Purification and Characterization of a New Cold-active Cellulolytic Enzyme Produced by *Pseudoalteromonas* sp. ArcC09 from the Arctic Beaufort Sea

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A cold-active endoglucanase-producing bacterium was isolated from the Beaufort Sea of the Arctic Ocean and identified as *Pseudoalteromonas* sp. ArcC09. Cellulolytic activity of ArcC09 reached a maximum of 60 U/mg when cultivated in ZoBell medium for 72 h at 15 °C. This purified endoglucanase, with a molecular mass of 28 kDa, exhibited maximum activity at pH 7.0 and 55 °C. The ArcC09 endoglucanase exhibited 10% and 36% of its maximal activity even at low temperatures of 5 °C and 15 °C, respectively. However, it showed lower thermal stability than a mesophilic cellulase, which is characteristic of a psychrophilic enzyme. The activity was inhibited by $CuSO_4$, and linear alkylbenzene sulfonate (LAS). These findings supplement the understanding of cold-active endoglucanases and may have commercial applications in enzymatic digestion of cellulosic biomass to fermentable sugars.

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Keywords: Arctic bacterium; Cellulolytic enzyme; Cold-active; Pseudoalteromonas; Purification

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INTRODUCTION

Cellulose is a polysaccharide comprised of glucose units connected *via* β-1,4,-glycosidic linkages. It is considered the most plentiful and renewable bio-resource in the world. The utility of lignocellulosic biomass has emerged and interest in the development of efficient cellulases has increased (Gomes *et al*. 2015). Cellulases have broad applications in a variety of fields, such as the pulp and paper, detergent, and biofuel industries (Srivastava *et al*. 2014). There are several types of cellulases, namely, 1,4-β-D-glucan-4-glucanohydrolases (EC 3.2.1.4, endoglucanases), 1,4-β-D-glucan glucano-hydrolases (EC 3.2.1.74, exoglucanases), 1,4-β-D-glucan cellobiohydrolases (EC 3.2.1.91, cellobiohydrolases), and β-glucoside glucohydrolases (EC 3.2.1.21, β-glucosidases) (Lynd *et al*. 2002). The hydrolysis of a cellulosic substrate into fermentable sugars requires three major cellulase systems, namely, endoglucanase (Sharma *et al*. 2016), cellobiohydrolases (Zhu and McBride 2017), and β-glucosidases (Parisutham *et al*. 2017).

Most cellulolytic producers reported to date are fungi, such as *Trichoderma reesei* (Bischof *et al*. 2016), *Neurospora crassa* (Dogaris *et al*. 2013), *Penicillium oxalicum* (Li *et al*. 2017), and *Aspergillus niger* (Stricker *et al*. 2008), and bacteria, including *Clostridium acetobutylicum* (Doi and Kosugi 2004), *Clostridium thermocellum* (Olson *et al*. 2010), *Bacillus halodurans* (Annamalai *et al*. 2013), *Acidothermus cellulolyticus* (Wang

et al. 2015a), *Paenibacillus* sp. (Kanchanadumkerng *et al*. 2017), and *Bacillus licheniformis* (de Marco *et al*. 2017). Most of these microorganisms produce cellulases requiring moderate or higher temperatures for optimal activity. Higher temperatures (60 to 70 °C) are necessary to saccharify the cellulose slurry, requiring more energy, which can be saved by using cold-active cellulases (Maharana and Ray 2015). According to a recent review, cold-active enzymes produced by psychrophiles from deep-sea areas are promising sources in industry, and it has been revealed that their flexible structure aids in maintaining their activity at low temperatures (Jin *et al*. 2019). There are several advantages of cold-active enzymes in industrial applications (Sarmiento *et al*. 2015). In terms of cost, a low-temperature process using a cold-active enzyme can reduce the processing time and energy needed to facilitate the reaction. Low-temperature reaction processes carried out in cold environments also reduce the incidence of contamination and, in some cases, reduce the production of unwanted byproducts. In addition, because of their low thermal stability, cold-active enzymes can be used when an enzyme is demanded for a particular process only, to avert an unwanted byproduct at higher temperatures. In addition, cold-active cellulases can be used to remove undesired fibrils from clothes in the textile industry (Ponnada *et al*. 2011). The cold-active enzymes can conserve energy, as their coverage temperature range is broad, including cold to warm conditions (Kasana and Gulati 2011). Therefore, a single enzyme could be applied to conduct reactions at various temperatures. Despite these advantages, the study of cold-active cellulases has been extremely limited (Yang and Dang 2011; Caf *et al*. 2014; Wang *et al*. 2015b; Souza *et al*. 2016). In the present study, a new cellulase-secreting bacterium was obtained from Arctic seawater and verified for cold-active cellulolytic activity.

