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# Purification and characterization of 1,3-β-D-glucanase from *Eisenia foetida*

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ABSTRACT

1,3-β-D-glucanase from the earthworm *Eisenia foetida* was purified to electrophoretically homogeneous state. The molecular weight of the purified enzyme was estimated 42,000 by SDS-PAGE. The N-terminal amino acid sequence of the enzyme was very similar to those of CCF-I from *E. foetida* and CCF-like protein from *Aprorrectodea caliginosa*. The enzyme was most active at pH 6.0 and 60 °C, and stable at pH 6.0–10.5 and 60 °C. The enzyme was inhibited by metal ions $Mn^{2+}$, $Cu^{2+}$, $Fe^{2+}$, $Al^{3+}$, and hydrolyzed 1,3-β-D-linked oligosaccharides (triose, tetraose, and pentose) into glucose, and biose as end products.



## 1. Introduction

Earthworm belonging to the phylum Annelida that are known to hydrolyze carbohydrates, suggesting their ability to digest leaf litter, roots, yeast, brown algae, and fungi in soil (Prat, Charrier, Deleporte, & Frenot, 2002). In recent year, interest on the application of earthworms in composting for the breakdown of a wide range of organic residues, including sewage sludge, animal waste, and industrial refuse has increased tremendously. We have studied cold adapted raw-starch digesting amylases, anti-plant viral serine protease, and cold adapted carboxymethyl cellulase from the cell-free extract of *Eisenia foetida* (Ueda, Asano, Nakazawa, Miyatake, & Inouye, 2008; Ueda et al., 2008; Ueda et al., 2010). In our screening program for glycoside hydrolases from earthworm, we have found 1,3-β-D-glucanase from the cell-free extract of the earthworm *E. foetida*.

1,3-β-D-glucanases represent a well-known class of enzymes wide spread in bacteria, fungi, plants, and marine animals. They are classified into two groups; exo-1,3-β-D-glucanase (EC 3.2.1.58) and endo-1,3-β-D-glucanase (EC 3.2.1.39). 1,3-β-D-glucanases catalyse the hydrolysis of 1,3-β-D-glucosidic linkages in 1,3-β-D-glucan. This polymer is a major component of fungal cell walls and a major structural and storage polysaccharides (in the form of laminarin) in marine macroalgae (Hong, Cheng, Huag, & Meng, 2005). The physiological functions of 1,3-β-D-glucanase are distinct and depend on their sources (Kikuchi, Shibuya, & Jones, 2005). In plants, these enzymes are believed to be involved in many important aspects of plant physiology and development, such as germination, growth defense against pathogens, flowering, cellular and tissue development and differentiation, and probably other roles. In fungi, several of these enzymes have roles during cell separation in unicellular organisms, and the development of cell wall architecture in yeasts and filamentous fungi. In bacteria, these enzymes take part in the degradation of polysaccharides that can be present in their natural environment and be used as an energy source. In animal, the 1,3-β-D-glucanases are commonly found in marine echinoderms which take part in digestion of algal food and also play some important roles in embryogenesis. In Annelida earthworm, 42 kDa protein named coelomic cytolytic factor 1 (CCF-1) has revealed significant homology with the putative catalytic region of 1,3-β-D- and 1,4-β-D-glucanases (Beschin et al., 1998). Beschin et al. showed that CCF-1 efficiently bind both 1,3-β-D-glucan and lipopolysaccharide. Although, neither native CCF-1or recombinant CCF-1 was shown to exert 1,3-β-D-glucanase. It suggests that the CCF-1 protein of *E. foetida* coelomic fluid likely plays a role in the protection of earthworms against microbes.

Little information about 1,3-β-D-glucanase from *E. foetida* has been known up to now. Therefore, we describe the isolation, purification, and characterization of 1,3-β-D-glucanase from *E. foetida*.

## 2. Materials and methods

### 2.1. Chemicals

Avicel was from Asahikasei Co. (Tokyo, Japan). Curdlan, xylan from *Betula pendula* and rice starch were from Sigma-Aldrich Co.

