefore, the herbivorous characteristics of these crabs might contribute to the matter cycle between seaside forests and coastal waters.

It is probable that the land crabs possess enzymes for biomass degradation, such as cellulase and lignin peroxidase. To date, active cellulases and laminarinases have been identified in the digestive juice, gut, midgut gland and hepatopancreas of a wide variety of crustacean species including land crabs (4). Since genes encoding endo-β-1,4-glucanase have been reported in several land crabs, endogenous synthesis of endo-β-1,4-glucanase may be largely responsible for the substantial cellulase activities reported in these species. Furthermore, β-1,4-glucosidase was recently identified in one land crab, *Gecarcoidea natalis* (5). However, little molecular evidence is available for the cellulase enzyme, cellobiohydrolase in land crabs. It is thought that a two enzyme system may be adequate for the catabolism of cellulose to glucose by crustaceans and other arthropods. On the other hand, a cellobiohydrolase is present in the wood boring isopod, *Limnoria quadripunctata* (6). Symbiotic bacteria living in guts may contribute to cellulase activity by producing cellulases containing cellobiohydrolase. Moreover, it is unclear whether lignin or tannin degradation occurs in the guts of land crabs. With respect to lignin degrading enzymes, phenol oxidase (laccases) and heme containing peroxidases, namely lignin, manganese and multifunctional (versatile) peroxidase have been extensively studied in fungi including white rot fungus and several bacterial species (7). However, there is little evidence for animal enzymes showing lignin degradation activity. If novel enzymes for biomass (especially lignin) degradation could be identified from the land crabs, these would contribute to the efficient use of biomass resources. In this study, we analyzed the biomass-degrading activities of the land crab,

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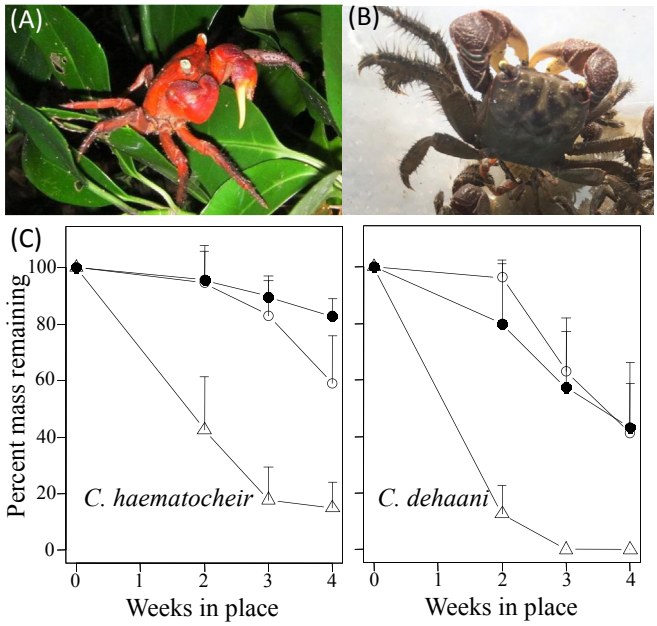

FIG. 1. (A) *C. haematocheir*. (B) *C. dehaani*. (C) Consumption of leaves by the land crabs. Land crabs were fed with green (open circles) and brown (closed circles) *Machilus* leaves and green Zelkova leaves (open triangles). Crabs with 2–3 cm body size were used. The bars indicate the standard errors of three independent feeding experiments.

C. haematocheir and *C. dehaani*. We demonstrate the occurrence of guaiacol oxidation activity, which might be related to lignin degradation in the guts of land crabs.

