Diversity of *Paenibacillus* sp. LLZ1 Cellulase and its Improved Enzyme Activity and Stability in the Ionic Liquid 1-Ethyl-3-methylimidazolium Diethyl Phosphate

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The stability of cellulase in the presence of ionic liquids (ILs) is pivotal for the *in situ* saccharification of cellulose. This study investigated the effects of various carbon sources on the cellulase production of *Paenibacillus* sp. LLZ1. The results showed that the activities of both the total cellulase and endoglucanase induced by microcrystalline cellulose were higher than those induced by other examined carbon sources. Simultaneously, a zymography analysis revealed the presence of seven protein bands (carboxymethylcelluloses 3, 4 and 6 through 10) with endoglucanase activity when MCC was used as the carbon source for culturing *Paenibacillus* sp. LLZ1. The endoglucanase induced by MCC showed stability in a 25% solids 1-ethyl-3-methylimidazolium diethylphosphate ([Emim]DEP) solution. Furthermore, the addition of metal ions and surfactants increased the endoglucanase activity in the [Emim]DEP solution, where 0.5 mM of Fe^{2+} and 0.1% of polysorbate (PSM 60) led to 11% and 29% increases in filter paper activity, respectively. Finally, three types of biomass (MCC, bagasse cellulose, and bagasse) were used as raw materials for *in situ* enzymatic hydrolysis when involved 5% [Emim]DEP and 0.1% polyoxyethylene (60) sorbitan monostearate (PSM 60). Fourier-transform infrared spectroscopy and specific surface area measurements were used to explain the effects of PSM 60 on the hydrolysis efficiency of these three biomasses.

Keywords: Biomass; Cellulase; Endoglucanase activity; Ionic liquid; Paenibacillus sp. LLZ1; Surfactant

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INTRODUCTION

The conversion of cellulosic materials to soluble sugars is one of the major approaches for sustainable energy generation (Zheng *et al.* 2017). However, cellulose is recalcitrant to enzymatic hydrolysis due to the extensive networks of inter- and intramolecular hydrogen bonds as well as van der Waals interactions (He *et al.* 2015; Hu *et al.* 2016a). Presently, ionic liquids (ILs) have been applied in lignocellulosic pretreatments as designable solvents to increase the accessibility to the enzyme, thereby promoting the hydrolysis efficiency of cellulose (Perez-Pimienta *et al.* 2013). However, the activity of a majority of the cellulolytic enzymes is impeded due to the presence of ILs (Hu *et al.* 2016a), and the enzymes may be partially or completely inactivated in the presence of low concentrations of ILs.

Therefore, an industrially feasible pretreatment and hydrolysis process requires robust cellulases that are stable and active in the presence of small amounts of ILs co-precipitated with recovered cellulose or for saccharification in the presence of ILs. To develop cellulases for enhanced hydrolysis of cellulose into monosaccharides in non-

natural IL environments, several IL-tolerant strains were selected from chemically polluted microhabitats or from culture media containing IL environments (Xu *et al.* 2016). Genomic studies have revealed that most of the microorganisms, such as *Trichoderma reesei* and *Aspergillus niger*, can produce a wide spectrum of cellulase (Brunecky *et al.* 2017). Moreover, cellulase-producing bacteria often produce diverse varieties of cellulase that can adapt to changing environmental conditions (Leis *et al.* 2017). Although several reports have been published on the diversity of cellulase, only a few studies addressed the IL tolerance of cellulases induced by different environmental conditions. To resolve the contradiction between the different effects of ILs on cellulose and cellulase, the immobilization of cellulase has been widely investigated. For example, the immobilization of cellulase onto PEGylated graphene oxide (GO) nanosheets was successfully achieved, and the PEGylated GO-Cellulase retained 61% of the initial activity in 25% (w/v) 1-butyl-3-methylimidazolium chloride ([Bmim]Cl) (Xu *et al.* 2016). In addition, different types of surfactants combined with IL pretreatment were also investigated. Recently, the nonionic surfactants were found to enhance the enzymatic hydrolysis of microcrystalline cellulose (Avicel PH101) by reducing the cellulase deactivation (Lou *et al.* 2018). To the best of the authors' knowledge, the hydrolysis of lignocellulose is catalyzed by microbial cellulases, including endoglucanase, *β*-glucosidase, and cellobiohydrolase. Another study suggested that a cellulase-producing strain, *Talaromyces amestolkiae*, could secrete high levels of β-glucosidase, *β*-1,4-endoglucanase, and exoglucanase when cultured in the presence of different carbon sources (Eugenio *et al.* 2017). In addition, various carbon sources induced the formation of various cellulases. Surprisingly, previous studies have seldom focused on the IL tolerance of cellulases induced by different carbon sources.