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(St. Louis, USA). Laminarin from *Eisenia bicyclis* was from Nacalai tesque (Kyoto, Japan). Carboxymethyl cellulose and laminarin from *E. bicyclis* were from Nacalai Tesque, INC (Kyoto, Japan). 1,3-β-D-linked oligosaccharides and chitin were from Seikagaku Co. (Tokyo, Japan). Chitosan 7B was from Funakoshi Co., Ltd. (Tokyo, Japan). Xylooligosaccharide was from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Pachyman from Icelandic Moss was from Megazyme International Ireland Ltd. (Wicklow, Ireland).

### 2.2. Animal strain, coelomic fluid excruding and homogenation

The experiments were done with earthworm *E. foetida* which were obtained from Nagane Industry Inc. (Sapporo, Japan) and those at nearly the same age group were used in the experiments. The earthworms were washed, conserved with wet filter paper and starved for 24 h at 20 °C. Then, they were freeze dried, pounded in mortar and the resulting powder (10 g) was suspended in pH 7.0 50 mM Tris–HCl buffer. The above process was done to introduce the earthworms to extrude coelomic fluid through epidermal dorsal pores. The suspension was gently mixed in a reciprocal shaker for 1 h at 4 °C.

### 2.3. Enzyme assay and protein determination

1,3-β-D-glucanase activity was measured by determining the amount of reducing sugar released from laminarin. The amount of enzyme activity required to form an amount of reducing sugar corresponding to 1 μmol of glucose per min of reaction time was regarded as one unit of enzyme activity. The reaction mixture consisted of 0.4% laminarin in pH 6.0 0.1 M acetate buffer and enzyme to give a final volume of 0.3 ml. After incubation for 20 min at 37 °C, the amount of reducing sugar contained in the sample was then determined according to the method of Somogyi-Nelson (Somogyi, 1952, standard assay method). Protein was assayed by the method of Bradford (Bradford, 1976) with bovine serum albumin as the standard. Protein contents in the effluents were monitored by measuring the absorbance at 280 nm.

### 2.4. Purification of 1,3-β-D-glucanase

All purification steps were done at 4 °C, unless otherwise noted.

*Step 1*: The homogenated suspension of *E. foetida* described in Section 2 was centrifuged at 27,000 × *g* for 30 min. Protease inhibitor cocktail at the concentration of 2% was added to the supernatant. The suspension was centrifuged at 27,000 × *g* for 30 min. The supernatant was added with 2% streptomycin sulfate and stirred for 30 min. The suspension was centrifuged at 27,000 × *g* for 30 min, and the supernatant was used as the starting material for purification.

*Step 2*: The supernatant was loaded onto a DEAE-Toyopearl 650 M column [2.5 cm (inner diameter) × 30 cm] equilibrated with 50 mM Tris–HCl buffer, pH 7.0 (buffer A). The bound enzyme was eluted with buffer A containing 0.3 M NaCl. The active fractions were dialyzed with Micro acylizer (Asahi kasei Co., Ltd.).

*Step 3*: The dialyzed enzyme solution was loaded onto a Resource Q column (the column volume, 6 ml) equilibrated with 50 mM Tris–HCl buffer, pH 7.0. The bound enzyme was eluted with 50 mM Tris–HCl buffer (pH 7.0) containing 0.6 M NaCl.

### 2.5. Effects of pH and temperature on enzyme activity

The enzyme activities were measured by the standard assay method with laminarin as substrate at various pHs and temperatures. The buffer systems used were 0.1 M sodium acetate buffer (pH 3.5–6.0), 0.1 M sodium phosphate buffer (pH 6.0–8.0), 0.1 M Tris–HCl buffer (pH 7.0–9.0), 0.1 M sodium carbonate-sodium hydrogen carbonate buffer (pH 9.0–10.0). The effect of temperature on enzyme activity was examined at 10–80 °C.