EXPERIMENTAL

Materials

Isolation of cellulolytic enzyme-producing bacteria

Over 4,600 microbes were isolated from CTD (conductivity, temperature, and depth) membranes, and the box core samples, head sea water, soil, and seawater were collected from the Arctic Beaufort Sea. Strains were cultured using Reasoner's 2A (R2A) broth (MB Cell, Seoul, South Korea), Marine Broth (BD Difco, Franklin Lakes, NJ, USA), ZoBell (0.5% [w/v] peptone and 0.1% [w/v] yeast extract in 75% [v/v] sea water), Super ZoBell (2% [w/v] glucose added to ZoBell media), International Streptomyces project (ISP) Medium 4 (BD Difco, Franklin Lakes, NJ, USA), and yeast extract, peptone, and glucose (YPG) media (0.5% [w/v] yeast extract, 0.5% [w/v] peptone, and 1% [w/v] glucose) plates. Endoglucanase-producing bacteria were isolated by culturing microbes on media plates containing 1% [w/v] carboxymethyl-cellulose (CMC, Sigma, St. Louis, MO, USA). Plates were incubated at 15 °C for 3 days, and bacteria having extracellular cellulolytic activity were selected by applying 0.1% [w/v] Congo red (Sigma-Aldrich, Munich, Germany) for 10 min followed by washing with 1 M NaCl (Teather and Wood 1982). Fifty-four strains were sorted by observing clear zones, which were light orange surrounding the colonies, with the size of the clear zones measured and listed in sequential order. Ten bacterial strains showing the largest clear zone measurements were selected and tested for cellulolytic activity using liquid culture broth (Table 1).

Bacterial Identification and Phylogeny Tree Construction

The isolated ten strains were identified using 16S rRNA PCR with 27F (5' AGA GTT TGA TCM TGG CTC AG 3') and 1492R (5' TAC GGY TAC CTT GTT ACG ACT T 3') primers and sequencing was performed with 518F (5' CCA GCA GCC GCG GTA ATA CG 3') and 800R (5' TAC CAG GGT ATC TAA TCC 3') primers. The sequenced contigs were aligned using the EzTaxon program (www.eztaxon.org) (Chun *et al*. 2007), and the closest matched strains were determined. The phylogenetic tree was constructed based on the 16S rRNA gene sequence data, using the neighbor-joining method (Saitou and Nei 1987) of the computer program Molecular Evolutionary Genetics Analysis 6 (MEGA6, version 6.0, State College, PA, USA) (Tamura *et al*. 2013). *Bacillus halodurans* was included as the outgroup.

Table 1. Sampling Sites and Source of the 10 Bacteria from Beaufort Sea*

Strain Code	Location	Depth (m)	Source
ArcC01	70°47'33.28 N, 135°34'04.92 W	42	CTD membrane
ArcC02	69°53.64.05 N, 138°16.45.98 W	25	CTD membrane
ArcC03	70°23.72.78 N, 135°18.74.83 W	30	CTD membrane
ArcC04	70°27.54.40 N, 134°33.66.64 W	15	CTD membrane
ArcC05	70°48.11.11 N, 136°05.98.11 W	740	Head sea water
ArcC06	69°42.06.81 N, 137°32.31.91 W	24	CTD membrane
ArcC07	70°39.34.56 N, 138°21.11.99 W	200	CTD membrane
ArcC08	70°39.34.56 N, 138°21.11.99 W	200	CTD membrane
ArcC09	70°39.34.56 N, 138°21.11.99 W	200	CTD membrane
ArcC10	69°42.06.81 N, 137°32.31.91 W	0	CTD membrane

*Samples were collected during the 2014 KOPRI-led Arctic Araon scientific cruise (Aug. 30 to Sep. 15, 2014)

Cultivation of bacteria and measurement of cellulolytic activity

Selected bacteria were cultivated in liquid ZoBell medium containing 1 g/L of Cellulose (9004-34-6, Carl Roth, Karlsruhe, Germany) for 3 days at 15 °C. The supernatant from 10 cultures were isolated by centrifugation (9,000 × g, 30 min), and their activities were determined through assaying CMC hydrolysis rates. Endoglucanase activity was assayed *via* addition 0.1 mL of each supernatant (enzyme solution) to 0.5 mL CMC (1% [w/v]) in 50 mM sodium acetate buffer (pH 5.0) followed by incubation at 37 °C for 15 min. The reaction was stopped *via* the addition of 0.6 mL of 3,5-dinitrosalicylic acid (DNS) reagent and the OD_{540} was measured using a Scinco S-3100 spectrophotometer (Scinco Co., Ltd., Seoul, Korea) (Caf *et al*. 2014). The unit of activity per milligram (U/mg) was defined as the amount required to release 1 µmol of reducing sugar for 1 minute per milligram of protein. Commercially available cellulase (Cellulase from *A. niger*, C1184, Sigma, Ronkonkoma, NY, USA) was used as a standard cellulolytic enzyme.

Purification of cellulolytic enzyme

The cultured broth was centrifuged at 9,000 × g for 30 min at 4 °C (RC-5C Plus, Sorvall, Waltham, MA, USA), and the supernatant was collected for protein precipitation.

Ammonium sulfate ($(NH_4)_2SO_4$) was added with gentle stirring at 4 °C until the solution reached 70% [w/v] saturation. The solution was centrifuged at 9,000 × g for 30 min, and the precipitate was dissolved in 10 mL of 20 mM potassium phosphate buffer (pH 7.0). Two milliliters of the crude enzyme extract were loaded onto a Phenyl-Sepharose column (HiTrap, General Electric, Boston, MA, USA; 5 × 5 mL). The enzyme was eluted using 20 mM potassium phosphate buffer (pH 7.0) added with 1% isopropanol at a flow rate of 1.0 mL/min. The active fractions were pooled and loaded onto a Superdex 75 (General Electric, Boston, MA, USA) column with 20 mM potassium phosphate buffer (pH 7.0), and active fractions were pooled and concentrated using Vivaspin 20 (10 kDa cut-off) (General Electric, Boston, MA, USA).

