Turkish Journal of Biology

Volume 37 | Number 4 Article 1

1-1-2013

Purification and characterization of a novel detergent- and organic solvent-resistant endo-beta-1,4-glucanase from a newly isolated basidiomycete Peniophora sp. NDVN01

DINH KHA TRINH

DINH THI QUYEN

THI TUYEN DO

NGOC MINH NGHIEM

TÜBİTAK

Turkish Journal of Biology

http://journals.tubitak.gov.tr/biology/

Research Article

Turk J Biol
(2013) 37: 377-384
© TÜBİTAK
doi:10.3906/biy-1207-37

Purification and characterization of a novel detergent- and organic solvent-resistant endo-beta-1,4-glucanase from a newly isolated basidiomycete *Peniophora* sp. NDVN01

Dinh Kha TRINH[1,2], Dinh Thi QUYEN[2,3,*], Thi Tuyen DO[2], Ngoc Minh NGHIEM[2]

[1]Department of Life Science, College of Sciences, Thai Nguyen University, Quyet Thang Commune, Thai Nguyen City, Vietnam
[2]Institute of Biotechnology, Vietnam Academy of Science and Technology, 10600 Hanoi, Vietnam
[3]Department of Biotechnology and Pharmacology, University of Science and Technology of Hanoi, 10600 Hanoi, Vietnam

Received: 19.07.2012 • **Accepted:** 09.11.2012 • **Published Online:** 30.07.2013 • **Printed:** 26.08.2013

Abstract: A novel extracellular endoglucanase from a basidiomycete strain *Peniophora* sp. NDVN01 was purified 2.8-fold to homogeneity through ammonium sulfate precipitation and gel filtration with Bio-Gel P-100 and Sephadex G-75. The endoglucanase had a specific activity of 163.8 U/mg protein and a molecular mass of 32 kDa. Optimum temperature and pH were at 60 °C and 4.5, respectively. The enzyme was stable at up to 42 °C and in the pH range of 3.5–5.5 with a residual activity of over 80% for 24 h of treatment. Ni^{2+} activated but other metal ions showed no or slight inhibitory effect on the enzyme activity. The endoglucanase showed a high resistance to most tested detergents and organic solvents. The endoglucanase catalyzed the hydrolysis of barley β-glucan and carboxymethyl cellulose (CMC), but not toward xylan, locust bean gum, and Avicel, typical substrates for xylanase, mannanase and exoglucanase, respectively. The kinetic parameters K_m, V_{max}, K_{cat}, and K_{cat}/K_m for barley β-glucan and carboxymethyl cellulose were 5.9 mg/mL, 9804 U/mg, 6.14 × 10^5 min^{-1}, and 1.04 × 10^5 and 34.8 mg/mL, 1825 U/mg, 1.14 × 10^5 min^{-1}, and 0.33 × 10^4, respectively. Hydrolysis of CMC liberated cellobiose, cellotriose, cellotetraose, and a detectable amount of glucose. These results suggest that the endoglucanase might potentially be used in enzymatic reactions and to investigate the efficacy of feed enzymes.

Key words: Characterization, endoglucanase, enzyme kinetics, detergent- and organic solvent-resistant, purification, *Peniophora* sp. NDVN01

1. Introduction

Cellulose, a polysaccharide of D-glucose units linked by 1,4-β-D-glycosidic bonds, is a major constituent of plant cell walls and the most abundant organic compound in the biosphere (1). Cellulolytic enzymes catalyze the hydrolysis of β-1,4-glycosidic linkages in plant polysaccharides (2). Cellulose is converted to glucose by a complex enzyme system called cellulase, comprising 3 hydrolytic enzymes: endo-1,4-β-D-glucanase (EG, EC 3.2.1.4; endoglucanase, endocellulase, endo-1,4-β-glucanase, carboxymethyl cellulase (CMCase), β-1,4-glucanase, β-1,4-endoglucan hydrolase, and celludextrinase), exo-1,4-β-D-glucanase (EXG, EC 3.2.1.91; cellobiohydrolase, exocellulase, microcrystalline cellulase, and avicelase), and β-glucosidase (BGL, EC 3.2.1.21; cellobiase). Endoglucanases cleave β-1,4-glycosidic bonds of cellulose chains randomly to produce new chain ends, while exoglucanases release cellobiose from reducing and nonreducing ends, generally from the crystalline parts of cellulose, and glucosidases hydrolyze cellobiose and short-chain cello-oligosaccharides to glucose (3).