MATERIALS AND METHODS

Dissection of land crabs, preparation of crude enzyme extracts *C. haematocheir* and *C. dehaani* were caught in Kashima forest, a maritime forest in Ishikawa prefecture, and maintained with leaves. For dissection, crabs were incubated for 1 h on ice to induce a state of apparent death. Then, crabs were soaked in 500-fold Osvan solution to sterilize their body surface. After washing with sterilized phosphate buffered saline (PBS), crabs were dissected. The stomachs, midgut glands and intestines were removed, and the guts were homogenized with a Potter–Elvehjem tissue grinder in 5 ml of PBS. In crabs, stomach exists just beneath mouth and midgut gland fills most of the area around the stomach. Intestine joins posterior part of stomach. It runs postero-medially in the abdomen and ends in rectum. In this study, rectum was dissected as a part of intestine. Crude extracts were used for enzyme assays after the centrifugation and filtering sterilization of supernatants. Protein concentrations were determined using the TaKaRa BCA Protein Assay Kit (Takara, Shiga, Japan).

Lignin degradation assays To investigate the lignin-degrading activity of land crab guts, we used the guaiacol oxidation method (8). The reaction was initiated by mixing 100 μl of enzyme sample with 900 μl of reaction reagent (4 mM guaiacol, 1 mM $MnSO_4$, 1 mM H_2O_2). To estimate the production of tetraguaiacol, absorbance at 465 nm was measured every minute for 10 min using a spectrophotometer SmartSpec Plus (Bio-Rad, Tokyo, Japan). To determine guaiacol oxidation activity, one unit was defined as the amount of enzyme required to produce 1 μmol of tetraguaiacol in 1 min (9). The molar extinction coefficient of 25,500 $M^{-1}cm^{-1}$ was used for tetraguaiacol. The effects of inhibitors, including EDTA, NaN_3, 2-mercaptoethanol and SDS on peroxidase activity were investigated by incorporating each inhibitor into the assay mixture. To study the pH profile associated with guaiacol oxidation activity, the pH of the reaction mixture was adjusted with modified universal buffer (MUB). The MUB stock solution was made by dissolving 6.3 g of boric acid, 14.0 g of citric acid, 11.6 g of maleic acid, 12.1 g of Trizma base, and 19.5 g of NaOH in 500 ml of deionized water. Aliquots (50 ml) were adjusted to the required pH (pH 2.5–11.0) with 1.0 M HCl or 1.0 M NaOH and diluted to 100 ml with deionized water. The buffer was diluted 5-fold in the final assay solution. For the optimum temperature, crude extracts of *C. haematocheir* midgut gland were diluted 100-fold to perform slow reactions. The reaction was performed for 10 min at various temperatures (5°C, 10°C, 15°C, 20°C, 25°C, 30°C, 35°C, 40°C, 45°C and 50°C).

Degradation of lignin was analyzed using size-exclusion high performance liquid chromatography (HPLC). Reactions were performed using commercial lignin (Kanto Chemical Co. Inc., Tokyo, Japan). Assay mixtures containing 0.5% lignin and crude extract were incubated at 37°C for 24 h in the presence of 1 mM H_2O_2. Then, 60 μl of reaction mixture was analyzed by HPLC. Chromatography was performed at a flow rate of 0.3 ml/min with an Asahipak GF-310 HQ column (7.5 × 300 mm) (Showa Denko, Tokyo, Japan), with PBS over 80 min. The molecular weights of reaction products were estimated based on molecular weight markers (polystyrene sulfonate 29,100 Da and 3610 Da; Polymer Standard Service, Mainz, Germany) and dNTPs of approximately 500 Da (Takara).

Specific activity staining of the gels Non-dissociating SDS-PAGE was performed using 10% polyacrylamide. The resolving buffer was Tris-HCl (pH 8.8) and the reservoir buffer was Tris-glycine (pH 8.3); both buffers contained 0.1% SDS. Samples were mixed 1:1 with Ez-Apply sample buffer (ATTO, Tokyo, Japan) (100 mM Tris-HCl, pH 8.8, 20% sucrose, 0.06% bromophenol blue, 2% SDS). Dithiothreitol (DTT) was omitted from the sample buffer. Molecular mass was calibrated using the Prism Protein Marker (Amresco, Solon, OH, USA). To perform the stain, SDS was removed by washing the gel for 30 min at room temperature in two changes of a solution containing 10 mM Tris-HCl, pH 7.5. Staining was performed by incubating the gel in a solution of 4 mM guaiacol and 1 mM H_2O_2, in 20 mM sodium phosphate, pH 6.0, at room temperature for at least 20 min.

