Purification and Characterization of a Thermostable Laminarinase from *Penicillium rolfsii* c3-2(1) IBRL

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A laminarinase (endo-*β*-1,3-glucanase) was purified to homogeneity from *Penicillium rolfsii* c3-2(1) IBRL, which was originally produced in liquid culture containing 1% xylan from birchwood, via anion-exchange chromatography, gel filtration on Sephacryl S-100, and hydrophobic interaction chromatography. A single protein band with a molecular weight of 75 kDa was detected by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, which had an optimum catalytic activity at pH 4.0 to 5.0 and 70 °C. This purified enzyme was most stable in the pH range 4 to 7, while it was thermostable up to 55 °C and retained up to 90% of its activity after 4 h pre-incubation. A substrate laminarin kinetic study yielded estimated K_m and V_{max} values of 0.0817 mg/mL and 372.2 µmol/min/mg, respectively. Laminari-oligosaccharide degradation, which was analyzed by thin layer chromatography, yielded the major hydrolysis products laminaribiose and glucose.

Keywords: β-1,3-glucanase; Laminarinase; Laminari-oligosaccharide; Penicillium rolfsii c3-2(1) IBRL; Thermostability

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INTRODUCTION

Laminarin is a major storage carbohydrate that is mostly found in various brown seaweeds and microalgae; it is primarily comprised of linear *β*-1,3-linked glucose residues and *β*-1,6-linkages, such as the laminarin from *Laminaria digitata* (Kumagai and Ojima 2010). Laminarin can be hydrolyzed by laminarinase, which yields glucose as the main product of hydrolysis (Nielsen 1963). The widespread occurrence of laminarinase (*β*-1,3-glucanase) has been reported in a great variety of microorganisms (Chesters and Bull 1963a-c), while it is also widely distributed in invertebrates, such as earthworms (Ueda *et al.* 2011), and higher plants (Boucaud *et al.* 1987). Laminarinases are capable of hydrolyzing *β*-1,3-glucans (Tangarone *et al.* 1989), and they can be categorized into two groups based on the mode of hydrolysis. Endo-glucanases usually catalyze the cleavage of internal bonds at random sites in the polysaccharide chain to yield mixtures of laminari-oligosaccharides, whereas exo-type enzymes produce glucose as the end product by sequentially cleaving glucose units from the nonreducing end (Noronha and Ulhoa 2000; Miyanishi *et al.* 2003).

The distinct and diverse physiological roles of laminarinases depend on their sources (Gueguen *et al.* 1997; Kikuchi *et al.* 2005; Li *et al.* 2007). The roles include cell growth development, defense, and parasitism. In fungi, *β*-1,3-glucanases play important

roles in morphogenetic processes, *β*-glucan mobilization, and fungal pathogen-plant interactions (Bachman and McClay 1996). A combination of chitinase and *β*-glucanase was found to have antimycotic effects as a biological control agent to combat plant diseases, such as fungal pathogens (Noronha and Ulhoa 1996; Zverlov *et al.* 1997). Laminarinase was also reported to have potential for commercial yeast extract production (Ryan and Ward 1985) as well as for algal biomass conversion to fermentable sugar (Sharma and Nakas 1987; Zverlov *et al.* 1997). Furthermore, laminarinase has numerous potential applications in malting and brewing processes (König *et al.* 2002), fungal and yeast cell wall degradation (Pitson *et al.* 1993), animal feed (König *et al.* 2002), medical fields (Pang *et al.* 2005), and bioethanol production (John *et al.* 2011) .

The present study reports the purification and characterization of a laminarinase produced by *Penicillium rolfsii* c3-2(1) IBRL. Physiochemical and enzymatic properties are reported for when the lamarinase was produced in a medium containing xylan derived from birchwood.

EXPERIMENTAL

Microorganism and Culture Conditions

P. rolfsii c3-2(1) IBRL was isolated from the soil in an oil-palm farm in the northern part of the Malaysian Peninsula. This strain was maintained in a potato dextrose agar (PDA) slant (Difco). The fungus was grown in a basal medium that contained sodium nitrate, 1.0 g; potassium dihydrogen phosphate, 1.0 g; magnesium sulfate heptahydrate, 0.5 g, and yeast extract, 0.5 g, which were dissolved in 1 L of distilled water at pH 5.0. The medium also contained 1% (w/v) xylan from birchwood (Sigma) to facilitate the production of enzymes. The culture was incubated with agitation at 200 rpm for 7 days at 30 °C.