The inactivation mechanism of *Paenibacillus* sp. LLZ1 cellulases (PCs) in the presence of 1-ethyl-3-methylimidazolium diethylphosphate ([Emim]DEP) has been preliminarily investigated (Hu *et al.* 2016a, 2016b). Herein, further attempts were made to investigate the influence of various carbon sources including microcrystalline cellulose (MCC), sucrose, carboxymethylcellulose (CMC), cellobiose, starch, and glycerol on the production of cellulase by *Paenibacillus* sp. LLZ1. Sodium dodecyl sulfate-polyacrylamide gel (SDS-PAGE) was used to reveal the diversity of *Paenibacillus* sp. LLZ1 endoglucanase induced *via* different carbon sources. Subsequently, the [Emim]DEP tolerance of cellulases induced by different carbon sources was explored. Finally, the addition of metal ions and surfactants improved the cellulase activity in the [Emim]DEP solution. Under optimal conditions, the MCC, bagasse, and bagasse cellulose (BC) were used as raw materials for *in situ* enzymatic hydrolysis in the reaction system with the [Emim]DEP solution and surfactant. Fourier-transform infrared spectroscopy (FTIR) and specific surface area were used to explore the underlying mechanism.

EXPERIMENTAL

Materials

The [Emim]DEP with > 99% purity was purchased from Chenjie Chemical Co., Ltd. (Shanghai, China). Both the microcrystalline cellulose (MCC) and Whatman Grade No. 1 filter paper were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Bagasse was obtained from the Guangdong province, China. It was washed with distilled water, dried to constant weight at 85 °C, crushed, and passed through 40-mesh sieve before usage; the purity was approximately 46%. Bagasse cellulose was extracted

with 90% purity following procedures described by Hu *et al.* (2016a) with some modifications. All other reagents were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

Bacteria strain and cellulase

The *Paenibacillus* sp. LLZ1 used in this study was previously isolated by the authors from soil samples (Hu *et al.* 2016a), and the strain was cultured in a liquid medium that contained (g/L): 3.0 dibasic sodium phosphate (Na_2HPO_4), 0.8 ammonium nitrate (NH_4NO_3), 0.5 magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$), 0.5 calcium chloride ($CaCl_2$), 2.0 yeast extract, and 5.0 of a carbon source. The six carbon sources selected were MCC, sucrose, CMC, cellobiose, starch, and glycerol. The strain was cultured for 7 days at 30 ℃ and 200 rpm, centrifuged at 6000 g for 10 min, and filtered through 0.22-μm nylon filters. The cell-free supernatant was used as a crude cellulase.

Methods

Enzyme assay

The filter paper activity (FPA; representing total cellulase activity) was determined by incubating the crude enzyme with Whatman No. 1 filter paper for 1 h at 40 ℃. A typical reaction mixture contained 0.5 mL of crude enzyme and 0.5 mL of 0.1 M acetic acid buffer (pH 6.0) soaked with 50 mg of Whatman No. 1 filter paper as a substrate. The crude cellulase was incubated with 2% CMC as the substrate at 40 ℃ for 30 min to analyze the CMC activity (CMCA; representing endoglucanase activity). The reaction mixture contained 0.5 mL of crude cellulase and 0.5 mL of 2% CMC substrate solubilized in 0.1 M of acetic acid buffer (pH 6.0). After incubation, the amount of liberated reducing sugar was quantified using the 3,5-dinitrosalicylic acid (DNS) method: Diluted in moderation, 1 mL of the diluted mixture was added to a 3 mL DNS boiling water bath for 5 min, and the absorbance was measured at 540 nm after cooling down to room temperature. The reducing sugar content obtained *via* the enzymatic reaction was calculated from the standard curve.

Electrophoresis and zymogram analysis of cellulase

A total of 50 μL of crude cellulase solution was incubated at 40 ℃ for 1 h. The samples for SDS-PAGE were prepared by mixing 8 μL of incubated cellulase solution and 2 μL of gel loading buffer without a reducing agent. The protein samples were resolved on 8% separating gel containing 0.1% CMC-Na as a substrate using the method described by Raddadi *et al.* (2013) with some modifications. After electrophoresis, the gel was incubated in renaturation buffer (0.1 M acetic acid buffer (pH 6.0) containing 2% polyethylene glycol octylphenol ether (Triton X-100) at 4 °C for 1 h and then soaked in the same buffer without Triton X-100 for 12 h at 4 °C. Finally, the gel was incubated at 40 °C for 1 h, stained in 0.1% Congo red for 30 min, and de-stained using 1 M of NaCl. The cellulolytic bands appeared as clear zones on the red background.