Cellulase activity For cellulase activity, we analyzed endoglucanase activity using carboxymethyl cellulose (CMC) as a substrate. The Somogyi-Nelson method was performed to estimate cellulase activities (10). Reaction mixtures (60 μl) containing 30 μl of 2% CMC in 50 mM potassium phosphate buffer (pH 7.0) and 30 μl of crude enzyme extracts were incubated at 37°C for 30 and 60 min. After adding 60 μl of Somogyi solution (Wako, Tokyo, Japan) to the reaction mixtures, the samples were boiled for 10 min. The samples were mixed with 60 μl of Nelson solution (Wako) and diluted with 1 ml of deionized water to analyze absorbance at 660 nm. One unit of enzyme activity was defined as 1 μmol of glucose-equivalent reducing sugar released per minute.

RESULTS

***C. haematocheir* and *C. dehaani* eat leaves** Because the ecology of the land crabs *C. haematocheir* and *C. dehaani* has not been fully studied, we first investigated whether these land crabs eat leaves. We reared these land crabs by providing *Machilus* and *Zelkova* leaves as bait for 1 month. *Machilus* is an evergreen tree and *Zelkova* is a deciduous tree. As shown in Fig. 1C, these land crabs were suggested to eat green and brown leaves. Although there seemed to be a preference for green leaves of *Zelkova*, the weight of all leaves decreased; however, the reason for this preference was not clear. Furthermore, according to the consumption rate of leaves, *C. dehaani* seems to have higher herbivorous character than *C. haematocheir*. This is consistent to our observation that *C. dehaani* crabs do not show cannibalistic behavior unlike *C. haematocheir*. Taken together, these findings clearly indicate that both land crabs should possess the ability to degrade biomass.

Guaiacol oxidation activity of crude extracts from *C. haematocheir* and *C. dehaani* guts To demonstrate the biomass-degrading ability of the land crabs, we studied the ability of crude gut extracts to degrade lignin and cellulose. We were especially interested in the lignin-degrading activity of the crabs, which is of high importance since lignin is thought to be a barrier to the utilization of cellulose and the activity of land crabs has not been reported before. To investigate lignin-degrading activities of gut extracts, we performed a guaiacol oxidation assay. This assay is considered a lignin peroxidase assay since the structure of guaiacol is often observed in lignin (8). The chemical can be also used as a substrate for laccase and manganese peroxidase. In this reaction, the substrate is converted to tetraguaiacol by tetramerization with oxidation (Fig. 2A). The red product was determined by measuring the absorbance at 465 nm. We dissected 21 *C. haematocheir* and 10 *C. dehaani* crabs and prepared crude extracts. Among these *C. haematocheir* crabs, several female crabs exhibited extremely strong guaiacol oxidation activity (Fig. 2A, Table 1). Conversely, only one male *C. haematocheir* crab among 9 males exhibited guaiacol oxidation