Methods

Gel electrophoresis and zymography

The molecular mass and purity of the cellulolytic enzyme was confirmed using sodium dodecyl sulfate - polyacrylamide gel electrophoresis (SDS-PAGE) and zymography. Samples were denatured at 100 °C for 10 min and separated by 8 to 16% SDS-PAGE gel (4561103, Bio-Rad, Hercules, CA, USA). Protein marker (Precision Plus ProteinTM Standards, Bio-Rad, Hercules, CA, USA) was used to determine the size of the target protein bands. Protein bands were visualized by staining SDS-PAGE gels with EZ-Gel staining solution (DoGenBio, Seoul, South Korea). For zymography, 50 mM Tris buffer-washed SDS-PAGE gel was put on to 15% [w/v] agar plate added with 1% [w/v] CMC. The plate was incubated at 15 °C for 1 h and stained with 0.1% [w/v] Congo red solution. After 10 min of staining, 1 M sodium chloride was used for de-staining.

Characterization of purified cellulolytic enzyme

Enzymatic activity was studied over a temperature range of 5 to 85 °C at 10 °C intervals. The effect of temperature was investigated after incubating the enzyme and CMC for 30 min at each temperature. Thermostability of the enzyme was determined under standard assay conditions after pre-incubating the purified enzyme for 1 h at the desired temperature. The effect of pH on enzyme activity was monitored within the range of 3.0 to 9.0. The activity was measured after pre-incubating the purified enzyme with various buffer systems for 1 h at their optimal temperature. The buffer systems (50 mM) used in this study were as follows: sodium acetate (pH 3.0 to 6.0), potassium phosphate (pH 6.0 to 8.0), and Tris-HCl (pH 7.0 to 9.0). The effect of various additives, such as metal ions (1 mM) as Cl_2 and SO_4 salts, as well as 1 mM of chemical reagents, on enzyme activity was investigated. Enzyme activity was determined in the presence of these additives. The metal ions and detergent additives were selected by referring to the study of Park *et al.* (2018).

RESULTS AND DISCUSSION

Identification and Selection of Cellulolytic Enzyme-producing Bacteria

Following incubation of the isolated strains (over 4,600) from the Arctic Beaufort Sea at 15 °C for 3 days on CMC plates, 54 bacterial strains were cellulase producers with a halo around the colonies on the plates. Among them, 10 of the strains produced relatively large halos, qualifying them as promising candidates for producing cold-active cellulases. Among the 10 candidate strains obtained, 9 strains belonged to the genus *Pseudo-alteromonas*, while the final strain was a member of the genus *Paraglaciecola* (Fig. 1).