Cellulases have a broad variety of applications in food, animal feed, brewing, paper pulp, detergent industries, textile industry, fuel, chemical industries, waste management, and pollution treatment (3–5). Due to their broad industrial applications, a variety of cellulases have been produced, purified, and characterized, most from filamentous fungi, including *Trichoderma* sp. (6,7), *Aspergillus aculeatus* (8), *A. nidulans*, *A. niger* (9), *A. awamori* (10), *A. oryzae* (11), and *A. terreus* (12), but also from several basidiomycetes including *Laetiporus sulphureus* (13), *Flammulina velutipes* (14), *Fomitopsis pinicola* (15), *Phanerochaete chrysosporium* (16), *Volvariella volvacea* (17), and *Moniliophthora perniciosa* (18). Due to the solubilizing hydrophobic substrates in enzymatic reactions and denaturing glycoproteins, the effects of organic solvents (19) and detergents (20) besides temperature, pH, and metal ions on cellulases were also reported. Even compatibility of cellulases with detergents

* Correspondence: quyen@ibt.ac.vn

was investigated because of the utilization for industrial purposes, especially for the detergent and laundry industry (21) and the modification of enzymes to enhance the stability in water-miscible organic solvents (22).

Peniophora is a genus of ligninolytic basidiomycetes that produce laccase (EC 1.10.3.2) (23), manganese-dependent peroxidase (EC 1.11.1.13) (24), pyranose oxidase (25), phytase (26), and alcohol oxidase (27). So far no studies have described the purification and characterization of a β-glucan–degrading enzyme from *Peniophora* sp. This paper, for the first time, describes the purification and characterization of physicochemical properties and enzyme kinetics of a novel endoglucanase from the newly isolated basidiomycete strain *Peniophora* sp. NDVN01 in Vietnam.

2. Materials and methods

2.1. Chemicals

Carboxymethyl cellulose (CMC) was acquired from BDH Prolabo Chemicals (a brand of VWR International S.A.S., Fontenay-sous-Bois, France); barley β-glucan, birchwood xylan, locust bean gum (LBG), SDS, Triton X-114, 3,5-dinitrosalicylic acid (DNS), glucose (G1), cellobiose (G2), cellotriose (G3), and cellotetraose (G4) from Sigma Aldrich Co. (St. Louis, MO, USA); Avicel from Taiwan (FMC BioPolymer); Sephadex G-75 from Pharmacia (GE Healthcare, Uppsala, Sweden); Bio-Gel P-100 from Bio-Rad (Hercules, CA, USA); Tween 20 and Tween 80 from Bio Basic Inc. (New York, USA); and Triton X-100 from Merck (Darmstadt, Germany). All other chemicals were of analytical grade unless otherwise stated.

2.2. Microorganism and growth medium

The basidiomycete strain *Peniophora* sp. NDVN01 from the Culture Collection of the Laboratory of Biology (Department of Life Science, College of Sciences, Thai Nguyen University, Vietnam) was identified based on morphology and the sequence variation region containing the 18S ribosomal RNA gene (partial sequence), internal transcribed spacer 1, 5.8S ribosomal RNA gene, internal transcribed spacer 2 (complete sequence), and 28S ribosomal RNA gene (partial sequence). It was deposited in GenBank with accession number JF925333 and used for EG production. The strain was grown in 250-mL Erlenmeyer flasks containing 50 mL of medium with 0.5% (w/v) pulp, 0.2% (w/v) $(NH_4)_2HPO_4$, 0.15% (w/v) KCl, 0.1% (w/v) $CaCO_3$, and 80% (v/v) potato infusion, pH 7. The cultivation was performed at 28 °C with agitation at 200 rpm for 7 days. The culture was centrifuged at 10,000 rpm for 10 min to obtain the crude enzyme.

2.3. Purification of endoglucanase

Ammonium sulfate was added slowly to 100 mL of crude enzyme solution with constant stirring in an ice water bath to reach 90% saturation (61.2 g per 100 mL enzyme solution). After the addition of the required amount of ammonium sulfate, the mixture was stirred for a further 30 min and centrifuged at 10,000 rpm for 15 min. The pellet was resuspended in 10 mL of sterile distilled water. The ammonium sulfate was removed by dialysis. From the dialysate, 8 mL was loaded into a Bio-Gel P-100 column (2.6 × 16 cm), preequilibrated with 50 mM sodium acetate buffer (pH 4.5) at a flow rate of 20 mL/h, and then washed with the same buffer. Proteins were eluted with 50 mM sodium acetate buffer (pH 4.5) at a flow rate of 20 mL/h, and fractions of 2 mL were collected. The 8 fractions that showed maximum enzyme activity were pooled and passed through a Sephadex G-75 column (2.6 × 40 cm), preequilibrated with 50 mM sodium acetate buffer (pH 4.5) at a flow rate of 20 mL/h, and then washed with the same buffer. Proteins were eluted with 50 mM sodium acetate buffer (pH 4.5) at a flow rate of 20 mL/h, and fractions of 2 mL were collected. The 4 fractions showing the maximum enzyme activity were pooled.

2.4. Endoglucanase activity

The EG activity was measured by the DNS method based on reducing sugars released (28). Reaction mixture containing 0.5 mL of appropriately diluted enzyme (0.53 µg for each reaction) and 1 mL of 1% (w/v) CMC prepared in 100 mM sodium acetate buffer, pH 5, was incubated at 50 °C for 10 min. The reaction was terminated by the addition of 1.5 mL of 1% (w/v) DNS solution, followed by boiling for 5 min to develop the red-brown color. Next, 0.5 mL of a 40% (w/v) potassium sodium tartrate (Rochelle salt) solution was added to stabilize the color. After cooling to room temperature in an ice-water bath, the absorbance of the solution was recorded at 575 nm on a UV-2500 spectrophotometer (Labomed Inc., Culver City, CA, USA). One unit (U) of enzyme activity is defined as the amount of the enzyme that liberates 1 µmol glucose from the substrate in 1 min under the standard assay conditions.