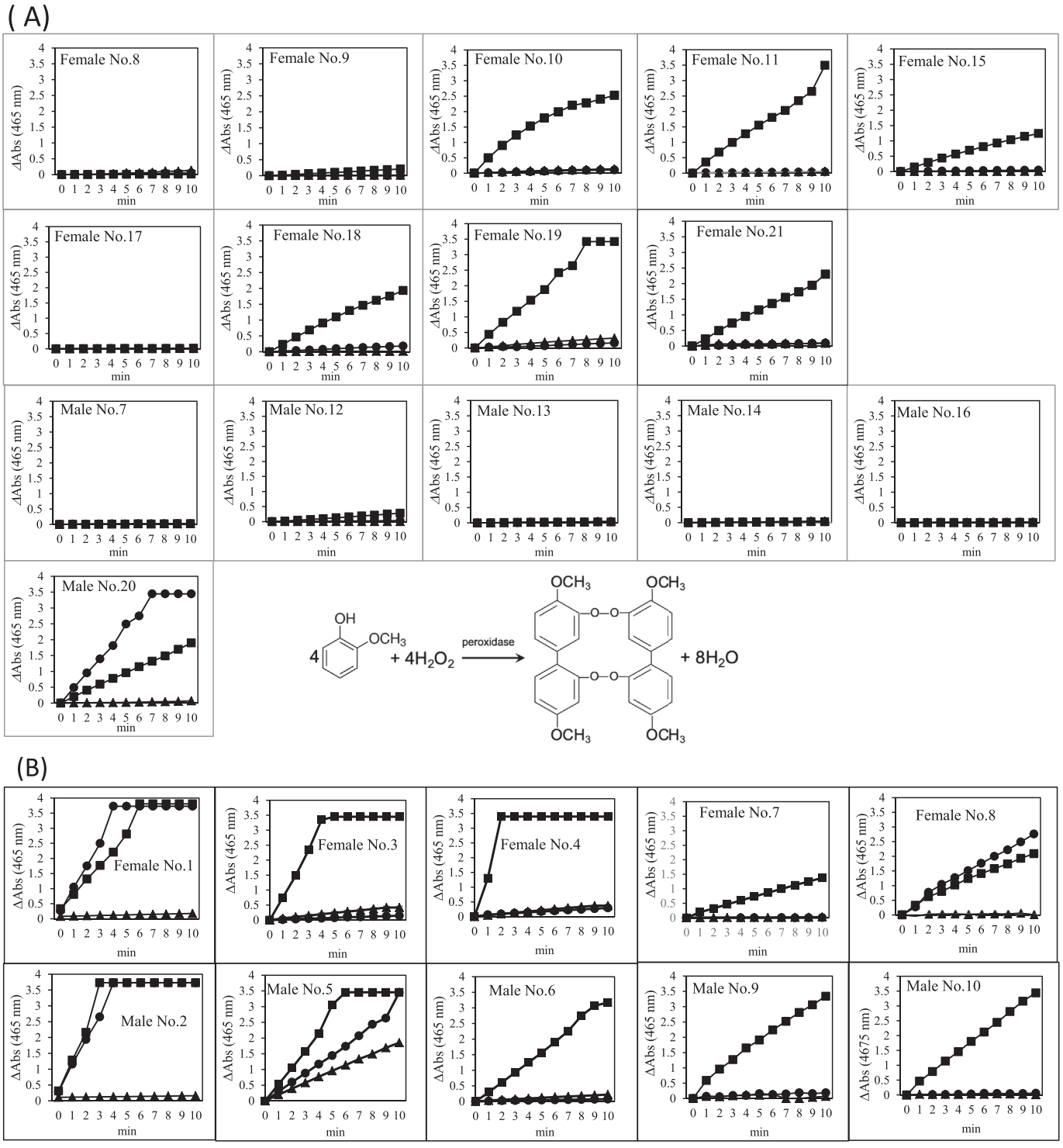

FIG. 2. Time course of guaiacol oxidation for *C. haematocheir* and *C. dehaani* land crabs. (A) Guaiacol oxidation reaction by peroxidases. Twenty-one *C. haematocheir* (A) and 10 *C. dehaani* (B) land crabs were dissected and crude extracts of stomachs (closed circles), midgut glands (closed squares), and hindguts (closed triangles) were analyzed.

activity, while 7 female crabs among 12 females showed activity (Figs. 2A and S1). This suggests that female crabs have a better ability to oxidize guaiacol. It is possible that the activity is related to higher preference of females for plant materials. In fact, a previous report suggested that females contained more plant food material in their foreguts than males did in other type of crab (11). We also studied another closely-related land crab, *C. dehaani*, which has more herbivorous characteristics than *C. haematocheir*. Ten *C. dehaani* crabs were analyzed (five males and five females). We detected guaiacol oxidation activity from all *C. dehaani* crabs (Fig. 2B). Table 1 shows the comparison of the guaiacol oxidation activities between several *C. haematocheir* showing the activity and all *C. dehaani*. Mean values were 0.355 U/mg protein and 1.321 U/mg protein for *C. haematocheir* and *C. dehaani*, respectively. According to the result of unpaired *t* test, the difference was significant ($p = 0.008$). Thus, the guaiacol

TABLE 1. Guaiacol oxidation activities of land crabs.