Enzyme Purification

All the purification steps were performed at 4 °C to conserve the enzyme activity, unless stated otherwise. The procedure used to purify laminarinase from *P. rolfsii* c3-2(1) IBRL involved the following 4 steps.

Step 1: Ammonium sulfate precipitation

After 1 week of fermentation, the culture broth was collected by filtration to remove the fungal mycelium. The supernatant was precipitated with 80% saturated ammonium sulfate at 4 °C. Precipitation was allowed to continue overnight, which was followed by centrifugation at 8000 rpm in a refrigerated centrifuge for 30 min. The precipitate was then dissolved in 50 mM sodium acetate buffer (pH 5.0) and any excess salt was removed using an Econo-Pac 10 DG desalting column (Bio-Rad). The filtrate was concentrated by centrifugal ultrafiltration (Vivaspin 20, 5 kDa cut-off, Sartorius) and used as the starting material for purification.

Step 2: Anion-exchange chromatography

The concentrated enzyme solution was applied to an anion-exchange column (RESOURCETM Q, 6 mL; GE Healthcare Bio-Sciences AB, Sweden), which was pre-equilibrated with 20 mM 3-morpholinopropanesulfonic acid (MOPS; Dojindo, Wako Pure Chemical Co. Ltd., Tokyo, Japan) buffer (pH 7.0) containing 1.0 M NaCl. The

sample was eluted at a flow rate of 4 mL/min. The eluted fractions were collected and screened to determine their laminarinase activities and protein content. The active fractions were pooled and concentrated by centrifugal ultrafiltration.

Step 3: Gel filtration

A portion of the pooled ion-exchange chromatography fractions was passed through a gel filtration column (HiPrepTM 16/60 Sephacryl S-100 High Resolution; GE Healthcare Bio-Sciences AB, Sweden). The gel filtration column was pre-equilibrated with 0.1 M phosphate buffer (pH 7.0) containing 0.1 M NaCl. The sample was eluted at a flow rate of 0.8 mL/min. The fractions were collected and analyzed to determine their laminarinase activity and protein content. The active fractions were pooled and used in the next purification step.

Step 4: Hydrophobic-interaction chromatography

The pooled fractions with laminarinase activities were dialyzed against 50 mM MOPS buffer containing 2.0 M ammonium sulfate (pH 7.0). Then, the pooled fraction was passed through a hydrophobic column (RESOURCETM PHE; GE Healthcare Bio-Sciences AB, Sweden) at a flow rate of 1 mL/min. The column was pre-equilibrated and eluted with 50 mM MOPS buffer (pH 7.0) with a linear ammonium sulfate gradient (0 to 2 M). The fractions were collected and analyzed to determine their laminarinase activity and protein content.

Enzyme Assay and Protein Determination

The laminarinase activity was assayed by determining the amount of reducing sugar released from laminarin derived from *Laminaria digitata* (Sigma). The reaction mixture comprised of 0.5% laminarin in pH 5.0 acetate buffer and enzyme to yield a final volume of 0.2 mL. After incubation for 15 min at 50 °C, the amount of reducing sugar present in the sample was then determined according to the method of Somogyi-Nelson (Somogyi 1945). One unit of laminarinase was defined as the amount of enzyme that catalyzed the release of 1 µmol of glucose-equivalent per min. The concentration of protein in the crude enzyme preparation was determined using the standard Lowry protein assay (Lowry *et al.* 1951), with bovine serum albumin as the standard. Broth containing the enzymes was used to determine the enzymatic activity and experiments were performed in triplicate.

Activity Detection by Zymography

Zymography was performed according to Zhang *et al.* (2009) with some modifications. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was used to determine the protein purity and the molecular weight of the purified enzyme in denaturing conditions. SDS-PAGE with a 10% polyacrylamide gel was performed after incorporating laminarin solution (final concentration = 0.1% w/v) in the separating gel, before the addition of ammonium persulfate and tetramethylethylenediamine (TEMED) to facilitate polymerization. Next, the gel was soaked with washing buffer and gently shaken for 15 min, and this washing procedure was repeated 4 times to remove the SDS. The gel was rinsed with distilled water and transferred to sodium acetate buffer for 30 min, which was repeated twice. The gel was rinsed with distilled water and incubated overnight in 50 mM sodium acetate buffer at 50 °C. The gel was stained with Congo red solution at room temperature for 30 min. The gel was rinsed with distilled water and

destained in 1 M NaCl solution at room temperature for 1 h. Finally, the gel was transferred to 5% (v/v) acetic acid to enhance the visualization of a clear zone band in the gel against a dark background.