Stability of PCs in [Emim]DEP solution

First, the PCs were produced in different culture media with various carbon sources. To investigate the stability of cellulase in ionic liquids, a certain amount of [Emim]DEP was solubilized in 0.1 M of acetate buffer. Then, 0.5 mL of the crude enzyme solution was added to 0.5 mL of the acetate buffer containing [Emim]DEP to achieve the IL concentrations of 0%, 5%, 10%, 15%, 20%, and 25% (v/v). Subsequently, 50 mg of filter paper was incubated in the reaction system for 1 h at 40 ℃. The altered FPA with different

concentrations of IL in the hydrolytic process was determined by the above enzyme analysis method. Similarly, the CMCA was measured, and the effect of ILs on the endoglucanase inactivation was evaluated.

Effect of metallic ions and surfactants on PCs

To investigate the effects of metal ions (Fe^{2+} and Zn^{2+}) on PCs, filter paper and CMC were selected as substrates in the reaction system within ILs. In this study, the FPA and CMCA were determined. The metal ions were added at concentrations of 0.5 or 1 mM. Moreover, the influence of a hydrophilic nonionic surfactant (polysorbate 60 (PSM 60; PSM 60) and alcohol ethoxylate-9 (AEO-9)) on PCs was evaluated with the addition of 0.05% and 0.1% (v/v), respectively.

In situ hydrolysis of biomass under optimal conditions

The conditions of the *in situ* hydrolysis of biomass were optimized in the authors' previous study (Hu *et al.* 2016a). The reaction temperature was 40 ℃ in the presence of 5% (v/v) [Emim]DEP concentration and 0.1% (v/v) of PSM 60 was used for the *in situ* hydrolysis of biomass. Herein, MCC, BC, and bagasse were selected as *in situ* enzymatic substrates. Before the *in situ* hydrolysis of biomass, a substantial amount of [Emim]DEP was used for the pretreatment of the above three biomasses with the addition of 0.1 M acetate buffer (pH 6.0), and the final [Emim]DEP concentration was 5% (v/v) in the reaction mixture. Specifically, 850 μL of the mixture was added to 150 μL of crude enzyme solution, and the final substrate content was 1 g/L. The process was monitored by withdrawing the samples from the reaction mixture at regular intervals. The conversion rate of cellulose was calculated according to Eqs. 1 through 3:

$$\text{Conversion rate (\%)} = 0.9 \times [\text{glucose weight (g)}] \times 100 / [\text{cellulose weight (g)}] \quad (1)$$

$$\text{BC: [cellulose weight (g)]} = [\text{BC weight (g)}] \times 90\% \quad (2)$$

$$\text{Bagasse: [cellulose weight (g)]} = [\text{Bagasse weight (g)}] \times 46\% \quad (3)$$

Analysis methods

The glucose released through *in situ* hydrolysis was quantified using a SBA-40C biological sensing analyzer (Biology Institute of Shandong Academy of Sciences, Jinan, China).

The variances of functional groups towards cellulose substrates MCC, bagasse, and BC were obtained using a Nicolet iS10 FTIR spectrometer (Nicolet Co., Waltham, MA, USA). The spectra were recorded in the range of 500 to 4000 cm^{-1} at a resolution of 0.5 cm^{-1}.

The specific surface areas of MCC, bagasse, and BC were calculated using the Quantachrome Autosorb® IQ surface and pore area analyzer (Quantachrome, Palm Beach County, FL, USA) by degassing the sample under vacuum at 90 °C for 12 h before analysis. The specific surface area was calculated using the Brunauer-Emmet-Teller (BET) method.

RESULTS AND DISCUSSION

Effects of Carbon Source on Cellulase Production by *Paenibacillus* sp. LLZ1

Cellulase is a complex enzyme system composed of various hydrolases, including endoglucanase, exoglucanase, and β-glucanosidase, which are diverse and inducible, and the biosynthesis of cellulase can be induced *via* polysaccharides, oligosaccharides, and monosaccharides (Shida *et al.* 2015). The present study aimed to examine the impact of various carbon sources (MCC, sucrose, CMC, cellobiose, starch, and glycerol) on the production of PCs. Figure 1 shows that CMCA (U/mL) was found in the following order: 1.19 MCC > 0.77 sucrose > 0.65 CMC > 0.53 cellobiose > 0.4 soluble starch > 0.33 glycerol. Furthermore, the order of FPA was similar to that of CMCA, and the FPA (U/mL) was measured at 0.53 MCC, 0.38 sucrose, 0.35 CMC, 0.31 cellobiose, 0.25 soluble starch, and 0.18 glycerol. The data in Fig. 1 indicated that the activities of both total cellulase (FPA) and endoglucanase (CMCA) induced by MCC were higher than those induced by the other examined carbon sources. However, using starch or glycerol as carbon sources induced low cellulase activity. This result was in agreement with a previous report; however, the difference in the CMC-induced cellulase activity was higher than that of MCC (Hong *et al.* 2013). In contrast, compared to the MCC as a carbon source, the activities of endoglucanases induced by sucrose, CMC, and cellobiose were approximately 65%, 55%, and 45%, respectively (Fig. 1). For all of the tested carbon sources, the CMCA was approximately twice as high as the FPA. This phenomenon was similar to the results previously described, wherein the endoglucanase activity induced by different carbon sources (such as CMC, ball-milling cellulose, or filter paper) was approximately 1.8-fold of FPA (Sohail *et al.* 2016).