	Midgut gland	Stomach	Intestine
C. haematocheir crab			
Female No. 3	0.17	–[a]	–[a]
Female No. 10	0.08	ND	ND
Female No. 11	1.29	ND	ND
Female No. 15	0.03	ND	ND
Female No. 18	0.23	ND	ND
Female No. 19	0.48	ND	0.01
Male No. 20	0.22	0.01	ND
C. dehaani crab			
Female No. 1	1.95	0.69	ND
Male No. 2	2.32	1.21	0.02
Female No. 3	1.31	0.02	0.01
Female No. 4	2.05	0.05	0.06
Male No. 5	0.96	0.46	0.29
Male No. 6	0.54	0.01	0.04
Female No. 7	2.15	0.01	0.04
Female No. 8	0.33	0.43	ND
Male No. 9	0.52	0.03	0.03
Male No. 10	1.08	0.02	ND

U/mg protein. ND, not detected.
[a] *C. haematocheir* crab No. 3 was not dissected into three digestive tracts.

oxidation activities of *C. dehaani* seems to be higher than those of *C. haematocheir*. This result also suggested that the guaiacol oxidation activity might play an important role in the herbivorous behavior of land crabs. Furthermore, this remarkable activities were detected mainly from midgut glands. The guaiacol-oxidizing enzyme might be expressed in the midgut gland, which is equivalent to the liver and pancreas of mammals, and be secreted to stomach for the digestion of plant refractory materials. In several land crabs, strong activities were also detected from the stomach fractions.

CMCase activity of land crabs We also studied another biomass-degrading activity, cellulase activity. To analyze this activity, CMC was used as a substrate. The CMCase activity is regarded as an endoglucanase activity. The results from the assay using the Somogyi-Nelson method are shown in Table 2. Unlike in the case of guaiacol oxidation assays, the endoglucanase activity was detected from almost all guts of all dissected land crabs. With regard to the enzyme activity, we were not able to detect any significant difference between species or sexes. We could not detect CMCase activity from intestines of 5 land crabs. This seems to be due to lack of contents in these intestines.

TABLE 2. CMCase activities of land crabs.

	Midgut gland	Stomach	Intestine
C. haematocheir crab			
Female No. 9	0.14	0.40	1.58
Female No. 10	0.10	0.17	1.60
Female No. 11	0.07	0.08	0.14
Male No. 12	0.80	0.60	0.26
Male No. 13	0.98	0.87	0.33
Male No. 14	0.34	0.60	ND
Female No. 15	0.10	0.05	0.12
Male No. 16	0.02	0.03	ND
Female No. 17	0.02	0.04	0.03
Female No. 18	0.38	0.62	ND
Female No. 19	0.42	1.33	0.54
Male No. 20	0.55	1.10	1.76
C. dehaani crab			
Female No. 1	0.53	0.21	0.76
Male No. 2	0.45	0.30	0.78
Female No. 3	0.91	0.91	1.26
Female No. 4	0.92	0.88	2.92
Male No. 5	2.80	0.68	2.35
Male No. 6	1.83	3.13	6.24
Female No. 7	0.46	0.64	0.94
Female No. 8	0.73	0.17	0.85
Male No. 9	0.49	0.50	ND
Male No. 10	0.65	0.76	ND

U/mg protein; ND, not detected.

Properties of guaiacol oxidation activity We next studied the characteristics of the guaiacol oxidation activity by adding several inhibitors or omitting several components to compare the activity to those of well known lignin-degrading enzymes such as laccase, Mn-dependent peroxidase and lignin peroxidase. As for laccase inhibitor, NaN_3, the inhibitor exhibited no inhibitory effect at 0.1 mM on the guaiacol oxidation activity of the land crab midgut gland (Fig. 3A), although this concentration has been reported to sufficiently inhibit fungal laccase (12). Conversely, 1 and 5 mM NaN_3 resulted in a significant reduction in guaiacol oxidation activity. Furthermore, the lack of H_2O_2 abolished the activity (Fig. 3B). Thus, the enzyme was not a typical laccase, since the enzyme normally does not require H_2O_2 for reaction. With respect to Mn-dependent peroxidase inhibitor, EDTA did not affect the activity (Fig. 3B). This result is consistent with the result that lack of Mn^{2+} in the reaction mixture had no effect on the peroxidase activity (Fig. 3B). It is probable that the enzyme is an Mn^{2+}-independent peroxidase, unlike other well-known lignin peroxidases. SDS showed an inhibitory effect (Fig. 3C), and 0.5% 2-mercaptoethanol and heating at 100°C for 10 min also abolished the enzymatic activity (data not shown). These results indicate that guaiacol oxidation is catalyzed by an enzyme but not caused by chemical reactions.