Effects of pH and Temperature on the Enzyme Activity and Stability

The laminarinase activity was measured under various pH conditions (pH 3.0, McIlavine buffer; pH 4.0 to 6.0, sodium acetate buffer; pH 7.0 to 8.0, sodium phosphate buffer; and pH 9.0 Tris-HCl buffer) according to the method described earlier. To determine the pH-dependent stability, the purified enzymes were diluted in each of the different buffers and incubated at 4 °C for 24 h. Next, the laminarinase activity was assayed after incubating at 50 °C for 15 min to compare the residual activity with the initial activity. To determine the optimal temperature, the laminarinase activity was investigated within a temperature range of 30 to 75 °C. To evaluate the thermal stability, the purified enzymes were incubated at different temperatures with the optimal pH for various time intervals up to 4 h. After incubation, the remaining activity was measured using the standard assay conditions.

Determination of Kinetic Parameters

The initial reaction rate (laminarinase activity) was determined using laminarin as the substrate at 0.5 to 10 mg/mL with 50 mM acetate buffer in the optimal assay conditions (70 °C, pH 5.0, 15 min). The kinetic constants *i.e.*, K_m and V_{max}, were estimated from the Michaelis–Menten plot and linear regression based on double-reciprocal plots according to the Lineweaver–Burk plot method using SigmaPlot 10. The maximum rate was expressed as μmol/min/mg of protein.

Substrate Specificity of Laminarinase

The activity of the purified laminarinase was tested using various polymers that contained *β*-glycosidic bonds such as laminarin from *Laminaria digitata* (Sigma), *β*-glucan from barley (Sigma), avicel (Sigma), chitin and chitosan (Sigma), carboxymethyl cellulose (Sigma), curdlan (Sigma), lichenan (Megazyme), red pullulan (Megazyme), and *p*-nitrophenyl *β*-D-glucopyranoside (Sigma). Enzymatic hydrolysis was conducted by incubating each selected substrate with the purified enzyme at the optimal pH and temperature. In each case, degradation was assayed based on the production of reducing sugars, which was measured as described earlier.

Analysis of the Hydrolysis Products

The hydrolysis products of laminaribiose, laminaritriose, laminaritetraose, laminaripentaose, and laminarihexaose (Megazyme) were analyzed by thin layer chromatography (TLC). The purified laminarinase (0.05 U) was added to 5 mg/mL laminaribiose, laminaritriose, laminaritetraose, laminaripentaose, and laminarihexaose, which were prepared in 50 mM sodium acetate buffer (pH 5.0), and incubated overnight (17 to 20 h) at 50 °C. Another tube was prepared containing glucose, which served as the control. The laminarinase hydrolysis products, as well as glucose, laminaribiose, laminaritriose, laminaritetraose, laminaripentaose, and laminarihexaose, were fractionated on a TLC plate (Silica gel 60; Merck; Darmstadt, Germany). The solvent used to develop the chromatogram was ethyl acetate:acetic acid:water (2:2:1), as reported by Kumagai and Ojima (2010). The products were visualized by spraying a chromogenic

reagent, which was a mixture of ethanol and sulfuric acid (95:5), onto the TLC plates, followed by drying at 100 °C for 5 to 10 min.

RESULTS AND DISCUSSION

The laminarinase activity was higher when *P. rolfsii* c3-2(1) IBRL was cultured with xylan (data not shown). Thus, xylan was used as the carbon source in the expression medium. The purification scheme (Table 1) resulted in a 21.3-fold purification of laminarinase relative to the crude broth and a substantial increase in the specific activity compared with the initial value, *i.e.*, from 8.42 U/mg to 179.37 U/mg of protein. The anion exchange chromatography elution profile of the ammonium sulfate-precipitated enzyme is shown in Fig. 1.