2.5. Gel electrophoresis and protein concentration

The homogeneity and molecular mass of the EG were determined by 12.5% SDS polyacrylamide gel electrophoresis (PAGE) (29) using Biometra equipment (Göttingen, Germany). Proteins were visualized by staining with 0.1% (w/v) Coomassie Brilliant Blue R-250. Protein concentrations were estimated by the Bradford method with bovine serum albumin as standard (30).

To detect glucanase activity, native PAGE was performed using 12.5% polyacrylamide containing 0.2% (w/v) CMC. The native gel was submerged in sterile distilled water for 30 min and then in 1% (w/v) Triton X-100 for 1 h at room temperature. The gel was then transferred into 50 mM

sodium acetate buffer, pH 4.5, for 30 min and incubated overnight at room temperature. The gel was stained in a solution of 1% (w/v) Congo Red for 30 min, and destained in 1 M sodium chloride for 15 min. The activity band was clearly visible as yellowish clearances against a deep red background after 10 min destaining.

2.6. Temperature and pH optimum

The pH and temperature optima of the EG were determined by measuring the activity of 0.53 µg EG for each reaction as described above using 100 mM sodium acetate buffer (pH 3–5.5) and 100 mM potassium phosphate buffer (pH 5.5–7.5) at 50 °C, and at the temperature range of 30 to 85 °C using 100 mM sodium acetate buffer (pH 5), respectively.

2.7. Thermal and pH stability

For the determination of thermal and pH stability, the purified enzyme, at 0.53 µg for each reaction, was preincubated at different temperatures ranging from 30 to 60 °C for 2–24 h and a pH range from 3 to 7.5 (100 mM sodium acetate, pH 3–5.5, and 100 mM potassium phosphate, pH 6–7.5) at 37 °C for 2–24 h, respectively. The residual activity was then determined.

2.8. Effect of metal ions, detergents, and organic solvents

The purified EG, 0.32 µg for each reaction, was incubated in the presence of 2–10 mM of various metal ions (Ag^+, K^+, Na^+, Ba^{2+}, Ca^{2+}, Cu^{2+}, Fe^{2+}, Mg^{2+}, Mn^{2+}, Ni^{2+}, and Zn^{2+}), EDTA, and β-mercaptoethanol, or in the presence of 1%–20% (w/v) of different detergents (Tween 80, Tween 20, SDS, Triton X-100, and Triton X-114), or in the presence of 1%–20% (v/v) of different solvents (methanol, ethanol, isopropanol, acetone, and 1-butanol) at 37 °C for 30 min. The residual activity was then determined.

2.9. Substrate specificity

To determine the substrate specificity of the EG from *Peniophora* sp. NDVN01, barley β-glucan, CMC, birchwood xylan, LBG, and Avicel were used as substrates.

2.10. Kinetic parameters

The Michaelis–Menten kinetic parameters (K_m and V_{max}) were determined against 1 to 10 mg/mL of barley β-glucan and 7 to 20 mg/mL of CMC as substrate using Lineweaver–Burk plots.

2.11. Enzymatic product analysis

For enzymatic hydrolysis of CMC, 900 µL of 1% (w/v) CMC substrate prepared in 100 mM sodium acetate buffer (pH 5) was incubated with 100 µL of 1.6 µg purified EG at 37 °C for 72 h. Products were detected by TLC (20 × 20 cm layer of silica gel 60, Merck), performed with a mobile phase of n-butanol/acetic acid/water (2:1:1, v/v/v). Carbohydrate products were visualized by spraying the TLC plate with 10% (v/v) sulfuric acid in ethanol and incubating at 120 °C for 15 min (31). A mixture of glucose (G1), cellobiose (G2), cellotriose (G3), and cellotetraose (G4) was used as the standard.

2.12. Statistical analysis

All measurements were carried out in triplicate with the resulting values being the mean of the cumulative data obtained.

3. Results and discussion

3.1. Enzyme purification and molecular mass

The glucanase from *Peniophora* sp. NDVN01 was purified to homogeneity through precipitation and gel filtration with Bio-Gel P-100 and Sephadex G-75 with a purification factor of 2.8 and a yield of 3.6% (Table 1). The purified EG exhibited a specific activity of 163.8 U/mg protein and an estimated molecular mass of 32 kDa (Figure 1, lane 3).