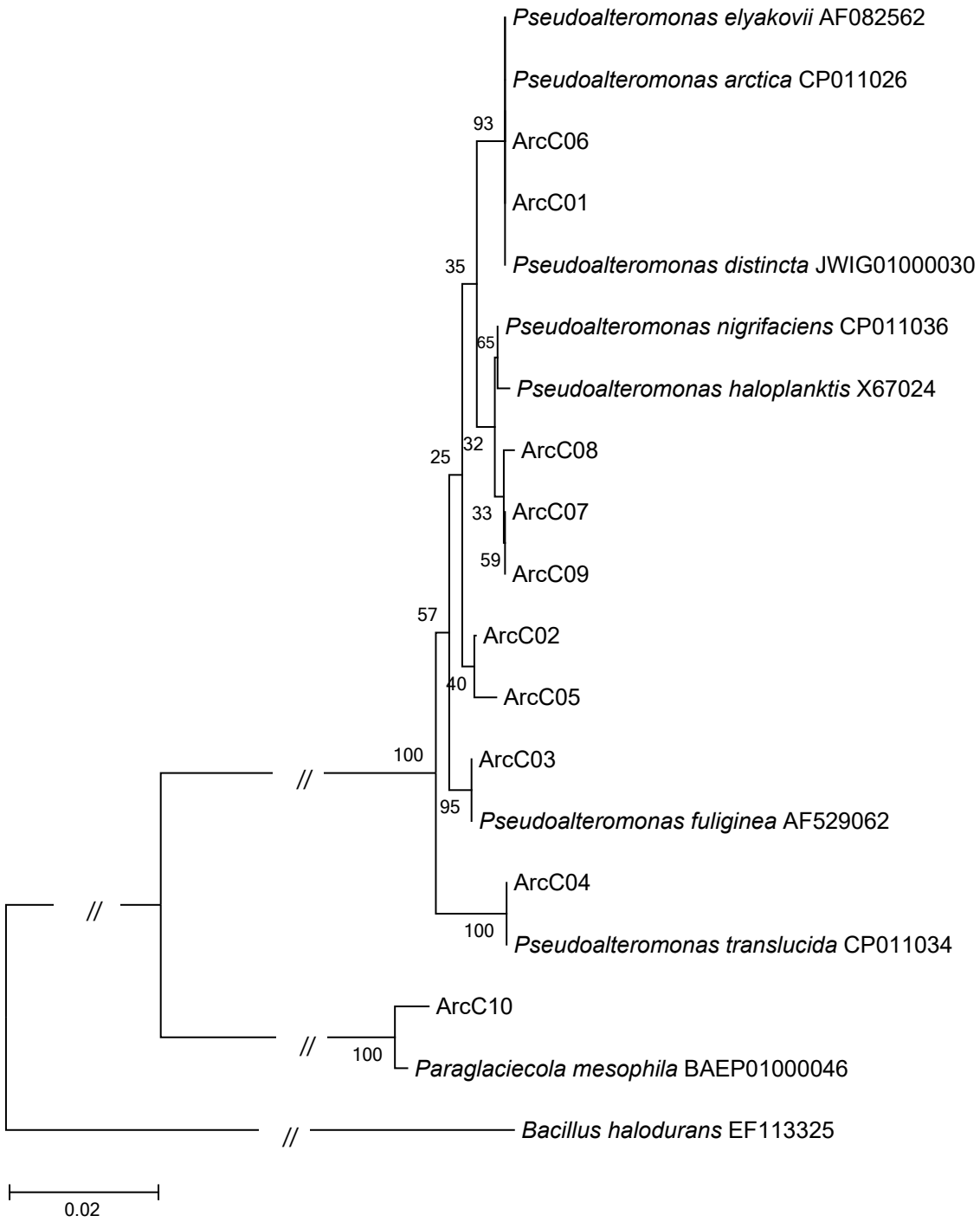

Fig. 1. Phylogenic tree of strains possessing cellulase activity. The phylogenic tree was prepared with MEGA6 software using the neighbor-joining method and 1,000 bootstrap replications. The scale bar indicates 0.02 substitutions per base position. *Bacillus halodurans* was used as an outgroup.

Phylogenetic analysis revealed that *Pseudoalteromonas* sp. ArcC01 and *Pseudoalteromonas* sp. ArcC06 were 99.73% and 99.66% similar to *Pseudoalteromonas arctica*, respectively, in the monophylogenic clade, and ArcC03, ArcC04, and ArcC10 were similar to *Pseudoalteromonas fuliginea* (99.86%), *Pseudoalteromonas translucida* (99.93%), and *Paraglaciecola mesophila* (99.51%), respectively, in the monophylogenic clade. The remaining isolates (ArcC02, 05, 07, 08, and 09) were not monophylogenic with any typical strain. The activity of cellulolytic enzymes secreted by the 10 candidates was evaluated using CMC as a substrate, to produce reducing sugars that were quantified by measuring the optical density at 540 nm. Specific activity units (U/mL) were calculated as the production of 1 µmol of reducing sugars per minute, per milliliter of cell culture broth. Three strains (ArcC02, 05, and 09) showed high activity (U/mL). The unit per milliliter of ArcC02 was 0.29 and ArcC05 was 0.27. The endoglucanase from ArcC09 (KCTC 14216BP) showed the highest activity (0.31 U/mL), and it was therefore selected for subsequent studies (Fig. 2).

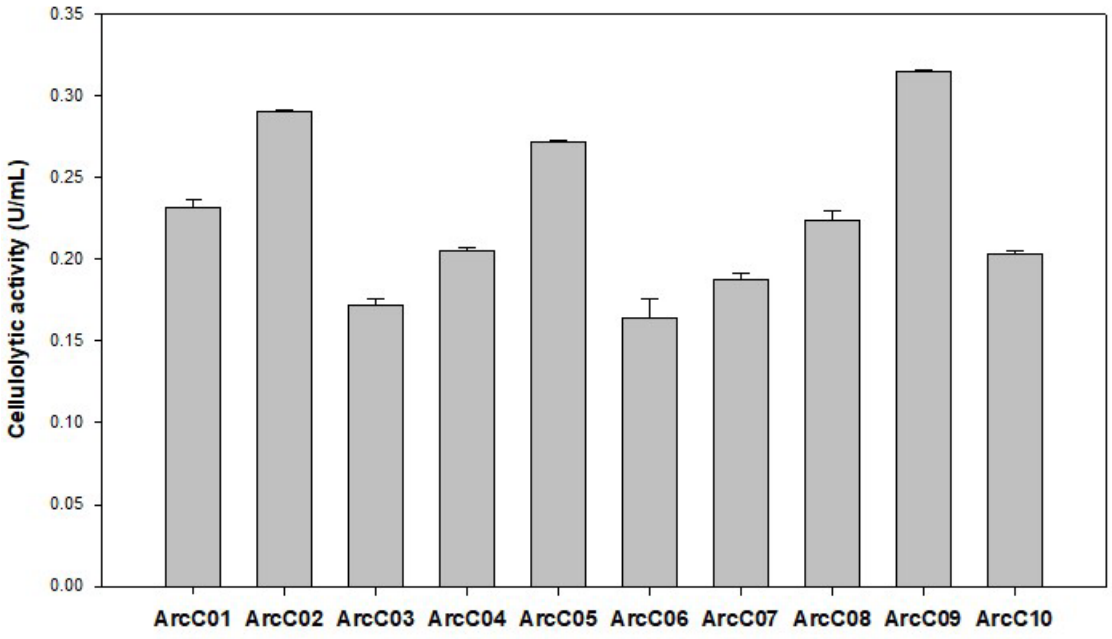

Fig. 2. Cellulolytic activity measured for each cell culture reaction solution. The assay was conducted at least three times, and the error bars indicate the standard deviation.