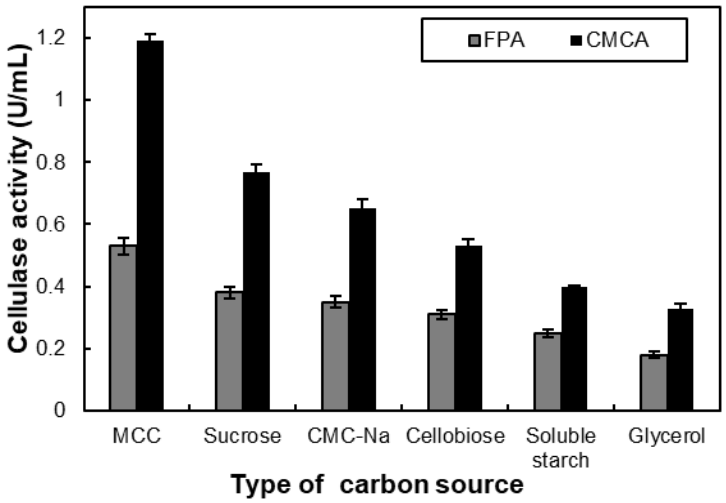

Fig. 1. Effects of different carbon sources on the cellulase activity; the error bars indicate the standard deviation from three independent experiments

In addition, cellulases have a natural diversity. For example, Pason *et al.* (2006) reported 17 proteins produced by *Bacillus curdlanolyticus* B-6, and the zymogram analysis revealed 9 CMCases. Similarly, in this study, zymogram analysis revealed the existence of multiple protein bands with endoglucanase activity. The results in Fig. 2 indicated that at least eight endoglucanases (CMC1 through CMC10) were detected from the gel.

Strikingly, the different carbon sources induced the expression of cellulose-producing genes in *Trichoderma rimannii* and produced different types of cellulases (Margolles-Clark *et al.* 1997). Herein, at least seven endoglucanases were produced when the carbon source was MCC (CMC3, CMC4, and CMC6 through CMC10). Additionally, the protein bands with endoglucanase activity in the media-containing sucrose, CMC, cellobiose, starch, and glycerol were 5 (CMC3 and CMC6 through CMC9), 4 (CMC5 and CMC7 through CMC9), 1 (CMC10), 4 (CMC1 through CMC3 and CMC8), and 3 (CMC4 through CMC6), respectively. Previous studies suggested that different protein bands in SDS-PAGE represent various endoglucanases, and the corresponding endoglucanase activities might be evaluated by the intensity of the protein bands (Raddadi *et al.* 2013; Hu *et al.* 2016b). Figure 2 shows only one bright band for when cellobiose was used as the carbon source. Moreover, the bands of endoglucanases induced by starch and glycerol were also few and fuzzy. These results were similar to those of the enzymatic activity obtained *via* endoglucanase analysis (Fig. 1) using the same three carbon sources (cellobiose, starch, and glycerol). Overall, the current results confirmed that various endoglucanases could be produced by *Paenibacillus* sp. LLZ1 when using different carbon sources in the fermentation medium. Similar results were also found by Pardo and Magnelli (1996), wherein the maximal yield of cellulase was achieved using MCC as the carbon source; however, CMC-Na induced the endoglucanase production. Therefore, the exploration and regulation of carbon sources seem to be plausible for improving the expression and activity of cellulase.