### 2.6. Effects of pH and temperature on enzyme stability

The pH effect on the enzyme stability was by incubating the enzyme for 30 min at 37 °C in the following buffers of 0.1 M: sodium acetate buffer (pH 3.5–6.0), sodium phosphate buffer (pH 6.0–8.0), Tris–HCl buffer (pH 7.0–9.0), glycine–NaOH buffer (pH 9.0–10.0). For measuring the thermal stability, the purified enzyme was incubated in 0.1 M Tris–HCl buffer (pH 8.0) for 30 min at various temperatures in a range of 20–80 °C. After the incubation, the remaining activity was measured under the standard assay conditions.

### 2.7. Molecular mass

The molecular mass was estimated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) following the method of Laemmli (Laemmli, 1970) with Page Ruler™ unstained protein ladder (Fermentas Life Sciences INC., Ontario, Canada). Protein bands were detected by staining with Coomassie Brilliant Blue R-250.

### 2.8. N-terminal amino acid sequence

Proteins were separated using SDS-PAGE and transferred onto a polyvinylidene fluoride membrane. The membrane was washed extensively with water, stained with 0.25% Coomassie Brilliant Blue R-250, 5% aqueous methanol, and 7.5% acetic acid for 5 min, and then de-stained with 90% aqueous methanol for 10 min. A portion of the membrane containing the desired protein band was cut out, and the protein was extracted from the membrane, followed by sequencing the N-terminal amino acid sequence by an automated protein sequencer (Applied Biosystems, Model Procise® Model 491HT, Foster City, CA, USA).

### 2.9. Substrate specificity

The activity of the purified 1,3-β-D-glucanase was tested on various polymers with β-glycosidic bonds such as laminarin from *E. bicyclis*, curdlan, pachyman, avicel, carboxy methyl cellulose, colloidal chitin, chitosan, xylan from *B. pendula*, xylooligosaccharide. In each case, degradation was assayed by the production of reducing sugars and measured as described above.

### 2.10. Thin-layer chromatography (TLC)

The hydrolysis products from laminarin and 1,3-β-D-linked oligosaccharides were analyzed as follows. Reaction mixtures consisting of 21.7 ml of 0.1% laminarin from *E. bicyclis* in 0.1 M acetate buffer (pH 6.0), 0.3 ml of enzyme solution (2.5 U/ml), and 20 μl of 2% sodium azide was incubated at 37 °C for 0.3, 0.7, 1, 1.5, 2, 3, 6, and 27 h. After each reaction time, the mixture was boiling for 10 min. Reaction mixtures consisting of 1 ml of 1 mg 1,3-β-D-linked oligosaccharides (biose, triose, tetraose, and pentose) in 0.1 M acetate buffer (pH 6.0), 50 μl enzyme solution (2.5 U/ml) were incubated at 25 °C for various time duration. The reaction products for laminarin and 1,3-β-D-linked oligosaccharides were identified by thin layer chromatography (TLC) with a precoated silica gel plate (Merck 60 HPTLC plate, Darmstadt, West Germany). Reaction products on TLC plate, which was developed with a solvent system (chloroform:acetic acid:water = 5:7:1), were detected by spraying the plate with 20% sulfuric acid/ethanol reagent and drying in an

**Table 1**
Purification of 1,3-β-D-glucanase.

| Step | Total protein (mg protein) | Total activity (units) | Specific activity (units/mg protein) | Recovery (%) | Purification (fold) |
|---|---|---|---|---|---|
| Cell free extract | 1990 | 9.93 | 0.005 | 100 | 1 |
| DEAE-Toyopearl 650 M | 17.7 | 6.68 | 0.378 | 67.3 | 75.6 |
| Resource Q | 0.433 | 1.87 | 4.22 | 18.9 | 845 |

oven at 180 °C for 3 min (Kusuda, Nagai, Hur, Ueda, & Terashita, 2003).

### 2.11. Effect of metal ions on purified enzyme activity

The purified enzyme was pre-incubated with 5 mM metals at 37 °C for 30 min in 0.1 M acetate buffer (pH 6.0). The metal ions were as follows: $Mn^{2+}$, $Mg^{2+}$, $Ca^{2+}$, $Cu^{2+}$, $Fe^{2+}$, $Al^{3+}$, $Co^{2+}$. All the metal ions were added as chloride salts. The 1,3-β-D-glucanase activity was determined by the standard assay as described above using laminarin as substrate. Activity without metal ions was set as 100%.