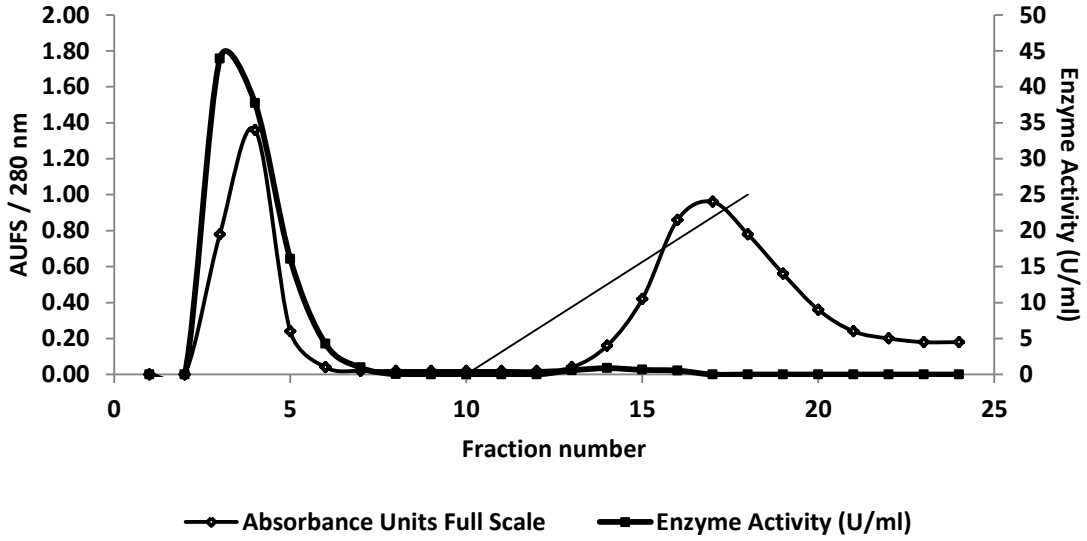

Fig. 1. Anion-exchange chromatography (RESOURCE™ Q, 6 mL) elution profile of protein and laminarinase activity. NaCl (1.0 M) gradient (continuous line)

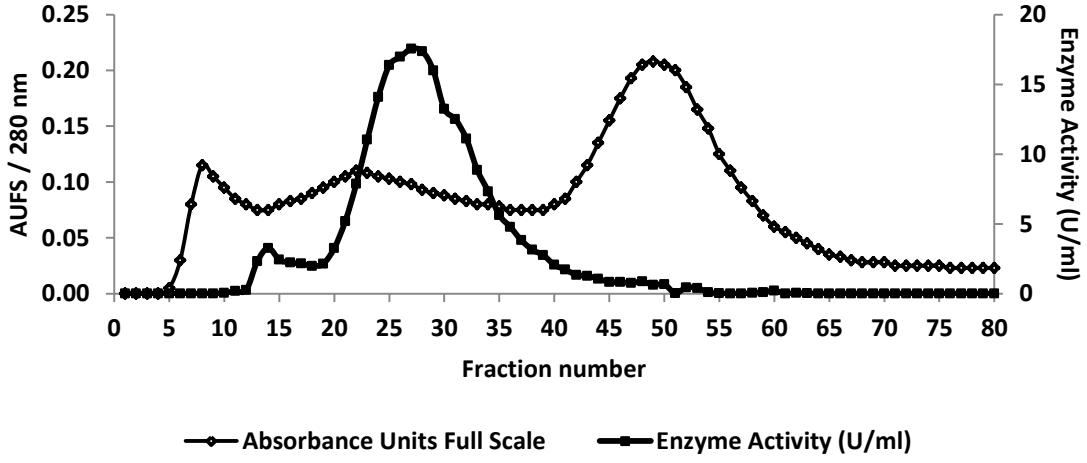

Fig. 2. Elution profile of laminarinase purification by gel filtration HiPrep™ 16/60 Sephacryl S-100 High Resolution

The single laminarinase activity peak corresponded to a major peak in protein fractions 3 to 5. These fractions did not adsorb to the positively charged solid support. The active fractions were initially eluted, before manipulating the gradient with increasing NaCl concentrations. Next, the active fractions were pooled and concentrated, before further purification by gel filtration on a Sephacryl S-100 column (Fig. 2). The gel-filtration chromatography showed that a single major laminarinase activity peak in fractions 20 to 37 corresponded to a minor peak in the protein fractions. Then, the active fractions were eluted using a phenyl–hydrophobic column (Fig. 3). The active enzymes in fractions 40 to 43 were desorbed from the column with a decreasing ammonium sulfate concentration.