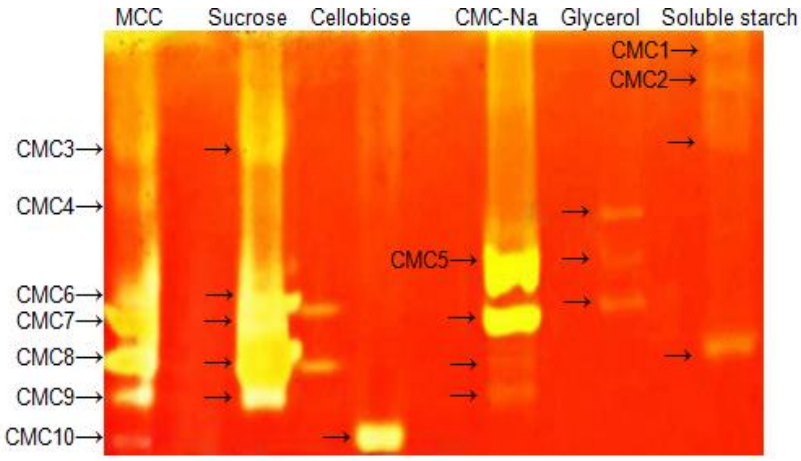

Fig. 2. Effects of different carbon sources on endoglucanase diversity of *Paenibacillus* sp. LLZ1

Stability of PCs in [Emim]DEP Solution

Although several studies have explored the enzymatic hydrolysis of biomass in systems with ILs, only a few studies found that cellulase could withstand a high concentration of ILs (Jaeger *et al.* 2015). In a previous study, a novel environmentally friendly aqueous-dimethyl sulfoxide/[Emim]DEP-cellulase system was established for the efficient *in situ* saccharification of cellulose (Hu *et al.* 2016a). Herein, the stability of PCs in the reaction system with various concentrations of [Emim]DEP was investigated. Figure 3 shows the altered activity of PCs in the reaction system with different concentrations of [Emim]DEP; six carbon sources were selected for the production of PCs. Similar to a majority of the cellulases, the activity of PCs induced *via* different carbon sources decreased with an increase in [Emim]DEP concentration (Fig. 3). The relative FPA induced

by MCC was reduced 32% (0.36 U/mL) in 5% (v/v) [Emim]DEP; however, the amount of relative FPA reduction induced by the other examined carbon sources was approximately 40%. When the [Emim]DEP concentration increased to 25% (v/v), the relative FPA induced *via* glycerol as the carbon source was reduced 92% (0.016 U/mL); however, the relative FPA induced *via* MCC was 19% (0.1 U/mL). Thus, compared with the FPA induced by the other carbon sources, the FPA induced *via* MCC was stable in the reaction system with a specific concentration of [Emim]DEP.

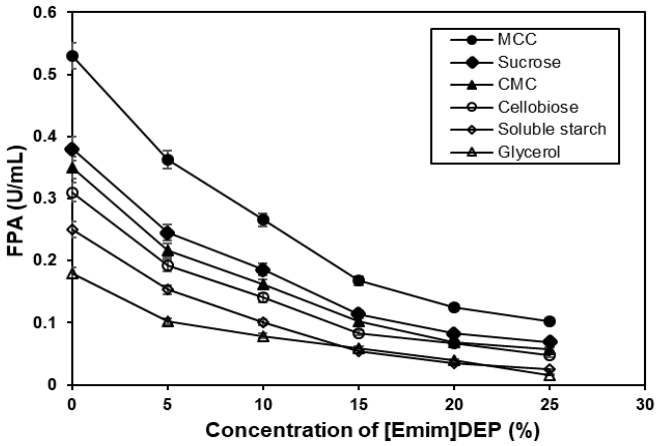

Fig. 3. Stability of *Paenibacillus* sp. LLZ1 cellulase induced by different carbon sources in [Emim]DEP solution; the error bars indicate the standard deviation from three independent experiments

Various effects were exerted on the individual cellulase components by the IL. A previous study indicated that the relative activities of endoglucanase, cellobihydrolase, and β-glucosidase in the presence of 10% 1,3-dimethylimidazolium dimethylphosphate ([Dmim]DMP) were 63%, 60%, and 34%, respectively (Engel *et al.* 2012). Therefore, the present study focused on the investigation of endoglucanase stability in the enzymatic hydrolysis system with [Emim]DEP.

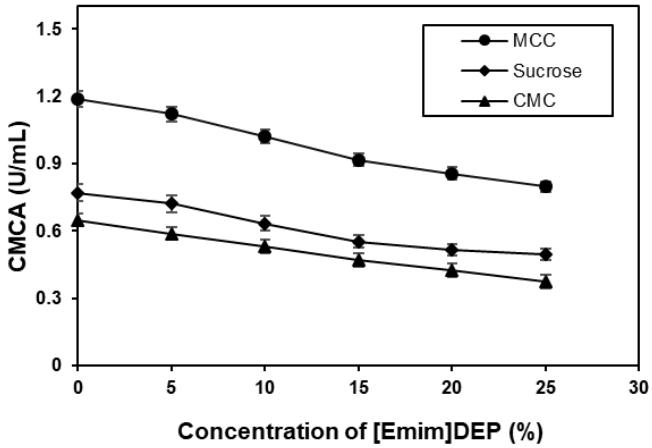

Fig. 4. Stability of *Paenibacillus* sp. LLZ1 endoglucanase induced by three carbon sources in [Emim]DEP; the error bars indicate the standard deviation from three independent experiments