The pH profile of the enzyme activity was also determined using universal buffer system. Enzyme activities were measured at pH 2.5–10.0. As shown in Fig. 3D, the optimum pH for the reaction was close to pH 6.0. The optimum pH corresponded to the pH of *C. haematocheir* land crab midgut gland, pH 6.3. Furthermore, this enzyme acted at 5–60°C and the optimum temperature was 25–55°C (Fig. 3E). The wide range of optimum temperatures may reflect the normal temperature range for land crabs (15–35°C).

To investigate the molecular weight of the enzyme, we performed a zymogram for guaiacol oxidation activity, as previously described for other oxidases (13). After non-dissociating SDS-PAGE was performed by omitting DTT from the sample buffer, the gel was stained with 4 mM guaiacol and 1 mM H_2O_2. Fig. 4 shows that a clear band could be observed from the midgut gland sample. No band was observed from the stomach and intestine samples. The stained band was estimated to be approximately 57 kD in size. Because only a single band was detected, the peroxidase activity of the midgut gland seemed to derive from a single enzyme.

Effect of *C. haematocheir* midgut gland extract on lignin To investigate the ability of the land crab midgut extract to react with lignin, we also analyzed the activity by using commercial lignin. Since lignin used in this assay is alkaline treated for solubilization, the structure may differ from that of natural lignin. Therefore, it is possible that reactivity of the solubilized lignin is lower than that of natural lignin. We estimated the activity of midgut gland by analyzing the change in molecular weight of the lignin substrate using gel-filtration HPLC. Lignin-derived products were detected by analyzing the UV absorption. Fig. 5 shows that lignin signals were somewhat affected following incubation with midgut gland extract for 24 h at 37°C. The main peak moved slightly to the low molecular-weight fraction compared to the heat-inactivated control sample. Although the degradation seems to be weak, this may be due to the altered structure of commercial lignin.

DISCUSSION

In this study, we were able to demonstrate the biomass-degrading activities of two closely related Japanese land crabs,