Purification of Cellulolytic Enzyme from ArcC09

Endoglucanase was purified from ArcC09 using protein precipitation, followed by two orthogonal chromatography methods. Protein was collected from the culture supernatant, the specific activity of which was 2.3 U/mg, by ammonium sulfate precipitation. Ammonium sulfate precipitation yielded 86.8% of the total activity with 13.9 U/mg of specific activity. When the ammonium sulfate precipitate was subjected to the phenyl sepharose column, one peak showing cellulolytic activity was eluted by 1% isopropanol in 20 mM tris-HCl (pH 7.0). The partially purified enzyme solution had 17.5 U/mg of specific activity with 7.6 of the purification fold. After gel filtration chromatography, a yield of 13.2% of total endoglucanase activity was finally obtained, along with a 10.6-fold purification improvement (Table 2). This is similar to the previously reported purification of a cellulase from *Aplysia kurodai*, resulting in an 8.4-fold increase in purity (Rahman *et al*. 2014).

Table 2. Purification Summary for ArcC09 Endoglucanase

Purification Steps	Total Protein (mg)	Specific Activity (U/mg)	Total Activity (U)	Yield (%)	Purification (Fold)
Culture supernatant	134	2.30	310	100	1.00
$(NH_4)_2SO_4$ precipitation	19.4	13.9	269	86.8	6.00
Phenyl sepharose	4.40	17.5	77.0	24.8	7.60
Superdex 75	1.70	24.6	40.8	13.2	10.6

The purity of the enzyme was confirmed using SDS-PAGE, where a single protein band was observed, and its enzyme activity was analyzed by zymography (Fig. 3). The number of protein bands on the gel decreased as the purification steps proceeded and finally a single band was dyed and observed. The molecular mass of the single band was approximately 30 kDa, which is between 25 and 37 kDa of the protein marker. The molecular mass of the purified protein was reaffirmed and calculated to be 28 kDa using Compute pL/Mw tool, Expasy (Artimo *et al*. 2012). The purified enzyme was used to determine the enzyme characteristics.

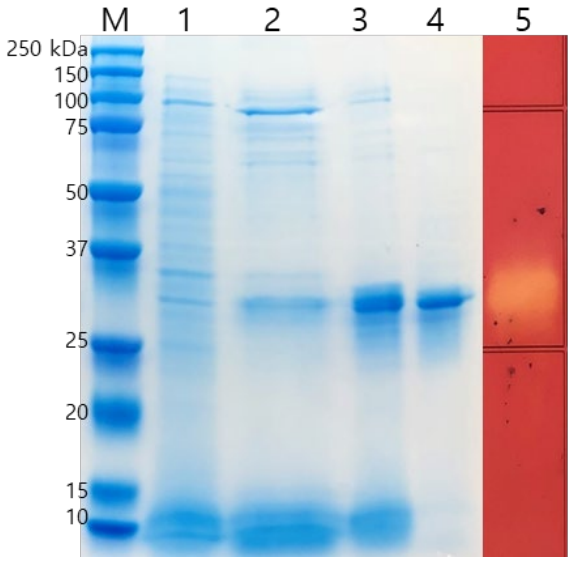

Fig. 3. The SDS-PAGE of endoglucanase at each step of the purification. Lane M: Protein marker; Lane 1: Crude supernatant; Lane 2: Protein collected with ammonium sulfate precipitation; Lane 3: Enzyme fraction purified with hydrophobic interaction chromatography; Lane 4: Enzyme fraction purified with size exclusion chromatography; and Lane 5: Zymography of the purified endoglucanase enzyme

Effect of Temperature and pH on Cellulolytic Enzyme Activity and Stability

The activity of the purified enzyme was analyzed at various temperatures and pH and was compared to commercial cellulase from *A. niger* (Fig. 4).