Figure 4 shows the changes in CMCA in the reaction system with different concentrations of [Emim]DEP, and the endoglucanase was induced by MCC, sucrose, and CMC. Furthermore, when the [Emim]DEP concentration was 5% (v/v), the relative CMCAs were 94% (1.12 U/mL, MCC), 93% (0.72 U/mL, sucrose), and 91% (0.59 U/mL, CMC). However, the corresponding FPA was < 70%, which showed that *Paenibacillus* sp. LLZ1 endoglucanase was stable in 5% [Emim]DEP solution. For example, by increasing the [Emim]DEP concentration from 5% to 25%, the residual relative CMCA persisted at > 58%. The residual CMCA induced *via* MCC was 67% (0.80 U/mL) in 25% [Emim]DEP solution, which demonstrated the tolerance of ILs among the three tested carbon sources.

To the best of the authors' knowledge, there has been a lack of studies demonstrating that endoglucanase obtained from commercial or microbial organisms is stable in ILs. Table 1 shows the stability data of several endoglucanases in different ILs. Interestingly, the *Paenibacillus* sp. LLZ1 endoglucanase showed an improved IL tolerance. For example, compared to the results of Johnson *et al.* (2016), the decrease in the specific activity of enzyme E1 was 39% in 25% [Emim]DEP solution, while that induced *via* MCC was only 33% (Table 1). In addition, compared with several other endoglucanases in the table, the endoglucanase produced in this experiment showed relatively good stability despite the different types of ionic liquids used. Thus, *Paenibacillus* sp. LLZ1 endoglucanase exists stably in 25% [Emim]DEP solution and has a better prospect for industrial application.

Table 1. Recently Published Stability of Endoglucanase in Ionic Liquids

Name	Organism	Carbon Source	IL	% IL	Activity Loss (%)	References
EG1	*Stachybotrys microspora*	Glucose and Cellulose	[Bmim]Cl	10	20	Benhmad *et al.* (2017)
				20	50	
E1	*Escherichia coli* (*E1* gene)	Glucose	[Bmim]Cl	5	33	Summers *et al.* (2017)
				20	85	
E1	*Escherichia coli* (DNA2.0, CA, USA)	Glucose	[Emim]DEP	25	39	Johnson *et al.* (2016)
			[Emim]Cl	25	48	
Cellulase 1	*Paenibacillus sp. LLZ1*	MCC	[Emim]DEP	5	6 ± 0.6	This study
				20	33 ± 0.5	

Stability of PCs in 5% [Emim]DEP Solution with Addition of Metal Ions or Surfactants

The cellulase induced *via* MCC showed enhanced stability in the [Emim]DEP solution. Moreover, some metal ions or surfactants effectively improved the cellulase activity under harsh conditions as previously described (Bharmoria *et al.* 2014; Akimkulova *et al.* 2016). The effects of metal ions (Fe^{2+} and Zn^{2+}) and nonionic surfactants (PSM 60 and AEO-9) on cellulases in the presence of 5% [Emim]DEP solution as the ionic liquid were investigated. As shown in Fig. 5, Fe^{2+}, PSM 60, and AEO-9 effectively promoted the FPA; however, Zn^{2+} slightly inhibited FPA. Compared to the control, when Fe^{2+} was added at 0.5 mM in the reaction system with 5% [Emim]DEP IL, the relative FPA (111%) was promoted. However, with increased Fe^{2+} concentration at 1 mM, the relative FPA (97%) was slightly inhibited. Furthermore, the addition of 0.05% PSM 60 or AEO-9

caused a 16% and 15% increase in the relative FPA, respectively. When PSM 60 increased from 0.05% to 0.1%, the relative FPA increased 29%. Moreover, in the presence of the Fe^{2+} at 0.5 mM, the relative CMCA (112%) was higher than the control or the other experimental groups. Similarly, 0.05% PSM 60 also promoted CMCA, which was 110% for the control. Additionally, Fe^{2+} improved the effect on CMCA as previously described (Hmad *et al.* 2017).