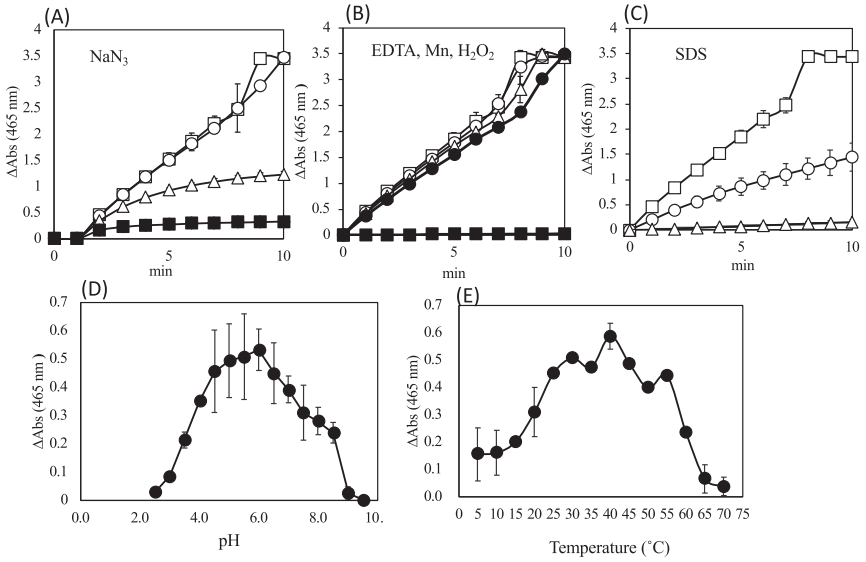

FIG. 3. Effect of inhibitors, components, pH, and temperature on guaiacol oxidation activity of midgut gland of *C. haematocheir* No.11. (A) Effects of NaN_3. Open squares, control; open circles, 0.1 mM; open triangles, 1 mM; closed squares, 5 mM. (B) Effects of EDTA. Open squares, control; open circles, 10 mM; open triangles, 25 mM. Reactions lacking Mn^{2+} (closed circles) or H_2O_2 (closed squares) were also performed. (C) Effects of SDS. Open squares, control; open circles, 0.1%; open triangles, 1%. (D) Guaiacol oxidation activities at various pH levels. (E) Guaiacol oxidation activity at various temperatures. The bars indicate the standard deviations of three independent reactions.

C. haematocheir and *C. dehaani*. To date, some previous studies have reported that herbivorous land crabs and crayfish, such as *G. natalis* and *Cherax destructor*, possess endo-β-1,4-glucanase, β-1,3-glucanase (laminarinase), and β-glucosidase (5,14–18). These previous studies demonstrated that land crabs and crayfish can produce endogenous enzymes for the degradation of major components of plant biomass, such as cellulose and hemicellulose. Since we were also able to detect cellulase activity from *C. haematocheir* and *C. dehaani*, it is possible that these land crabs also produce endogenous cellulase enzymes, as observed with *G. natalis* (Christmas island red crab). With respect to the degradation of lignin or tannin, which are also major components of plant biomass, few studies have been conducted on land crab enzymes compared with those on cellulases. Several land crabs, including *G. natalis*, *Neosarmatium smithi* and *Birgus latro*, can assimilate or modify plant tannins (19–21). However, the mechanism through which this occurs is still poorly understood, although several factors such as high gut pH, oxidative conditions, redox potential and enzymes have been considered. Therefore, the findings of the present study might represent the first report of lignin or tannin related enzyme in land crab guts. In the case of *C. haematocheir*, the guaiacol oxidation activity was mainly detected from female land crabs. Since a previous report showed that female land crabs are more herbivorous than male crabs are (12), the enzyme activity detected in this study might be correlated to dietary preference. Furthermore, this activity was

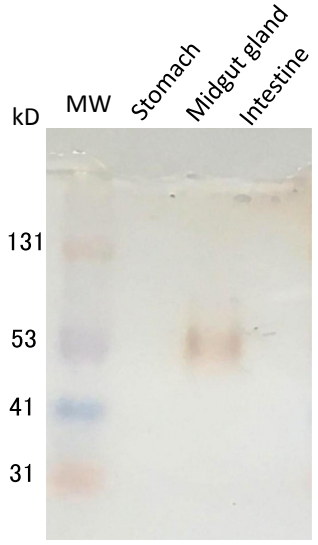

FIG. 4. Electrophoretic analysis of the guaiacol oxidation activity in the stomach, midgut gland and intestine of *C. haematocheir* No. 10. SDS-PAGE (10%) was run under non-dissociating conditions in the absence of DTT and stained with a solution of 4 mM guaiacol and 1 mM H_2O_2 in 20 mM sodium phosphate, pH 6.0. Pre-stained molecular markers were obtained from Amresco.