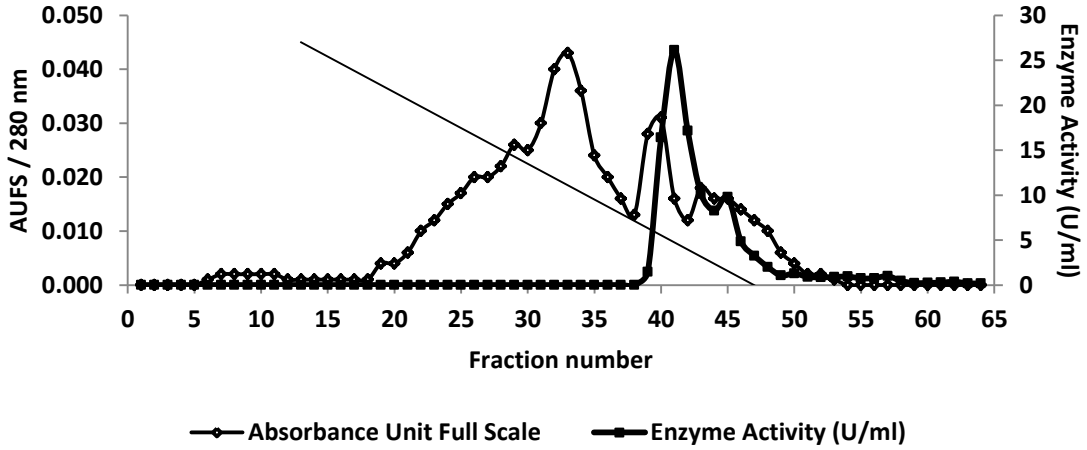

Fig. 3. Hydrophobic-interaction chromatography of laminarinase on phenyl Resource™ PHE column after gel-filtration column chromatography. $(NH_4)_2SO_4$ (2.0 M) gradient (continuous line)

Table 1. Summary of Purification Scheme for the Laminarinase of *P. rolfsii* c3-2(1) IBRL

Fraction	Volume (mL)	Total Protein (mg)	Activity (units)	Total Activity (units*mL)	Specific Activity (units/mg)	Fold Purification	% Yield
Cell free supernatant	5.0	80.0	126.27	631.35	8.42	1.0	100.0
RESOURCE™ Q, 6 mL	1.0	21.03	283.82	283.82	13.50	1.60	45.0
HiPrepTM 16/60 Sephacryl S-100 High Resolution	5.0	2.63	11.61	58.05	22.07	2.62	9.2
RESOURCE™PHE	4.0	0.19	8.52	34.08	179.37	21.30	5.4

Coomassie brilliant blue staining of 6 µg of the purified enzyme in SDS-PAGE with a 10% polyacrylamide gel detected a homogeneous single protein band with an estimated molecular weight of 75 kDa (Fig. 4). The zymogram of the laminarinase preparation demonstrated the presence of a zone of hydrolysis. According to Copa-Patiño *et al.* (1989), the denatured molecular weight of *P. oxalicum* was found to be 79 kDa,

which is similar to the molecular weight of the laminarinase from *P. rolfsii* c3-2(1) IBRL. A laminarinase with a molecular weight of 65 kDa was also detected in *P. italicum* by SDS-PAGE (Sánchez *et al.* 1982).

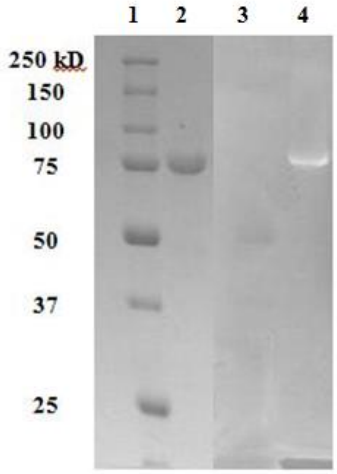

Fig. 4. Development of laminarinase zymogram. Lanes 1 and 3 were loaded with Protein Marker (Precision PlusProtein™ Standards – BIO-RAD); lanes 2 and 4 with 6 µg of purified enzyme treated with sample buffer (AE-1430 EzApply; ATTO Corporation). Lanes 1 and 2 were stained with Coomassie brilliant blue; lanes 3 and 4 were developed as zymogram with gel impregnated 0.1% laminarin, stained with congo-red solution followed by 5% of acetic acid (v/v).

The effects of pH and temperature on the activity and stability of the laminarinase from *P. rolfsii* c3-2(1) IBRL are shown in Figs. 5 and 6. The optimum pH for the purified enzyme was 4.0 to 5.0. Similar results were reported for the laminarinase produced by *P. oxalicum* (pH 4.0) (Copa-Patiño *et al.* 1989), whereas the laminarinase from *P. italicum* had an optimum pH of 6.0 (Sánchez *et al.* 1982). Laminarinases with an optimum of pH 5.0 are produced by a wide variety of organisms (Bara *et al.* 2003; Chipoulet and Chararas 1984; Kulminskaya *et al.* 2001; Sena *et al.* 2011).