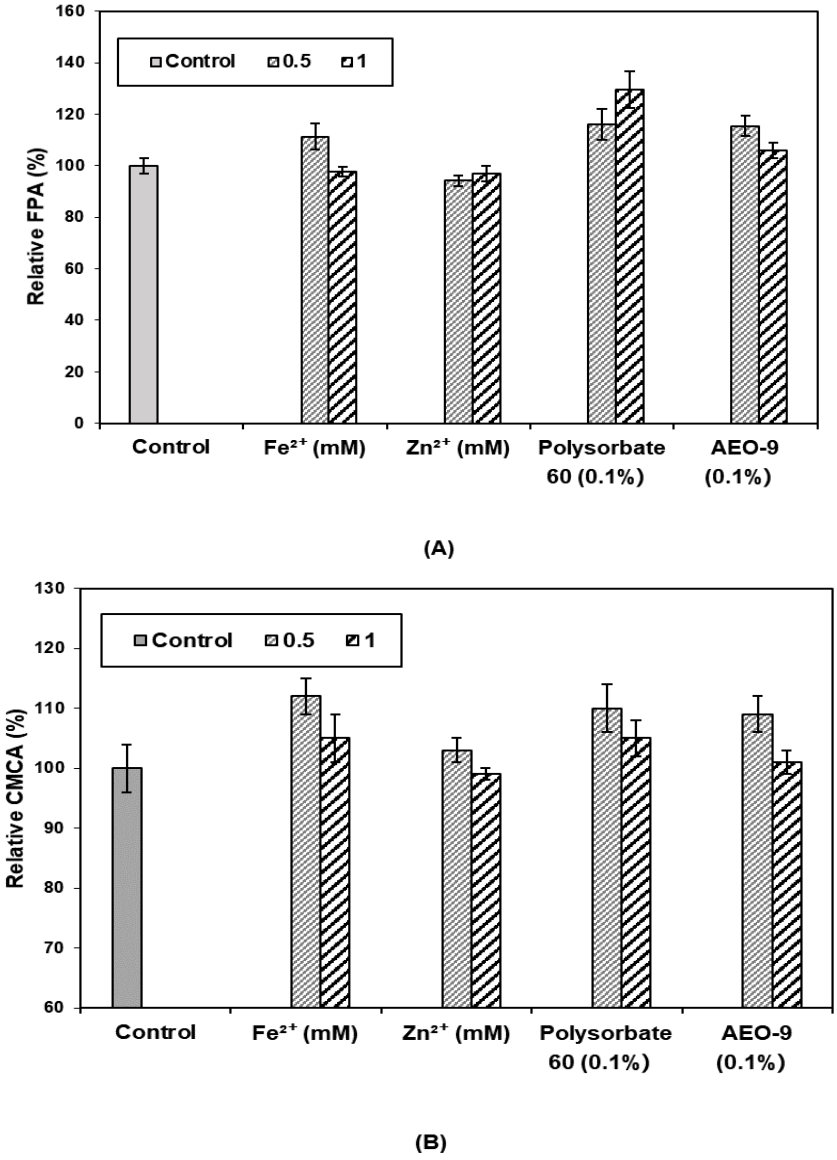

Fig. 5. Effect of metal ion or surfactant supplements on cellulase activity (A) and endoglucanase activity (B); the error bars indicate the standard deviation from three independent experiments

Time Course of *in situ* Saccharification with PSM 60 Addition

Because 0.1% PSM 60 enhanced the FPA, this concentration was selected to explore the influence on biomass hydrolysis in the reaction system with 5% [EMIM]DEP. Three kinds of biomass (MCC, BC, and bagasse) were used as the raw materials for *in situ* enzymatic hydrolysis. The *in situ* hydrolysis system of [Emim]DEP with PSM 60 exhibited a better conversion rate and efficiency as compared to that without the PSM 60 addition

(Fig. 6). With the addition of 0.1% PSM 60, the amount of glucose released in the initial 12 h from MCC, BC, or bagasse was 0.30 g/L, 0.31 g/L, and 0.19 g/L, which was 107%, 119%, and 126% of the control, respectively. Therefore, this result also confirmed that the stability of PCs in the IL system was improved successfully *via* the addition of 0.1% PSM 60. After 108 h of enzymolysis, the conversion rates of MCC, BC, and bagasse were 77.4%, 92%, and 95.8%, respectively, in the hydrolysis system with 0.1% PSM 60 addition, while that without PSM 60 were 73.8%, 88%, and 91%, respectively, in the reaction system.