## 3. Results and discussion

### 3.1. Purity, molecular weight, and N-terminal amino acid sequence

The yield and purity of the 1,3-β-D-glucanase at each purification steps are summarized in Table 1. 1,3-β-D-glucanase thus obtained were purified 845-fold with recoveries of 18.9%. As shown in Fig. 1, 1,3-β-D-glucanase was shown to be homogeneous electrophoretically by SDS-PAGE with molecular mass estimated to be 42,000 daltons. The value was similar to those of CCF-1 (42 kDa) from *E. foetida* (Beschin et al., 1998), CCF-like protein (42 kDa) from *Aporrectodea caliginosa* (Silerova et al., 2006), and 1,3-β-D-glucanases (37.5–46.2 kDa) from *Cryptopygus antarcticus* (Song et al., 2010), *Stichopus japonicus* (Zhu et al., 2008), *Periphaneta americana* (Genta, Terra, & Ferreira, 2003), *Cherax destructor* (Allardyce & Linton, 2008). The N-terminal amino acid sequence of the enzyme was determined to be FTDWDQYHKVWTDGFDYFDG- (Table 2), and this showed the homology with CCF-1 of *E. foetida* (Beschin et al., 1998), CCF-like protein of *A. caliginosa* (Silerova et al., 2006), and α-amylase family protein of *Geobactor metallireducens* GS-15 (GenBank accession No. ABB31075).

In our previous paper, we have reported that EF-CMCase25 (25 kDa) occurs as a complex with β-D-glucosidase (32 kDa), 1,3-β-D-glucanase (40 kDa), and β-D-xylosidase (Ueda et al., 2010). In this study, we purified the 1,3-β-D-glucanase (42 kDa) from the same cell free extract of *E. foetida*. It is considered that there are several 1,3-β-D-glucanase isozymes in *E. foetida*. We are studying the cloning of 1,3-β-D-glucanase genes of *E.foetida*.

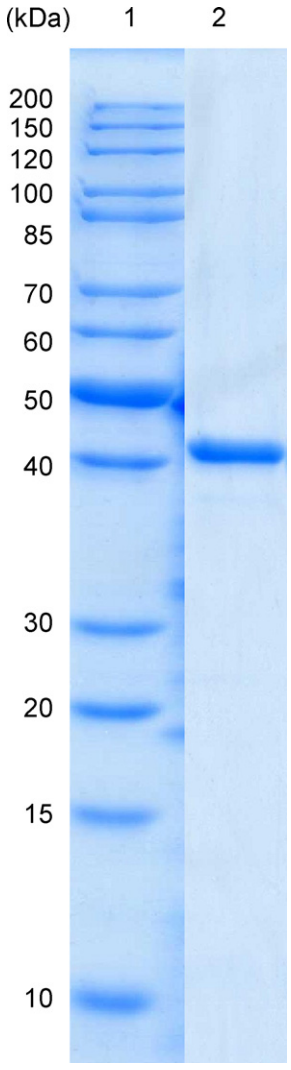


**Fig. 1.** SDS-PAGE pattern of the purified *E. foetida* 1,3-β-D-glucanase. (1) PageRuler™ Unstained Protein Ladder (Fermentas Inc., Ontario, Canada). (2) Purified 1,3-β-D-glucanase.

**Table 2**
Comparison of N-terminal and internal amino acid sequences.

| | | Amino acid sequences | |
|---|---|---|---|
| 1,3-β-D-glucanase[a] | 1 | **FTDWDQYH**K**VW**T**DGFDYFDG**- | 20 |
| CCF1[b] | 18 | **FTDWDQYH**I**VW**Q**DEFDYFDG**- | 37 |
| CCF-like protein[c] | 18 | **FTDWDQYH**I**VW**Q**DEFDFFEG**- | 37 |
| α-amylase[d] | 240 | IR**DWD**R**YH**E-YL**D**G-**DFFD**L- | 257 |

The identical residues are marked in bold letters.
[a] This study.
[b] CCF1 (Beschin et al., 1998).
[c] CCF-like protein (Silerova et al., 2006).
[d] α-amylase family protein: [*Geobacter metallireducens* GS-15, accession No. ABB31075].