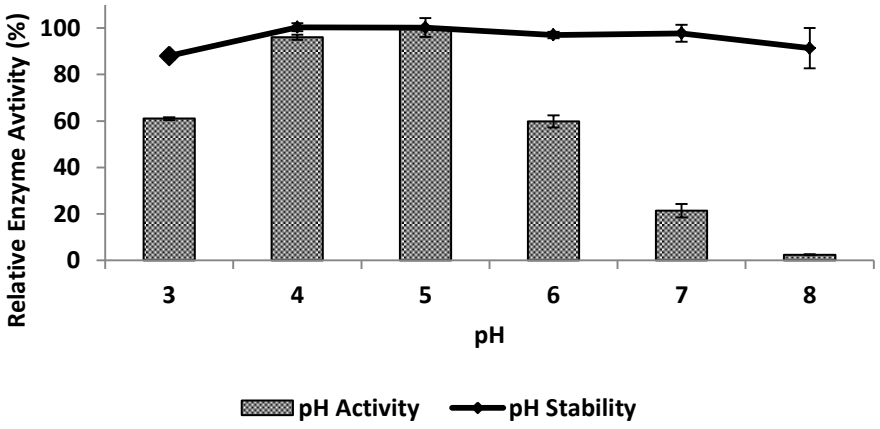

Fig. 5. Effect of pH on the activity and its stability of *P. rolfsii* c3-2(1) IBRL laminarinase. The activity of purified enzyme was estimated by using laminarin at 50 °C in various buffers. For pH stability study, the purified enzymes were pre-incubated without substrate with different buffers (pH 3.0 to 8.0), at 4 °C for 24 h.

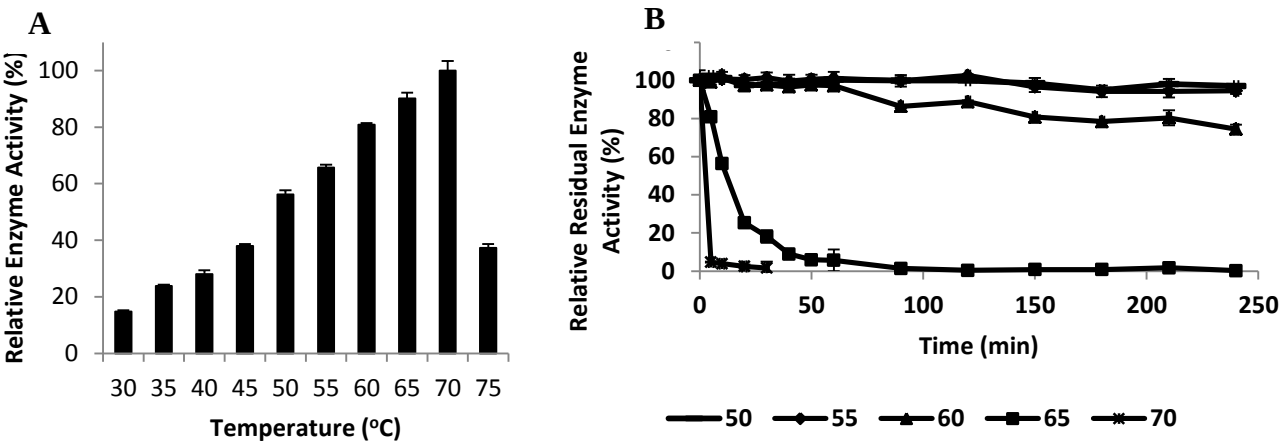

Fig. 6. Influence of temperature on activity (A) and its thermal stability (B) of *P. rolfsii* c3-2(1) IBRL laminarinase. (A) The activity of purified enzyme was estimated by laminarin at pH 5.0 and various temperatures incubation. (B) The thermal stability was investigated by incubating the purified laminarinase in 50 mM acetate buffer (pH 5.0) for up to 4 hours in the absence of substrate at 50 to 70 °C. The remaining activity was measured using laminarin at 50 °C. Each data represents the mean of triplicate measurements.

A previous report suggests that the optimal pH range for *β*-1,3-glucanase activity is usually 4.0 to 6.0 (Pitson *et al.* 1993). However, the laminarinase activity might extend over the pH interval 2.25 to 7.6, although it is very weak below pH 3.0 and above 7.4 (Nielsen 1963). The pH stability analysis of *P. rolfsii* c3-2(1) IBRL laminarinase activity showed that this enzyme was stable in the pH range 4.0 to 7.0 after overnight incubation, which is a wider range of pH stability compared with the laminarinase from *P. oxalicum* (pH 5.0 to 6.0). This purified laminarinase exhibited 97 to 100% relative activity of its maximum stability in the range pH 4.0 to 7.0. However, it gradually lost its enzymatic stability below pH 4.0 (88% relative activity) as well as above pH 7.0 (91% relative activity).