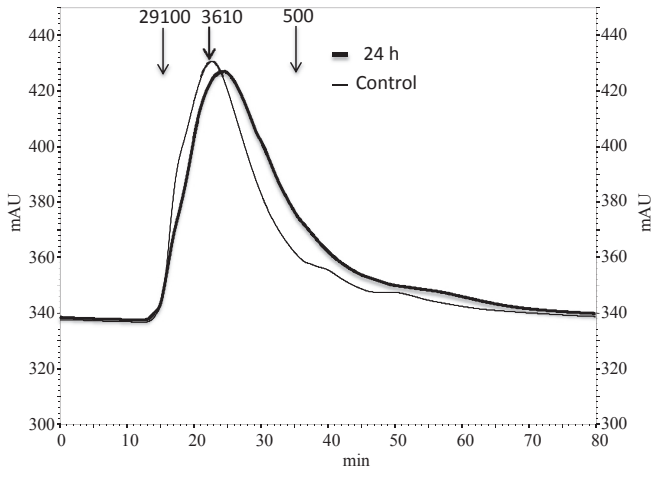

FIG. 5. Gel permeation chromatography of commercial lignin after incubation with crude extracts of midgut gland from *C. haemtocheir* No. 19 crab. GF-310 HQ column (7.5 × 300 mm) was eluted with PBS. Polystyrene sulfonate standards and dNTPs were used as molecular weight markers. Peaks from the enzymatic reaction are compared with those from a heat-inactivated control sample.

detected from all *C. dehaani* land crabs, that are more herbivorous than *C. haematocheir*, and mean value of the activities was significantly higher than that of *C. haematocheir*. These results might also reflect the relationship between the guaiacol oxidation activity and herbivorous characters of land crabs. However, it is still unclear whether the activity is cause or result of the herbivory. Conversely, we could not show that cellulase activity affects the feeding behavior of land crabs, because cellulase activities were detected from all dissected crabs and guts irrespective of sexes or species. Though CMCase activities of *C. dehaani* appear to be higher than those of *C. haematocheir*, it is difficult to estimate the difference because of considerable variation between crabs. This result seems to be consistent to the previous report that cellulase activity is not correlated with the dietary preference of land crabs (16).

We used guaiacol as a substrate to study the lignin related activity. While crude extracts prepared from midgut glands of *C. haematocheir* and *C. dehaani* exhibited high guaiacol oxidation activity, the enzymes showed weak activity against 2,6-DMP (data not shown), which is often used as a substrate in lignin peroxidase assays (13). This result suggests that the enzyme from these land crabs specifically catalyzes guaiacol oxidation. The guaiacol oxidation activity might not cause the complete degradation of lignin, since the guaiacol oxidation activity did not exhibit high reactivity with commercial lignin. However, this weak reactivity may be sufficient to separate cellulose from natural lignin in biomass by altering its structure. Alternatively, the guaiacol oxidation activity may act well to natural lignin, although we could not use the natural substrate in this study because of difficulty in its extraction.

We could not rule out the possibility that symbiotic microorganisms express the guaiacol oxidation enzyme, because it was impossible to remove microorganisms from guts of land crabs. However, we are thinking that the possibility is low so far. We already isolated 34 bacterial strains from *C. haematocheir* guts and these bacteria did not show any guaiacol oxidation activity (data not shown). Furthermore, we also studied the number of microorganisms from each organ of the land crabs. The number of colonies isolated from midgut gland was estimated to be about one hundredth of those from stomach and intestine. Since only midgut glands showed high guaiacol oxidation activity in many crabs, the activity seems not to be caused by microorganisms. Previous report also suggested that symbiotic microorganisms do not play major roles in biomass digestion because space for microorganisms should be insufficient and ingested bacteria are effectively digested in land crabs (4). Thus, the guaiacol oxidation activity studied in this study seems to be mainly produced by land crab itself.

Guaiacol oxidation activity has also been studied in many plants (22–25). This enzyme is known to be involved in auxin and ethylene metabolism, redox reactions in plasma membranes, and cell-wall modifications (lignification and suberization). Therefore, it is possible that the enzymatic activity detected in the land crab guts plays an important role in lignin degradation or modification.

Here, we report for the first time the guaiacol oxidation activity of land crab guts. This activity may be responsible for lignin degradation and the assimilation of plant biomass. We are now performing RNA-seq analysis for midgut gland of *C. haematocheir* to obtain the gene coding for the guaiacol peroxidase, and transcripts for several laccases, cellulases and hemicellulases were already discovered. Since we could determine the molecular weight of the enzyme in this study, it would help us to identify cDNAs for the peroxidase. If the guaiacol peroxidase gene would be cloned, further molecular analyses and application to biotechnology field will become possible.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jbiosc.2019.02.012.

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