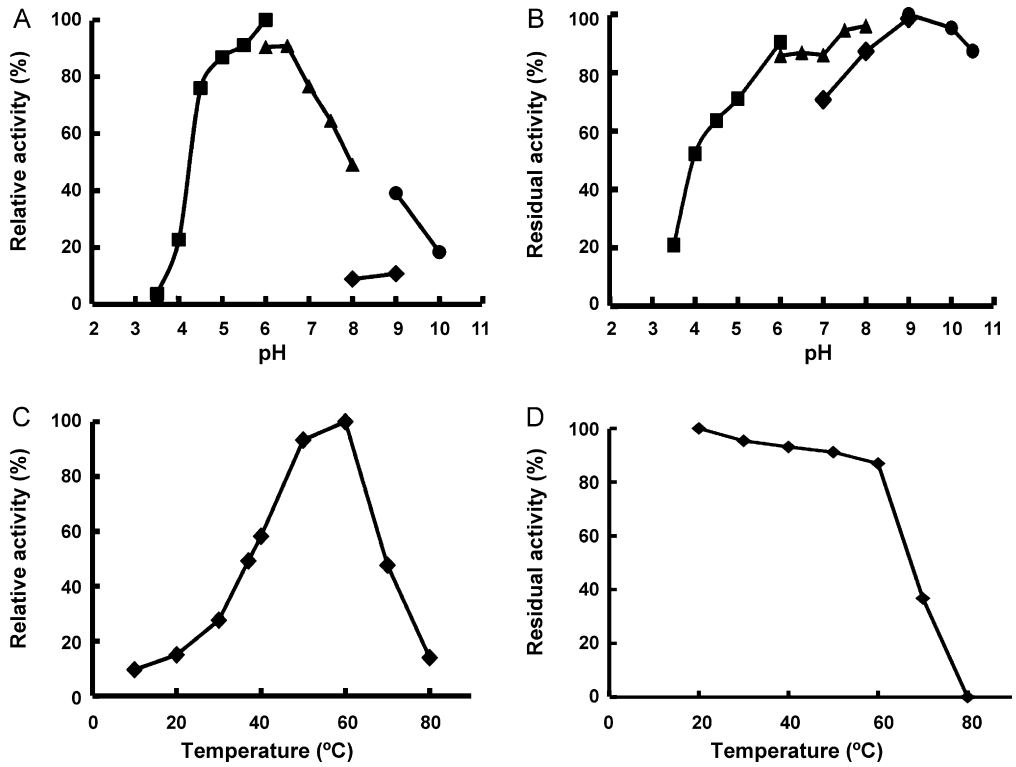


**Fig. 2.** Effects of pH on the activity (A) and stability (B), and effects of temperature on activity (C) and stability (D) of *E. foetida* 1,3-β-D-glucanase. (A) The activity of purified enzyme was estimated by using laminarin at 37 °C in various buffers. (B) The effect of pH on the enzyme stability was elucidated by incubating the enzyme for 30 min at 37 °C in various buffers. The remaining activity was measured using laminarin at pH 6.0. (C) The effect of temperature on the 1,3-β-D-glucanase activity was studied at 10–80 °C. (D) The thermal stability was investigated by incubating the purified 1,3-β-D-glucanase in 50 mM phosphate buffer (pH 8.0) for 30 min at 20–80 °C. The remaining activity was measured using laminarin at 37 °C. Each data represents the mean of duplicate measurement, and standard deviations are within the symbols.

