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Short genome communications

Complete genome sequence of *Bacillus* sp. 275, producing extracellular cellulolytic, xylanolytic and ligninolytic enzymes

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ABSTRACT

Technologies for degradation of three major components of lignocellulose (e.g. cellulose, hemicellulose and lignin) are needed to efficiently utilize lignocellulose. Here, we report *Bacillus* sp. 275 isolated from a mudflat exhibiting various lignocellulolytic activities including cellulase, xylanase, laccase and peroxidase in the cell culture supernatant. The complete genome of *Bacillus* sp. 275 strain contains 3832 protein cording sequences and an average G + C content of 46.32% on one chromosome (4045,581bp) and one plasmid (6389bp). The genes encoding enzymes related to the degradation of cellulose, xylan and lignin were detected in the *Bacillus* sp. 275 genome. In addition, the genes encoding glucosidases that hydrolyze starch, mannan, galactoside and arabinan were also found in the genome, implying that *Bacillus* sp. 275 has potentially a wide range of uses in the degradation of polysaccharide in lignocellulosic biomasses.

Lignocellulosic biomass is one of the most attractive carbon resources due to its abundance and renewability. The degradation of recalcitrant lignocellulosic biomass is the first obstacle encountered toward the production of alternative renewable fuels and chemicals. Furthermore, the utilization of all the major components of lignocellulose, cellulose, hemicellulose and lignin, is essential for the efficient and complete use of lignocellulose (Beckham et al., 2016; Brown and Chang, 2014; Moreira and Filho, 2016). Among the lignocellulose degradation technologies, microbial degradation is one of the promising methods for the use of cellulose, xylan and lignin (Mathews et al., 2015; Oh et al., 2015; Perez et al., 2002; Salvachúa et al., 2015).

Generally, *Bacillus* is a genus of rod-shaped, Gram-positive, aerobic or facultative anaerobic bacteria. Also, the genus *Bacillus* consists of a very diverse group of over 300 species (Euzéby, 2017; Feng et al., 2016). Several *Bacillus* strains have been reported to degrade lignin or lignin model compounds (Bandounas et al., 2011; Huang et al., 2013; Zhu et al., 2014). Moreover, *Bacillus* sp. 55S5 isolated from peat exhibited not only the capability of lignin modification but also cellulase and xylanase activities (Maki et al., 2012). Similarly, *Bacillus* sp. R2 isolated from the Red Sea also had cellulase, xylanase, pectinase and peroxidase activities (Khelil et al., 2016). Those results imply that *Bacillus* species have potential in degrading lignocellulose components including not only lignin but also cellulose and hemicellulose.

In this study, we report a newly isolated *Bacillus* sp. 275 as a potential lignocellulose-degrading bacterium producing extracellular cellulolytic, xylanolytic and ligninolytic enzymes. The complete genome of *Bacillus* sp. 275 was also sequenced and investigated to find the genes related to lignocellulose degradation. *Bacillus* sp. 275 isolated from a mudflat showed relatively high lignocellulolytic activities such as cellulase, xylanase and ligninase on solid agar plates compared to 88 other bacterial isolates belonging to *Bacillus*, *Streptomyces*, *Burkholderia* and *Pseudomonas* (Gong et al., 2017). To analyze the amount of extracellular enzymes and lignocellulolytic activities, *Bacillus* sp. 275 was grown in LB medium containing 10 g/L of tryptone, 5 g/L of yeast extract and 5 g/L of NaCl for 24 h at 30 °C and a cell-free supernatant was collected. As shown in Table 1, the lignocellulolytic activities of *Bacillus* sp. 275

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Table 1
Lignocellulolytic enzyme activities in the cell-free supernatant of *Bacillus* sp. 275 strain.

Enzyme feature	Activity (U/g protein)
Cellulase[a]	1245.8 ± 7.2
Xylanase[b]	138.4 ± 0.8
Laccase[c]	1.3 ± 0.3
Peroxidase[c]	0.5 ± 0.1

Protein concentration (74.48 ± 0.96 mg/L) in the supernatants was measured using the Bradford protein assay reagent (Bio-Rad, CA, USA).

[a] Cellulase (endo-cellulase) activity was quantified with the Cellulase assay kit (CELLG5 method) (Megazyme, Ireland).

[b] Xylanase activity was measured by the 3,5-dinitrosalicylic acid (DNS) colorimetric method (Teather and Wood, 1982) after adding xylan (10 g/L) as a substrate to the supernatant and reacting at 50 °C for 10 min.

[c] Peroxidase and laccase activities were assayed by the detecting oxidation of (2,2-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) [ABTS] (ε_{420nm} = 3.6 m^{-1} cm^{-1}) in 100 mM acetate buffer (pH 3.0) at room temperature with or without H_2O_2. Peroxidase activity was calculated by subtracting the oxidation activity without H_2O_2 from that with H_2O_2. One unit of enzyme activity was defined as the amount of enzyme required to release one micromole of product per minute. All experiments were performed in triplicate.

Table 2
Genome features of *Bacillus* sp. 275 strain.