The optimum temperature for the enzyme activity was found to be 70 °C at pH 5.0, which has not been reported for laminarinases from other fungi. The enzyme was stable up to 55 °C and it retained 90% of its activity even after 4 h pre-incubation at this temperature. This enzyme retained up to 70% of its activity after 4 h at 60 °C. After 1 h of incubation, it retained up to 90% of its activity, which was similar to that reported for the laminarinase produced by *Eisenia foetida* (Ueda *et al.* 2011), *Chaetomium thermophilum* (Li *et al.* 2007), and *Trichoderma longibrachiatum* (Tangarone *et al.* 1989). The use of thermostable enzymes in industrial applications facilitates increased reaction rates, higher substrate solubility, lower media viscosity, and longer enzyme shelf life at normal storage temperatures, as well as a lower risk of microbial contamination when reactions are performed at higher temperatures (Querol *et al.* 1996). The remarkable thermal tolerance, as well as the appearance of heat-resistance at a relatively high temperature, exhibited by the laminarinase from *P. rolfsii* c3-2(1) IBRL, enables it to be advantageous in the industrial enzyme market.

Based on the analysis of a Michaelis–Menten plot and a Lineweaver–Burk plot (Fig. 7), the hydrolysis rate of the laminarin from *L. digitata* linearly increased with its concentration to approximately 0.5 mg/mL, with a maximum rate at 2.0 mg/mL. The K_m and V_{max} values were estimated at 0.0817 mg/mL and 372.2 µmol/min/mg, respectively.

This was substantially lower than the K_m value of 0.26 mg/mL for the laminarinase from *P. oxalicum* (Copa-Patiño *et al.* 1989). However, it was higher than the K_m (0.04 mg/mL) of the laminarin produced by *P. italicum* (Sánchez *et al.* 1982). There are few research reports on the purification of the laminarinases produced by *Penicillium* fungal strains. However, the estimated value of K_m (0.0817 mg/mL) was similar to those reported for *T. asperellum* (0.087 mg/mL) (Bara *et al.* 2003) and *T. harzianum* (0.1 mg/mL) (Ramot *et al.* 2000).

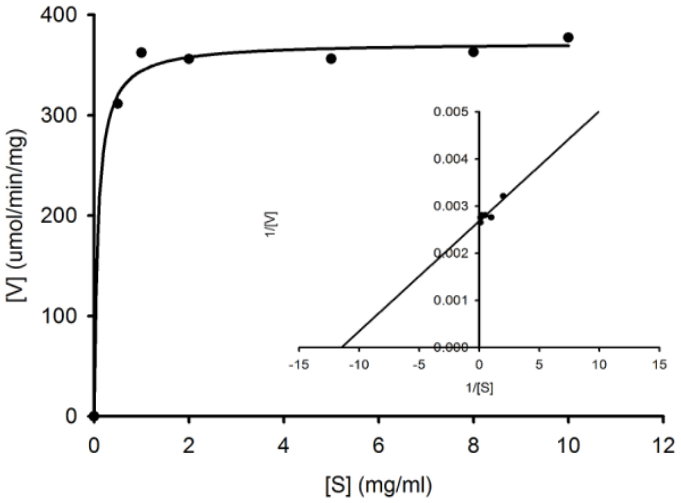

Fig. 7. Michaelis-Menten plot and Lineweaver-Burk plot for determining the V_{max} and K_m values of laminarinase of *P. rolfsii* c3-2(1) IBRL.

The mode of action of the purified laminarinase was investigated by TLC using a series of 1,3-*β*-D-linked bioses and hexaoses as substrates. Glucose was generated as part of the hydrolysis product from laminaribiose and laminarihexaose, according to the enzymatic activity patterns detected on the TLC plate (Fig. 8).

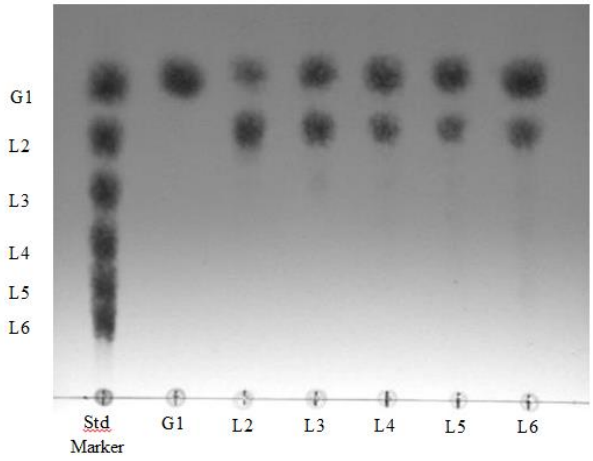

Fig. 8. TLC analysis for hydrolysis products released from laminari-oligosaccharides (L2–L6) with concentration of 5 mg/mL and enzyme (0.05 U) in the reaction mixture for overnight incubation at 50 °C. Laminari-oligosaccharides (G1, glucose; L2, laminaribiose; L3, laminaritriose; L4, laminaritetraose; L5, laminaripentaose; L6, laminarihexaose). Solvent system: ethyl acetate : acetic acid : H_2O; 2:2:1