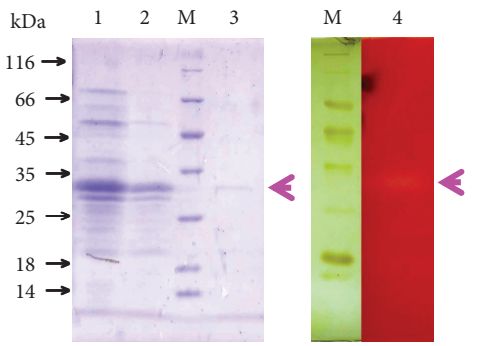

Figure 1. SDS-PAGE of the purified EG from *Peniophora* sp. NDVN01 through Bio-Gel P-100 and Sephadex G-75; lane M: molecular mass marker; lane 1: culture supernatant, lane 2: eluate through Bio-Gel P-100, lane 3: eluate through Sephadex G-75; lane 4: the EG activity staining with Congo Red.

Table 1. Purification steps of endoglucanase produced by *Peniophora* sp. NDVN01

Purification step	Total protein (mg)	Total activity (unit)	Specific activity (U/mg)	Recovery (%)	Purification fold
Crude enzyme	38.7	2271.3	58.7	100	1.0
Precipitation with $(NH_4)_2SO_4$	13.3	1159.6	87.2	51.1	1.5
Gel filtration, Bio-Gel P-100	6.1	715.0	117.2	31.5	2.0
Gel filtration, Sephadex G-75	0.5	81.9	163.8	3.6	2.8

The molecular mass of this glucanase (32 kDa) was lower than that of the EG from *V. volvacea* (42 kDa (17)), but higher than that of the EG from *L. sulphureus* var. *miniatus* (26 kDa (13)) and *P. chrysosporium* strain K3 (28 kDa (16)). EGs were purified 16-fold with a yield of 5% and a specific activity of 29 U/mg protein (*L. sulphureus* var. *miniatus* (13)) and 42-fold with a yield of 1.5% and a specific activity of 324 U/mg protein (*V. volvacea* (17)).

3.2. Temperature and pH optima

The EG activity increased gradually from 247 U/mg (32%) at 30 °C to the maximum of 761 U/mg (100%) at 60 °C (Figure 2A), and then decreased gradually to 388 U/mg (51%) at 85 °C. The EG activity gradually increased from 577 U/mg (75%) at pH 3 to the maximum of 767 U/mg (100%) at pH 4.5, and then decreased dramatically to 201 U/mg (26%) at pH 7 and was completely lost at pH 7.5 (Figure 2B).

The optimum temperature (60 °C) and optimum pH (4.5) for the EG from *Peniophora* sp. NDVN01 agreed with the cellulase produced by other basidiomycetes including *F. pinicola* KMJ812 (50 °C and pH 4.5 (15)), *L. sulphureus* var. *miniatus* (75 °C and pH 4 (13)), *F. velutipes* (60 °C and pH 6.1 (14)), and *V. volvacea* (55 °C and pH 7.5 (17)).

3.3. Temperature and pH stability

Thermostability is an important characteristic for potentially applicable enzymes in the biotechnology industry. The enzyme was temperature-stable at up to 42 °C and pH stable in the pH range of 3.5–5.5. The activity was retained over 70% after incubation at 30–42 °C for 24 h (Figure 3A) and over 77% after incubation at pH 3.5–5.5 at 37 °C for 24 h (Figure 3B).

The EG from *Peniophora* sp. NDVN01 was less thermostable than the β-1,3-1,4-glucanase from *L. sulphureus* var. *miniatus* (13) and the β-1,4-glucosidase from *F. pinicola* KMJ812 (15).

3.4. Effect of various metal ions

The effects of metal ions and other reagents on the endoglucanase activity were examined, each at the concentration of 2–10 mM (Table 2). The addition of Ni^{2+} increased the enzyme activity by up to 47%–68%; this suggested that the enzyme required divalent cations of Ni^{2+} for optimal activity. The addition of Ag^{+} and Cu^{2+} ions at the concentration of 4–10 mM completely inhibited the EG activity. Metal ions K^{+}, Ca^{2+}, Zn^{2+}, and Ba^{2+} showed no or slight effect on the EG activity (Table 2). Metal ions Na^{+}, Fe^{2+}, Mn^{2+}, Mg^{2+}, and EDTA showed a moderate inhibition.

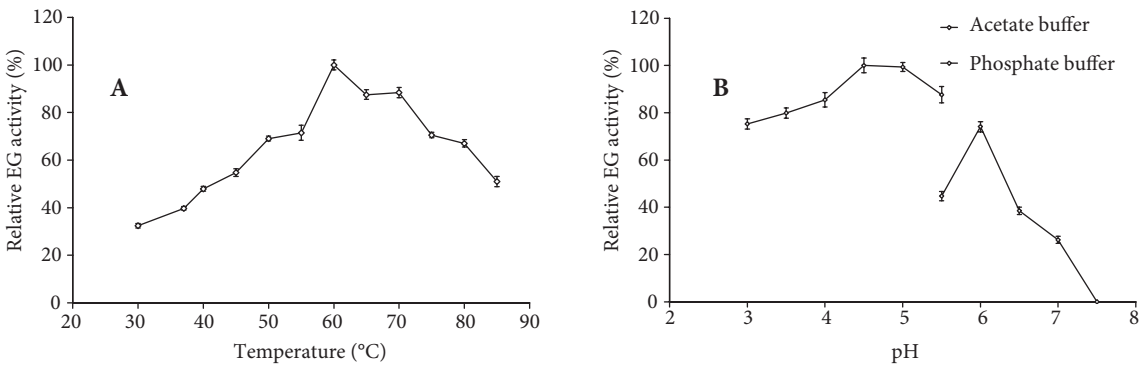

Figure 2. Temperature (A) and pH (B) optima.