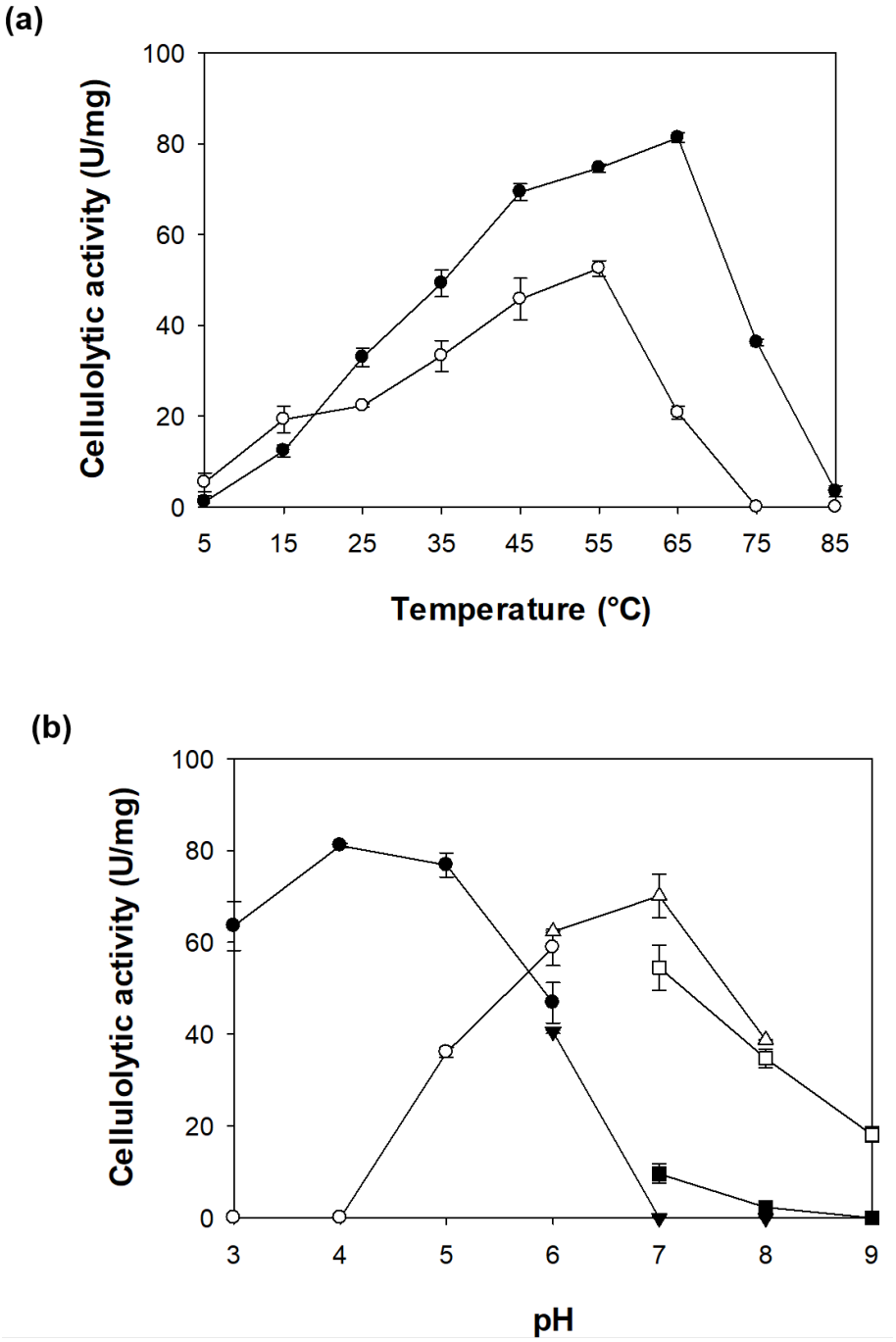

Fig. 4. Effect of temperature and pH on cellulolytic enzymes (**a**) Cellulolytic activity (U/mg) of purified endoglucanase from ArcC09 (○) and a commercially available cellulase from *Aspergillus niger* (●) measured over a temperature series. Each enzyme was evaluated at its optimal pH. (**b**) Effect of pH on the activity of purified endoglucanase from ArcC09 (at 55 °C) and a commercially available cellulase from *A. niger* (at 65 °C). The activity of purified ArcC09 endoglucanase is shown using open markers (○, △, and □), whereas filled markers represent the commercial cellulase (●, ▼, and ■). The following buffers were used to achieve the range of pH values tested: 50 mM sodium acetate buffer pH 3.0 to 6.0 (○/●), 50 mM potassium phosphate buffer pH 6.0 to 8.0 (△/▼), and 50 mM Tris-HCl buffer pH 7.0 to 9.0 (□/■). The assay was conducted three times, and the error bars indicate the standard deviation.

Microorganisms are a common source of cellulases, and especially fungi such as genera *Trichoderma* and *Aspergillus* are regarded as a practicing source (Sohail *et al*. 2009). Cellulase from *Aspergillus* sp. (Carezyme®) was commercialized by an enzyme global company Novozyme Corp. and garnered attention recently. In the present study, the commercial cellulase presented the highest activity at pH 4.0 and 65 °C. Wang and Hsu (2006) reported that the optimal pH of the commercial cellulase is 5.0, but it was more stable at pH 7.0 than under acidic conditions. Sulyman (2020) suggested that the optimal pH of purified cellulase from *A. niger* is 4.0, and it is consistent with the value obtained in the present study. *Aspergillus niger* endo-1,4-β-glucanase ENG1 from *Saccharomyces cerevisiae* presented an optimal activity at pH 6.0, which shows that the enzyme is more active under acidic conditions than under basic conditions (Taipakova *et al*. 2015). The optimal temperature differed from that of Sulyman's purified cellulase (40 °C), but it was close to that of Taipakova's expressed protein (60 °C).

The cellulolytic activity of the purified endoglucanase was highest at approximately 55 °C, but it rapidly decreased at temperatures above 55 °C. The enzyme from ArcC09 exhibited relative activities of 10.3% (5.4 U/mg) and 36.4% (19.3 U/mg) at 5 °C and 15 °C, respectively, compared to 100% (52.5 U/mg) at 55 °C. In contrast, commercial cellulase maintained some level of cellulolytic activity over the temperature range investigated, with 65 °C as the optimal temperature. It was noteworthy that absolute activity of ArcC09 endoglucanase under cold conditions (below the temperature of 15 °C) was higher than that of the commercial enzyme (1.2 and 12.4 U/mg at 5 and 15 °C, respectively). This demonstrated that ArcC09 endoglucanase has cold-active enzyme characteristics. Similarly, a cold-active cellulase, isolated form the deep sea, belonging to the same genus (*Pseudoalteromonas* sp. DY3), demonstrated 15% of its maximum cellulase activity at 5 °C (Zeng *et al*. 2006). The ArcC09 endoglucanase exhibited its maximum activity at pH 7, while the optimal pH for the commercial enzyme was pH 4. The optimum pH for the Arc09 cellulase was neutral, within the range of 6.0 to 7.0. This is a shared characteristic of secreted cellulase enzymes, having been observed previously in bacteria such as *Bacillus coagulase* Co4 (Adeleke *et al*. 2012), *B. subtilis* YJ1 (Yin *et al*. 2010), *B. vallismortis* RG-07 (Gaur and Tiwari 2015), and fungi *Acremonium alcalophilum* (Hayashi *et al*. 1996). Cellulase from *B. doagulase* Co4 showed the optimum pH at 7.5, *B. subtilis* YJ1 cellulase had its optimal pH at 6.0 to 7.5. *B. vallismortis* RG-07 and *A. alcalophilum* cellulase was most active at pH 7.0.