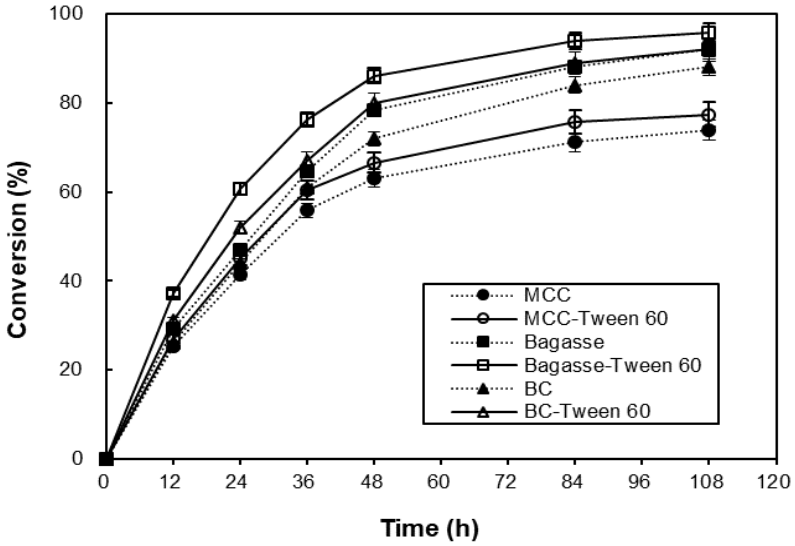

Fig. 6. The course of *in situ* enzymatic saccharification; the error bars indicate the standard deviation from three independent experiments

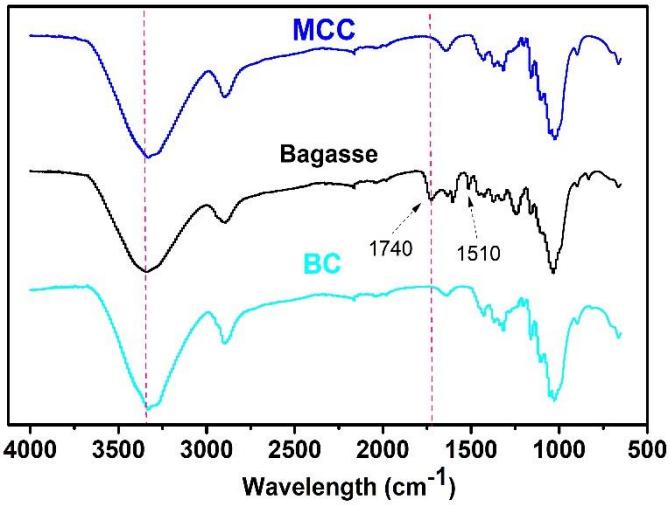

Fig. 7. FTIR patterns of MCC, BC, and bagasse

Finally, the effects of PSM 60 on the hydrolysis efficiency, FTIR, and specific surface area measurement of the three biomasses were assessed. The FTIR results in Fig. 7 show the absorption peaks of aromatic lignin rings (1510 cm^{-1}) and the carbonyl or acetyl absorption peaks (1740 cm^{-1}) of hemicellulose in bagasse. However, these peaks weakened or disappeared in BC, indicating the removal of lignin and hemicellulose in BC. The

specific surface area (m^2/g) is shown in Table 2. The order from greatest to least was bagasse (6.778) > BC (3.208) > MCC (0.921). Because all three kinds of biomass are insoluble solids, the enzymatic hydrolysis is closely related to the cellulase adsorption. The enzymatic hydrolysis of biomass is also related to the cellulose purity.

The addition of PSM 60 stimulated the efficiency of bagasse hydrolysis, and the addition of PSM 60 released the non-productive adsorption of cellulase and lignin in the biomass. Moreover, the large specific surface area of bagasse was beneficial for the adsorption of enzymes. Furthermore, the addition of nonionic surfactants can compete with the enzyme and reduce the amount of enzyme exposed in the air-liquid interface or reduce the cellulase deactivation caused by the air-liquid interface (Lou *et al.* 2018). Thus, this aspect might explain the promotion of PSM 60 in *in situ* enzymatic cellulose.

Table 2. Specific Surface Area of MCC, BC, and Bagasse

	Constant	Surface Area (m^2/g)
MCC	27.597	0.921
BC	34.715	3.208
Bagasse	6.280	6.778

CONCLUSIONS

1. In this study, the effects of various carbon sources (MCC, sucrose, CMC, cellobiose, starch, and glycerol) on the cellulase production by the strain *Paenibacillus* sp. LLZ1 were investigated in detail. The results showed that both filter paper activity (FPA) and CMCA induced *via* MCC were clearly higher than the other examined carbon sources.
2. Zymography analysis revealed the existence of seven protein bands (CMC3, CMC4 and CMC6 to CMC9) with endoglucanase activity when MCC was used as the carbon source for the culture of *Paenibacillus* sp. LLZ1.
3. The endoglucanase of *Paenibacillus* sp. LLZ1 that was induced by MCC showed good stability in 25% [Emim]DEP solution. Therefore, it shows biotechnological and industrial potential in the breakdown of lignocellulosic biomass.
4. The addition of metal ions and surfactants improved the endoglucanase activity in the [Emim]DEP solution, where 0.5 mM of Fe^{2+} and 0. 05% of PSM 60 led to 12% and 10% increases in endoglucanase activity, respectively.

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