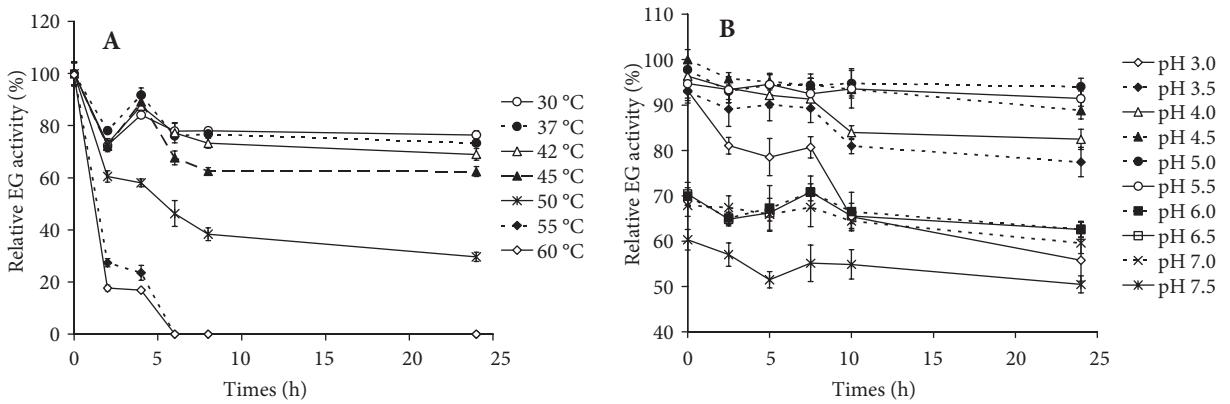

Figure 3. Temperature (A) and pH (B) stability. The relative EG activity in the control reaction at 37 °C and at pH 4.5 for time zero was defined as 100% for temperature and pH stability, respectively.

Table 2. Effect of metal ions and some other reagents on EG activity.

Metal ions and other reagents (mM)	Activity remaining (%)				
	2	4	6	8	10
Ag^{+}	72 ± 2.0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
K^{+}	91 ± 3.0	94 ± 2.6	93 ± 3.1	94 ± 3.3	91 ± 3.0
Na^{+}	86 ± 5.8	85 ± 1.8	81 ± 3.5	94 ± 2.8	97 ± 3.0
Ba^{2+}	98 ± 4.8	115 ± 1.8	93 ± 3.5	92 ± 3.2	91 ± 2.1
Ca^{2+}	102 ± 2.3	100 ± 2.9	99 ± 2.0	92 ± 2.4	86 ± 2.8
Cu^{2+}	53 ± 2.2	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Fe^{2+}	70 ± 2.8	77 ± 3.2	92 ± 2.4	93 ± 2.6	101 ± 2.5
Mg^{2+}	79 ± 2.8	74 ± 3.4	78 ± 2.3	80 ± 2.5	79 ± 3.1
Mn^{2+}	65 ± 3.2	84 ± 2.4	95 ± 2.6	95 ± 3.2	97 ± 3.2
Ni^{2+}	168 ± 1.6	147 ± 1.4	108 ± 3.1	105 ± 3.1	104 ± 2.6
Zn^{2+}	106 ± 2.8	97 ± 2.5	99 ± 2.9	99 ± 1.8	99 ± 1.2
EDTA	61 ± 1.1	65 ± 2.4	66 ± 3.0	71 ± 2.2	67 ± 3.1
2-Mercaptoethanol	115 ± 2.6	108 ± 3.2	103 ± 2.3	100 ± 2.5	94 ± 1.2

The relative EG activity in the control reaction without addition of any factor was defined as 100%.

An activity increase in the presence of 2-mercaptoethanol suggested the absence of a thiol group.

The finding that Ni^{2+} stimulated the EG from *Peniophora* sp. NDVN01 was coincident with the Ni^{2+} stimulation of the β-1,3-1,4-glucanase from *L. sulphureus* var. *miniatus* (13). The enzyme inhibition in the presence of EDTA suggested that the EG might contain inorganic groups forming inactive complexes with EDTA (32).

3.5. Effect of detergents

The effects of ionic (SDS) and nonionic detergents (Tween 20, Tween 80, and Triton X-100, Triton X-114), currently used for denaturing glycoproteins, on EG activity were tested. Tween 20, Tween 80, and Triton X-100 at the concentration of 1%–20% (w/v) and Triton X-114 at the concentration of 1%–5% (w/v) increased the EG activity by up to 55% (Figure 4A). However, the enzyme activity was inhibited by Triton X-114 at the concentration of 10%–20% (w/v) and completely blocked by SDS (Figure 4A).