The two cellulolytic enzymes demonstrated different characteristics with regard to temperature and pH stability (Fig. 5). When ArcC09 endoglucanase was incubated at above 55 °C for 1 h, the activity almost disappeared, whereas the activity of cellulase from *A. niger* remained 40% even after 1 h of incubation at 85 °C (Fig. 5a). The purified enzyme from ArcC09 lost its activity at both pH extremes, while the activity of commercial cellulase from *A. niger* remained relatively high up to pH 9.0, after which it began to decrease (Fig. 5b). The thermal stability of the enzyme was evaluated and deteriorated rapidly above 45 °C, where enzyme activity was reduced, and this might be a general feature of enzymes derived from psychrophilic organisms (Feller *et al*. 1996). Considering the industrial application, the above-mentioned characteristics of the purified cellulase, such as maximum activity at neutral pH, high overall cellulolytic activity, and stability at cold temperatures can be applied to processes such as cleaning devices covered with cellulose-containing substances, where it is necessary to minimize the formation of unwanted byproducts, as well as in the detergent industry (Kasana and Gulati 2011).

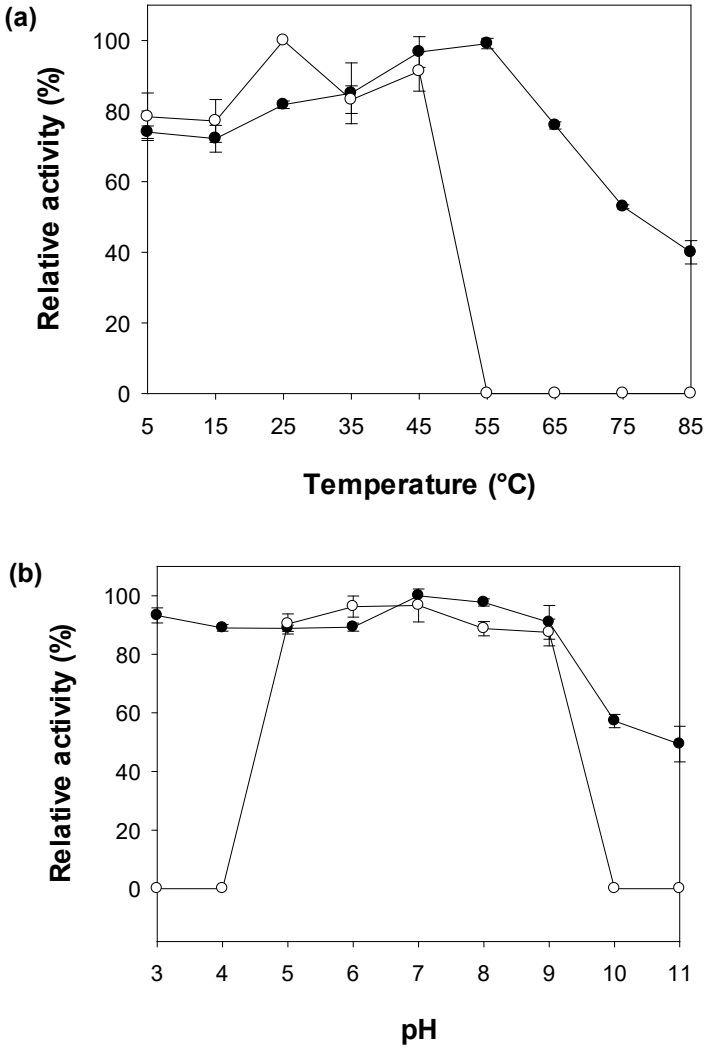

Fig. 5. Temperature and pH stability of cellulolytic enzymes (**a**) thermostabilities of purified endoglucanase from ArcC09 (○) and the commercial cellulase from *Aspergillus niger* (●) were evaluated over a temperature series (5 to 85 °C). The enzymes were pre-incubated at each temperature for 1 h at pH 7.0 for ArcC09 endoglucanase and pH 4.0 for *A. niger* cellulase. Thereafter, the remaining activities were measured at 55 °C for ArcC09 endoglucanase and 65 °C for *A. niger* cellulase. The measurement time was 30 min for each enzyme. (**b**) pH stabilities of purified endoglucanase from ArcC09 (○) and the commercial cellulase from *A. niger* (●) were evaluated over a range of pH conditions (pH 3 to 11). The enzymes were pre-incubated at each pH buffer for 1 h at 4 °C. Then, the remaining activities were measured at 55 °C for ArcC09 endoglucanase and 65 °C for *A. niger* cellulase. The measurement time was 30 min for each enzyme. The assay was conducted three times, and the error bars indicate the standard deviation.