In addition, the purified laminarinase had hydrolytic activity against *p*-nitrophenyl *β*-D-glucopyranoside, which is a typical substrate of exo-glucanases (Table 2), and this finding was in contrast with the results reported in another study (Lépagnol-Descamps *et al.* 1998). These results suggested that the enzyme purified in the present study acted in manner similar to an exo-enzyme. However, the results obtained using the TLC plate showed that not all of the laminaribiose was converted into glucose. Thus, it is possible that this enzyme had an endo-splitting activity against laminari-oligosaccharides (L2 to L6), as well as an exo-enzyme action. The mode of action of the laminarinase derived from *P. rolfsii* c3-2(1) IBRL requires further study to elucidate its mode of action.

The purified enzyme was highly active only against the laminarin from *L. digitata* where the ratio of *β*-1,3-linkages to *β*-1,6-linkages was 1:7 (Kulminskaya *et al.* 2001), (Table 2). A lower level of enzymatic activity was found with *β*-glucan from barley, curdlan, lichenan, and red pullulan. There were no reactions with avicel, chitin, chitosan, and carboxymethyl cellulose. Laminarin was more efficiently hydrolyzed by the purified enzyme than the other polymers with *β*-1,3-D-glycosidic bonds. The weak activity against *β*-glucan from barley and lichenan was probably due to the presence of 1,3-linkages in their molecular structures. According to Krah *et al.* (1998), bacterial and plant laminarinases exclusively cleave *β*-1,3-linkages adjacent to other *β*-1,3-linkages and cannot cut *β*-1,4-linkages if the *β*-1,3-linkages are mixed with other linked *β*-glucans. This might explain the relatively low specific activity against the *β*-glucans from barley and lichenan compared with laminarin. Similar results were obtained with a laminarinase derived from *Pyrococcus furiosus*, where the highest hydrolytic activity was against the *β*-1,3-glucose polymer laminarin, with weak activity against the *β*-1,3-1,4-glucose polymers lichenan and barley *β*-glucan (Gueguen *et al.* 1997).

Table 2. Relative substrate Specificity of Purified Laminarinase from *P. rolfsii* c3-2(1) IBRL

Substrate (1%)	Main linkage	Specific activity (U/mg)	Relative Activity (%)*
Laminarin from *Laminaria digitata*	*β*-1,3 and *β*-1,6	373.29±13.98	100 ± 3.7
β-glucan from Barley	*β*-1,3 and *β*-1,4	3.58±1.53	1 ± 0.4
Avicel	*β*-1,4	0	0
Chitin	*β*-1,4	0	0
Chitosan	*β*-1,4	0	0
CMC	*β*-1,4	0	0
Curdlan	*β*-1,3	11.94±1.99	3 ± 0.5
Lichenan	*β*-1,3 and *β*-1,4	2.86±0.65	1 ± 0.2
Red pullulan	*α*-1,4 and *α*-1,6	35.44±7.34	9 ± 2.0
p-nitrophenyl *β*-D-glucopyranoside		4.21±0.84	1 ± 0.2

* The purified enzyme was assayed in the standard assay condition with various compounds. After the reactions, reducing sugar released by enzymatic hydrolysis was determined. The amounts of reducing sugar released per min are shown as percentages of that measured for laminarin, which was defined as 100%. Each value represents the mean of triplicate measurements.

CONCLUSIONS

The present study showed that *P. rolfsii* c3-2(1) IBRL can produce laminarinase during submerged fermentation. To the best of our knowledge, this is the first report of the purification and characterization of a laminarinase from *P. rolfsii* c3-2(1) IBRL, and the enzyme has a molecular weight of 75 kDa according to SDS-PAGE analysis. The laminarinase of *P. rolfsii* c3-2(1) IBRL is potentially applied in the field of bioethanol production based on its enzymatic hydrolysis activity to produce fermentable sugar. The acidophilic and broad pH stability of the purified enzyme put its advantages appropriate for applications in the feed and food industries. The purified enzyme has relatively good thermostability and it could be used in industrial applications in the future, such as in the food, biotechnological, medical, and other industries.

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