Tween 20, Tween 80, and Triton X-100 stimulated the EG from *Peniophora* sp. NDVN01, but inhibited the EG from *A. oryzae* VTCC-F045 (11) and *A. awamori* VTCC-F099 (10). SDS completely inhibited the EG from *Peniophora* sp. NDVN01, but partially inhibited the EG from *A. oryzae* VTCC-F045 (11), *A. awamori* VTCC-F099 (10), and *A. niger* VTCC-F021 (33).

3.6. Effect of organic solvents

Organic solvents are used for solubilizing hydrophobic substrates in enzymatic reactions; thus, we tested the effects of various organic solvents (Figure 4B). Acetone stimulated the enzyme activity up to 21%. The addition of 1%–5% (v/v) of other organic solvents increased the EG activity by up to 20% whereas the addition of 10%–20% (v/v) led to the loss of up to 36% in the EG activity.

The EG from *Peniophora* sp. NDVN01 showed high resistance to organic solvents but the EG from *Aspergillus* species (11,10) was inhibited. This finding suggested that the enzyme might be used for transglycosylation in media containing organic solvent (19).

3.7. Substrate specificity

To determine the substrate specificity, the endoglucanase activity towards barley β-glucan, CMC, xylan, LBG, and microcrystalline cellulose (Avicel) was measured. The enzyme displayed the highest activity towards barley β-glucan (5478.8 ± 14.7 U/mg), 4.56 times as high as towards CMC (1202.2 ± 17.3 U/mg). In contrast, no activity towards xylan, LBG, and Avicel was observed.

The substrate specificity of this endoglucanase was coincident with other endoglucanases from *L. sulphureus* var. *miniatus* (13) and *A. terreus* (12), which also showed the highest catalytic activity towards barley β-glucan and almost no activity toward Avicel. In contrast to the endoglucanase,

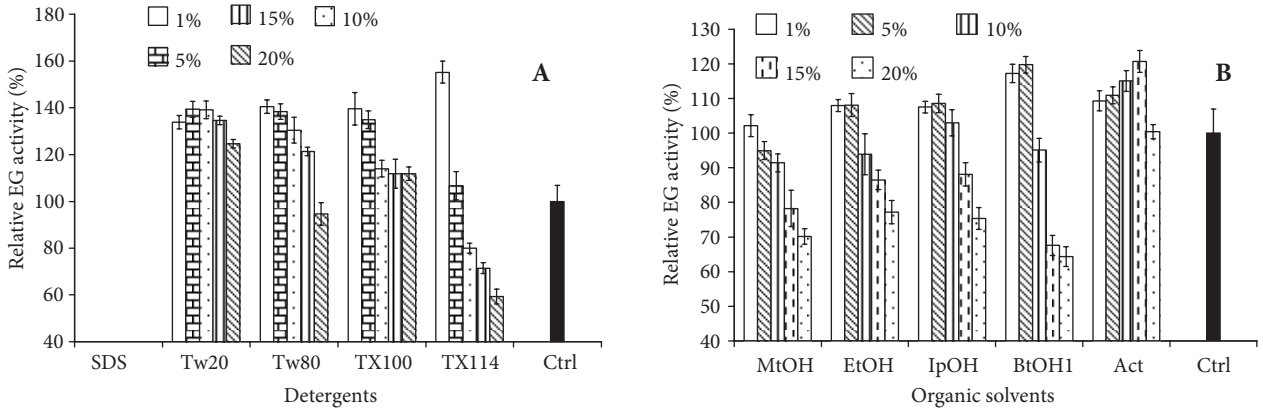

Figure 4. Effect of detergents (A) and organic solvents (B) on EG activity for Tween 20 (Tw20), Tween 80 (Tw80), Triton X-100 (TX100), Triton X-114 (TX114), control (Ctrl), methanol (MtOH), ethanol (EtOH), isopropanol (IpOH), 1-butanol (BtOH1), and acetone (Act). The relative EG activity in the control reaction without addition of any factor was defined as 100%.

the β-1,4-glucosidase from *F. pinicola* KMJ812 showed catalytic activity toward cellobiose and Avicel, but no catalytic activity toward CMC (15). The exo-β-1,3-1,6-glucanase from *F. velutipes* showed the highest activity on laminarin from *Laminaria digitata*, which consists of main chain β-1,3-glycosidic linkages with β-1,6-branches, but no activity on Avicel and CMC (14). The substrate specificity for cellulases comprising 3 hydrolytic enzymes (endoglucanase, exoglucanase, and glucosidase) confirmed that the enzyme from *Peniophora* sp. NDVN01 was an endoglucanase.

3.8. Kinetics

The Michaelis constant K_m, V_{max}, turnover number (k_{cat}), and catalytic efficiency (k_{cat}/K_m) obtained for the β-glucanase from *Peniophora* sp. NDVN01 with barley β-glucan and CMC as substrates are shown in Table 3. The enzyme showed lower K_m, higher k_{cat}, and higher k_{cat}/K_m values for barley β-glucan than for CMC.