### 3.2. Effects of pH and temperature on the activity and stability of 1,3-β-D-glucanase

Laminarin from *Eisenia bicyctis* was used as the substrate in the assay of the effects of pH and temperature dependence of the enzyme activity. As shown in Fig. 2A, the overall pH-activity profiles of the 1,3-β-D-glucanase appeared similar with optimum pH at 6.0. The enzyme showed at least 90% of their maximum activities over a pH range from pH 7.5 to 10.0 (Fig. 2B). The optimum pH of 1,3-β-D-glucanase was similar to those reported for *C. antarcticus* (Song et al., 2010), *S. japonica* (Zhu et al., 2008), *C. destructor* (Allardyce & Linton, 2008), *P. america* (Genta et al., 2003), *Abracris flavolineata* (Ferreira, Marana, Silva, & Terra, 1999), *Rhagium inquisitor* (Chipoulet & Chararas, 1994) (Table 4). The enzymes showed maximum activity at 60 °C and were stable up to 60 °C when incubated for 30 min (Fig. 2C and D), suggesting that 1,3-β-D-glucanase from *E. foetida* are moderately thermostable enzymes as those of *Bursaphelenchus xylophila* (Kikuchi et al., 2005), *Euphausia superba* (Suzuki, Horii, Kikuchi, & Ohnishi, 1987), and *Paecilomyces thermophila* (Yang, Quaojuan, Jiang, Fan, & Wang, 2008) (Table 4).

**Table 3**
Effects of various metal ions on the 1,3-β-D-glucanase activities.

| Metal ions | Residual activity (%) |
|---|---|
| No addition | 100 ± 0.01 |
| $Al^{3+}$ | 0 |
| $Ca^{2+}$ | 115 ± 2.6 |
| $Co^{2+}$ | 112 ± 0.11 |
| $Cu^{2+}$ | 0 |
| $Fe^{2+}$ | 0 |
| $Mg^{2+}$ | 112 ± 2.3 |
| $Mn^{2+}$ | 0 |
| $Zn^{2+}$ | 109 ± 0.45 |

Activity without metal ions was set as 100%. The results are from duplicate measurement.

### 3.3. Substrate specificity

The purified enzyme was active only toward glucans containing 1,3-β-D-linkages, such as laminarin (4.22 units/mg protein), curdlan (0.92 units/mg protein), pachyman (0.45 units/mg protein). The enzyme hydrolyzed laminarin more efficiently than other polymers with 1,3-β-D-glycosidic bonds. No reaction was observed toward chitosan, CM-cellulose, colloidal chitin, chitosan 7B, xylan, xylooligosaccharides. This result is similar to those of 1,3-β-D-glucanases from *B. xylophilus* (Kikuchi et al., 2005), and *Agaricus brasiliensis* (Shu, Xu, & Lin, 2006).

### 3.4. Action pattern of 1,3-β-D-glucanase on laminarin and 1,3-β-D-linked oligosaccharides

To clarify the mode of action of the purified 1,3-β-D-glucanase, laminarin from *E. bicyclis* was hydrolyzed at various time durations, and the products were analyzed by TLC. The sugars produced after 1 h were glucose, 1,3-β-D-linked triose, tetraose, and oligosaccharides of higher degree of polymerization. The sugars produced after 27 h were almost the same as the products after 1 h (Fig. 4 (1)). Laminarin from *E. bicyclis* (molecular weight: 6170) has 1,3-β-D- and 1,6-β-D-linkage as the main type of linkage. The ratio of 1,3-β-D- and 1,6-β-D-linkage type is 3:2, and laminarin from *E. bicyclis* has a highly 1,6-β-D-branched structure (Pang et al., 2005). Laminarioligosaccharides of higher degree of polymerization than laminaritriose have 1,6-β-D-branches in *E. bicyclis*. It is considered that the 1,3-β-D-glucanase cannot hydrolyze the triose with 1,6-β-D-branch.