Effects of Metal Ions and Reagents on the Activity of Cellulolytic Enzyme

To evaluate the effect of ions and other additives on both the Arc09 and commercial cellulases, the authors measured their activity under a variety of conditions. It was observed that the Arc09 enzyme lost the majority of its activity in the presence of both Cu^{2+} (7.9%) and linear alkylbenzene sulfonate (LAS) (2.7%) (Fig. 6a). A slight increase in enzyme activity was observed in the presence of Na^{2+} (118.3%), Ba^{2+} (114.8%), sodium dodecyl

sulfate (SDS) (117.9%), and ethylenediaminetetraacetic acid (EDTA) (114.9%). A negligible decrease was demonstrated in the presence of H_2O_2 (80.8%). In contrast, the activity of the commercial cellulase from A. niger showed no effect in the presence of Cu^{2+}(104%), while Ba^{2+} (88.6%), Zn^{2+} (77.6%), SDS (68.8%), and H_2O_2 (76.9%) reduced its cellulolytic activity, and a complete loss was observed in the presence of LAS (0%) (Fig. 6b). The presence of Mn^{2+} (142.9% for ArcC09 and 123.0% for *A. niger* cellulase) increased the activity of both cellulases. Tejirian and Xu (2010) showed that Ca^{2+} improved and Cu^{2+}, Mn^{2+}, and Zn^{2+} lowered the activity, whereas for ArcC09 endoglucanase Mn^{2+} and Zn^{2+} exhibited positive effects and Cu^{2+} showed negative effects on its activity. Compared with other cold-active cellulases such as an endoglucanase from *Paenibacillus* sp. BME-14 (Fu *et al*. 2010), the cold-active enzyme in the present study showed less reactivity to metal ions. Therefore, these characteristics could be useful to explore in industrial fields.

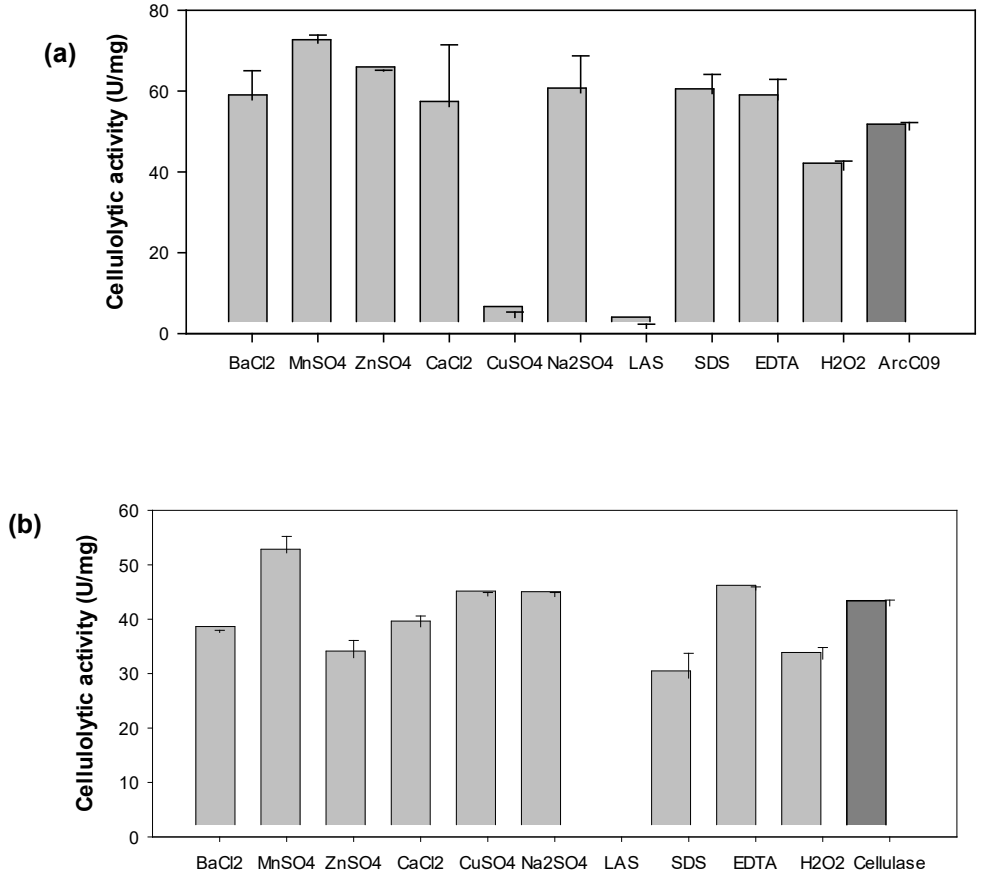

Fig. 6. Effects of metal ions, detergents, EDTA, and H_2O_2 on purified endoglucanase from ArcC09 (**a**) and cellulase from *A. niger*; (**b**) cellulolytic activity was measured in U/mg. Enzyme activity under standard conditions (*i.e.*, enzyme reactions without additives) is shown as the rightmost bar in each graph. The assay was conducted at least three times and the error bars indicate the standard deviation.

CONCLUSIONS

1. A novel marine bacterium *Pseudolateromonas* sp. isolated from the Arctic Beaufort Sea secreted a cellulolytic enzyme, and its purified form had a molecular mass of 28 kDa.
2. The cellulolytic enzyme was cold-active with 10% and 36% of its maximal activity remaining at 5 °C and 15 °C, respectively. It showed its maximum activity at pH 7 and was stable at near-neutral pHs.
3. Cellulolytic activity was considerably inhibited by Cu^{2+} but activated by Mn^{2+} and Zn^{2+}. Detergent linear alkylbenzene sulfonate (LAS) exhibited strong inhibition, whereas SDS and EDTA increased the activity.
4. The advantageous characteristics of the cellulolytic enzyme can be further developed *via* bioengineering techniques for its application to low temperature industrial processes.

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