Similar to the glucanase from *L. sulphureus* var. *miniatus* (13) and *A. terreus* (12), the EG from *Peniophora* sp. NDVN01 preferred hydrolysis of barley β-glucan to hydrolysis of CMC. This was proven by the higher V_{max} (the maximal rate), k_{cat} (the turnover number), and k_{cat}/K_m (the specific constant) values and lower K_m value (the Michaelis–Menten constant) toward barley β-glucan than those toward CMC.

3.9. Hydrolysis products

The hydrolysis products of CMC by the purified EG from *Peniophora* sp. NDVN01 were separated and detected with TLC (Figure 5). The major product of the CMC hydrolysis was cellobiose (G2) and cellotriose (G3), whereas glucose (G1) and cellotetraose (G4) were obtained in almost equal amounts. In addition, oligomers larger than G4 were also observed.

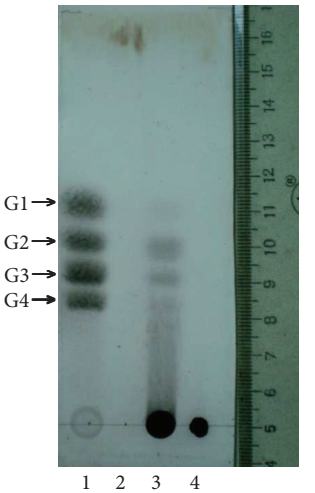

Figure 5. TLC analyses of hydrolyzed products. Lane 1: oligosaccharide standards, G1: glucose, G2: cellobiose, G3: cellotriose, G4: cellotetraose; lane 2: the purified EG; lane 3: denote hydrolyzed products of CMC after 72 h; lane 4: substrate 1% CMC (w/v).

The hydrolysis products of CMC were usually reducing sugars, including glucose. The hydrolysis of CMC and barley β-glucan EG of the from *A. terreus* also yielded cellobiose and cellotriose as the main products (12). The production

Table 3. Kinetic constants of endoglucanase from *Peniophora* sp. NDVN01.

Substrate	K_m (mg/mL)	V_{max} (U/mg protein)	K_{cat} (min^{-1})	K_{cat}/K_m
Barley β-glucan	5.9	9804	6.14×10^5	1.04×10^5
CMC	34.8	1825	1.14×10^5	0.33×10^4

of a relative amount of products indicates that the internal/central linkages are the preferred point of cleavage. This clearly demonstrates the classical interpretation of random hydrolysis, i.e. susceptibility of all bonds to cleavage.

In conclusion, biochemical properties of the extracellular endoglucanase from the newly isolated basidiomycete strain *Peniophora* sp. NDVN01 suggested that the endoglucanase is a potential enzyme for chemical reaction and additive enzyme for monogastric animals.

Acknowledgment

The study was supported by the Master Program of Development and Application of Biotechnology in Agriculture and Rural Development Towards 2020, Vietnam Ministry of Agriculture and Rural Development (Project: *Production and application of highly qualitative multi-enzyme products by recombinant microbes to improve the effective use of animal feed*, 2007–2010).