**Table 4**
Properties of 1,3-β-D-glucanases from invertebrates.

| Origins | Molecular mass (kDa) | Optimum pH | Optimum temperature (°C) | pH stability | Thermostability (°C) | Inhibitors etc. | References |
|---|---|---|---|---|---|---|---|
| *E. foetida* | 42 | 6.0 | 60 | 7.5–10 | ~60 °C | $Al^{3+}$, $Cu^{2+}$, $Fe^{2+}$, $Mn^{2+}$ | Present study |
| *Cryptopygus antarcticus* | 46 | 6.0 | 50 | ND | ~40 | ND | Song et al. (2010) |
| *Stichopus japonicus* | 37.5 | 5.5 | 40 | 5.0–8.0 | ~40 | $Cu^{2+}$, $Ag^{+}$ | Zhu et al. (2008) |
| *Bursaphelenchus xylophilus* | ND | 4.9 | 65 | ND | ND | ND | Kikuchi et al. (2005) |
| *Periplaneta americana* | 46.2 | 6.5 | ND | ND | ND | ND | Genta et al. (2003) |
| *Abracris flavolineata* | 146 | 5.7 | ND | ND | ND | ND | Ferreira et al. (1999) |
| *Rhagium inquisitor* | 95–100 | 5-6 | ND | ND | ND | ND | Chipoulet & Chararas (1994) |
| *Euphausia superba* | 65–70 | 4.3–5 | 65 | ND | ND | $Hg^{2+}$, $Fe^{3+}$, gluconolactone, Nbromosuccin-imide | Suzuki et al. (1987) |
| *Gecarcoidea natalis* | 41 | 7.0 | ND | ND | ND | ND | Allardyce and Linton (2008) |
| *Cherax destructor* | 41 | 5.5 | ND | ND | ND | ND | Allardyce and Linton (2008) |

ND, not determined.

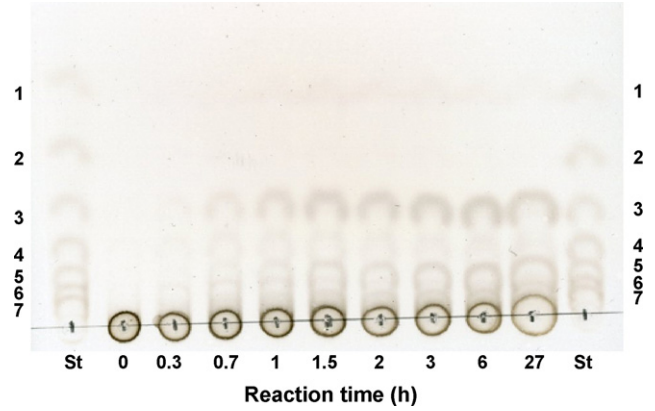


**Fig. 3.** Thin-layer chromatogram of the products from laminarin by 1,3-β-D-glucanase. Reaction products from the degradation of laminarin by the enzyme were detected by TLC as described in Section 2. Symbols: G1–G7 a series of authentic sugar standards of glucose, 1,3-β-D–linked biose, triose, tetraose, and pentose, hexaose, and heptaose, respectively.

We also used from 1,3-β-D-linked biose to pentaose as substrates to understand the mode of action of the 1,3-β-D-glucanase. The major hydrolysis products from 1,3-β-D-linked triose, tetraose, and pentaose were glucose and biose (Fig. 4 (2), (3), (4)). The biose was not degraded by the 1,3-β-D-glucanase. From these results, it is considered that this enzyme is endo-type 1,3-β-D-glucanase.

### 3.5. Effect of metal ions on the enzyme activity

The enzyme activity was slightly enhanced by $Mg^{2+}$, $Ca^{2+}$, $Co^{2+}$, and $Zn^{2+}$, whereas the enzyme was completely inhibited by $Mn^{2+}$, $Cu^{2+}$, $Fe^{2+}$, and $Al^{3+}$ (Table 3). It was found that $Ca^{2+}$ activated the activity of 1,3-β-D-glucanases from *Flavobacterium* sp. (Rasmussen, Madsen, Stougaard, & Jonsen, 2008) and *Agaricus bisporus* (Shu et al., 2006). $Mn^{2+}$ acted as inhibitor in decreasing the 1,3-β-D-glucanase activity produced by *Trichoderma longlbrachlatum* (Sharma & Nakas, 1987).