References

1. Hong J, Tamaki H, Akiba S et al. Cloning of a gene encoding a highly stable endo-1,4-glucanase from *Aspergillus niger* and its expression in yeast. J Biosci Bioeng 92: 434–441, 2001.
2. Han S, Yoo Y, Kang H. Characterization of a bifunctional cellulase and its structural gene. J Biol Chem 270: 26012–26019, 1995.
3. Bhat MK, Bhat S. Cellulose degrading enzymes and their potential industrial applications. Biotechnol Adv 15: 583–620, 1997.
4. Kirk O, Borchert TV, Fuglsang CC. Industrial enzyme applications. Curr Opin Biotech 13: 345–351, 2002.
5. Anish R, Rahman MS, Rao M. Application of cellulases from an alkalothermophilic *Thermomonospora* sp. in biopolishing of denims. Biotechnol Bioeng 96: 48–56, 2007.
6. de la Cruz J, Pintor-Toro JA, Benítez T et al. Purification and characterization of an endo-β-1,6-glucanase from *Trichoderma harzianum* that is related to its mycoparasitism. J Bacteriol 177: 1864–1871, 1995.
7. El-Zawahry YA, El-Mougith AA, El-Saadani MA et al. Partial purification and characterization of two endo-β-1,4-glucanase from *Trichoderma* sp. (Shmosa tri). Aust J Basic Appl Sci 4: 4559–4568, 2010.
8. Chen G, Du J, Zhuang L et al. Purification and properties of endoglucanases from *Aspergillus aculeatus* SM-L22. Wei Sheng Wu Xue Bao 41: 469–474, 2001.
9. Ali UF, El-Dein HSS. Production and partial purification of cellulase complex by *Aspergillus niger* and *A. nidulans* grown on water hyacinth blend. J Appl Sci Res 4: 875–891, 2008.
10. Nguyen VT, Quyen DT. Purification and properties of a novel thermoactive endoglucanase from *Aspergillus awamori* VTCC-F099. Aust J Basic Appl Sci 4: 6211–6216, 2010.
11. Nguyen HQ, Quyen DT. Purification and properties of an endoglucanase from *Aspergillus oryzae* VTCC-F045. Aust J Basic Appl Sci 4: 6217–6222, 2010.
12. Nazir A, Rohit S, Saini HS et al. Purification and characterization of an endoglucanase from *Aspergillus terreus* highly active against barley β-glucan and xyloglucan. World J Microbiol Biotechnol 25: 1189–1197, 2009.
13. Hong MR, Kim YS, Joo AR et al. Purification and characterization of a thermostable β-1,3-1,4-glucanase from *Laetiporus sulphureus* var. *miniatus*. J Microbiol Biotechnol 19: 818–822, 2009.
14. Fukuda K, Hiraga M, Asakuma S et al. Purification and characterization of a novel exo-β-1,3-1,6-glucanase from the fruiting body of the edible mushroom Enoki (*Flammulina velutipes*). Biosci Biotechnol Biochem 72: 3107–3113, 2008.
15. Joo AR, Marimuthu J, Lee KM et al. Purification and characterization of a β-1,4-glucosidase from a newly isolated strain of *Fomitopsis pinicola*. Appl Microbiol Biotechnol 83: 285–294, 2009.
16. Henriksson G, Nutt A, Henriksson H et al. Endoglucanase 28 (Cel12A), a new *Phanerochaete chrysosporium* cellulase. Eur J Biochem 259: 88–95, 1999.
17. Ding SJ, Wei G, Buswel JA. Endoglucanase I from the edible straw mushroom, *Volvariella volvacea*. Purification, characterization, cloning and expression. Eur J Biochem 268: 5687–5695, 2001.
18. Sena AR, Júnior GLV, Neto AG et al. Production, purification and characterization of a thermostable β-1,3-glucanase (laminarinase) produced by *Moniliophthora perniciosa*. An Acad Bras Ciênc 83: 599–609, 2011.
19. Oikawa T, Tsukagawa Y, Chino M et al. Increased trans-glycosylation activity of *Rhodotorula glutinis* endo-β-glucanase in media containing organic solvent. Biosci Biotechnol Biochem 65: 1889–1892, 2001.
20. de Marco JL, Felix CR. Purification and characterization of a β-glucanase produced by *Trichoderma harzianum* showing biocontrol potential. Braz Arch Biol Technol 50: 21–29, 2007.
21. Iqbal HMN, Ahmed I, Zia MA et al. Purification and characterization of the kinetic parameters of cellulase produced from wheat straw by *Trichoderma viride* under SSF and its detergent compatibility. Adv Biosci Biotechnol 2: 149–156, 2011.
22. Siddiqui KS, Shemsi AM, Anwar MA et al. Partial and complete alteration of surface charges of carboxymethyl cellulase by chemical modification: thermostabilization in water-miscible organic solvent. Enzyme Microb Technol 24: 599–608, 1998.
23. Niku-Paavola M-L, Fagerström R, Kruus K et al. Thermostable laccases produced by a white-rot fungus from *Peniophora* species. Enzyme Microb Technol 35: 100–102, 2004.
24. Machado KMG, Matheus DR, Bononi VLR. Ligninolytic enzymes production and remazol brilliant blue R decolorization by tropical Brazilian basidiomycetes fungi. Braz J Microbiol 36: 246–252, 2005.

25. Danneel HJ, Roessner E, Zeeck A et al. Purification and characterization of a pyranose oxidase from the basidiomycete *Peniophora gigantea* and chemical analyses of its reaction products. FEBS Eur J Biochem 214: 795–802, 1993.

26. Ullah AHJ, Sethumadhavan K. PhyA gene product of *Aspergillus ficuum* and *Peniophora lycii* produces dissimilar phytases. Biochem Biophys Res Commun 303: 463–468, 2003.

27. Danneel HJ, Reichert A, Giffhorn F. Production, purification and characterization of an alcohol oxidase from the ligninolytic fungus *Peniophora gigantea*. J Biotechnol 33: 33–41, 1994.

28. Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing sugars. Anal Chem 31: 426–428, 1959.

29. Laemmli UK. Cleavage of structure proteins during the assembly of the head of bacteriophage T4. Nature 227: 680–685, 1970.

30. Bradford MM. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72: 248–254, 1976.

31. Nishida Y, Suzuki K, Kumagai Y et al. Isolation and primary structure of a cellulase from the Japanese sea urchin *Strongylocentrotus nudus*. Biochimie 89: 1002–1011, 2007.

32. Bakare MK, Adewale IO, Ajayi A et al. Purification and characterization of cellulase from the wild-type and two improved mutants of *Pseudomonas fluorescens*. Afr J Biotechnol 4: 898–904, 2005.

33. Pham HT, Quyen TD, Nghiem MN. Purification and properties of a thermoactive endoglucanase from *Aspergillus niger* VTCC-F021. Turk J Biol 36: 694–701, 2012.