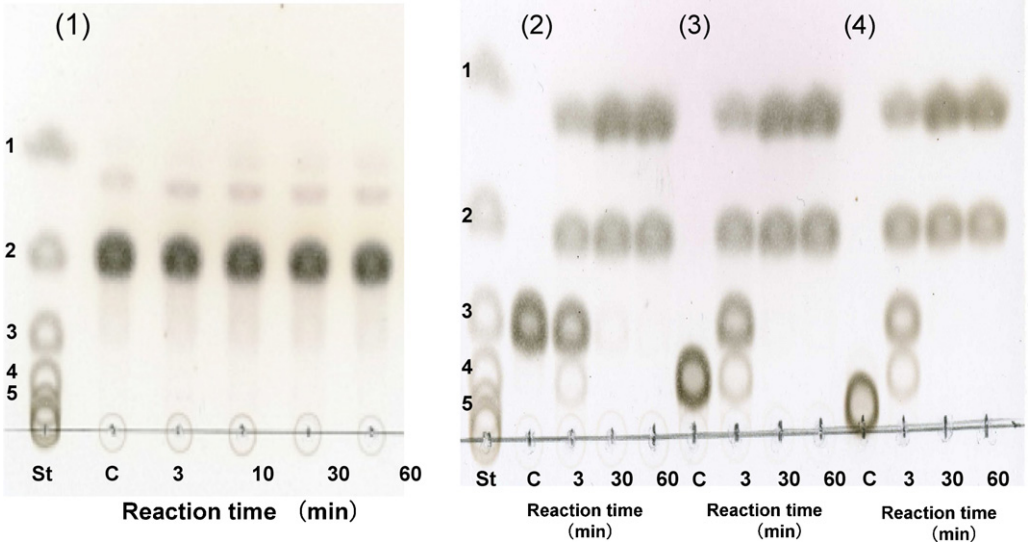


**Fig. 4.** Thin-layer chromatogram of the products from 1,3-β-D-linked oligosaccharide by 1,3-β-D-glucanase. Reaction products from the degradation of (1) 1,3-β-D-linked biose, (2) 1,3-β-D-linked triose, (3) 1,3-β-D-linked tetraose, and (4) 1,3-β-D-linked pentose by the enzyme were detected by TLC as described in Section 2. Symbols: G1–G5 a series of authentic sugar standards of glucose, 1,3-β-D-linked biose, triose, tetraose, pentaose, and hexaose, respectively. C: 0.1 M acetate buffer and each oligosaccharide.

## 4. Conclusion

To our knowledge, this is the first report about purification and characterization of 1,3-β-D-glucanase in the earthworm *E. foetida*. We identified a 42 kDa 1,3-β-D-glucanase with sequence similarity to previous characterized CCF-1 from *E. foetida*. The CCF-1 did not exhibited 1,3-β-D-glucanase activity. Pauchet et al. identified a glucan-binding protein family from Lepidoptera had glucanase activity (Pauchet, Freitak, Heidel-Fischer, Heckel, & Vogel, 2009). This glucanase was not detected in hemolymph. Its mRNA is constitutively and predominantly expressed in the midgut and is induced there when larvae feed on a diet containing bacteria. It suggest that *Eisenia* 1,3-β-D-glucanase might be also expressed in midgut. To clear the difference between CCF-1 and 1,3-β-D-glucanase in *E. foetida*, we continue to study on the structural elucidation of the protein and gene involved in *Eisenia* 1,3-β-D-glucanase.

In this study, it found that the oligosaccharides with a degree of polymerization higher than triose from laminarin are presented in the hydrolyzate (Fig. 3). The oligosaccharides exhibit varied biological activities such as antitumor and antiviral activities (Pacheco-Sanchez, Boutin, Angers, Gosselin, & Tweddell, 2006). We continue to study the use of 1,3-β-D-linked oligosaccharides in the areas of pharmaceuticals, cosmetics and foods.

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