Features	Chromosome	Plasmid
Genome size (bp)	4,045,581	6389
G + C content (%)	46.33	43.25
Total number of genes	4017	6
Protein-coding genes (CDS)	3827	5
rRNA genes	27	0
tRNA genes	86	0

were observed in the supernatant of the liquid culture broth. The activity of cellulase and xylanase from 74.48 mg/L of extracellular proteins were 1245.8 U/g and 138.4 U/g, respectively. Regarding the lignin degradation, the activities of laccase and peroxidase, which were known to be related to the degradation of lignin or lignin model compounds (Rahmanpour et al., 2016; Roth and Spiess, 2015; Yoshida and Sugano, 2015), were detected.

The genome of *Bacillus* sp. 275 was sequenced using a combination of the PacBio RSII system (Pacific Biosciences, CA, USA), Illumina MiSeq (100-bp paired-end) and the Roche 454 sequencing TITAN technology. All the reads were assembled using the Roche gsAssembler 2.6, CLC Genomics Workbench 6.5.1 and PacBio SMRT Analysis 2.1 with a genome coverage of 276 folds. The complete genome of *Bacillus* sp. 275 consisted of one 4,045,581 bp chromosome and one 6389 bp plasmid with 3832 protein cording sequences (CDS), 86 tRNA genes, 27 rRNA genes and an average G + C content of 46.32% (Table 2 and Fig. 1). The NCBI Prokaryotic Genomes Annotation Pipeline (PGAP) was used to annotate the genes of *Bacillus* sp. 275.

Strain 275 was initially classified as *B. siamensis* based on the most hit taxon strain with the 16S rRNA sequence data in the Eztaxon server (http://www.ezbiocloud.net) (Kim et al., 2012) compared to *B. siamensis* KCTC 13613^T with a pairwise similarity of 99.93% (e.g., 1471 bp matching out of 1472 bp). However, the number of mismatching nucleotide between the strain 275 and *B. velezensis* CR-502^T was also only one bp (e. g., 1402 bp matching out of 1403 bp) as same as the *B. siamensis* KCTC 13613^T. Meanwhile, the average nucleotide identity (ANI) values of the strain 275 compared to *B. velezensis* FZB42 were found to be over 98%. *B. velezensis* FZB42 had formerly classified as *B. amyloliquefaciens* subsp. *plantarum* FZB42^T and it had been the closest type strain to *Bacillus* sp. 275 based on the 16S rRNA sequences until *B. amyloliquefaciens* subsp. *plantarum* FZB42^T was re-classified to *B. velezensis* FZB42 (Dunlap et al., 2016). Based on these results that the closest relative to the strain 275 could not be clearly defined, the strain 275 was named as *Bacillus* sp. 275.

A phylogenetic tree of *Bacillus* sp. 275 based on 16S rRNA sequences is shown in Fig. 2. *Bacillus* sp. 275 was closely related to *B. siamensis* KCTC 13613^T and *B. velezensis* CR-502^T as described earlier. Furthermore, the tree showed that *B. amyloliquefaciens* DSM 7^T formed a distinct branch together with *B. siamensis* KCTC 13613^T, *B. velezensis* CR-502^T and *Bacillus* sp. 275.

The complete genomes of *B. siamensis* KCTC 13613^T and *B. velezensis* CR-502^T have not been reported. Therefore, instead of *B. siamensis* KCTC 13613^T and *B. velezensis* CR-502^T, *B. siamensis* SDLI1 (the only *B. siamensis* strain with the complete genome sequences available) and *B. velezensis* FZB42 whose complete genome sequences have been reported were selected for the comparative genome analysis with *Bacillus* sp. 275. Additionally, the complete genome of *B. amyloliquefaciens* DSM 7^T was also compared with that of *Bacillus* sp. 275.

The COG functional categories of the four complete genome sequences are shown in Table 3. Among the 3832 CDS of *Bacillus* sp. 275, 3431 CDS were classified into COG categories (Tatusov et al., 2000). The major categories of *Bacillus* sp. 275 were transcription (K), amino acid transport and metabolism (E), carbohydrate transport and metabolism (G), energy production and conversion (C), cell wall/membrane/envelope biogenesis (M) and inorganic ion transport and metabolism (P). Especially, the number of genes in carbohydrate transport and metabolism (G) related to lignocellulose degradation were 241. This value is similar to that of *B. velezensis* FZB42, but higher than those of *B. siamensis* SDLI1 and *B. amyloliquefaciens* DSM 7^T by 9% and 3%, respectively.

The genes related to the degradation of lignocellulose were detected in the genome of *Bacillus* sp. 275 (Table 4). Endoglucanase, β-glucanase, glucohydrolase and glucosidase were found in the complete genome of *Bacillus* sp. 275. Regarding xylan degradation, glycoside hydrolase 43 family protein (1,4-β-xylosidase), arabinoxylan arabinofuranohydrolase, glucuronoxylanase and α-*N*-arabinofuranoside were found. The genes encoding deferrochelatase (dye decolorizing peroxidase) and laccase involved in lignin degradation were also observed in the genome of *Bacillus* sp. 275. In addition, *Bacillus* sp. 275 has other glycosidases including α-amylase, β-mannosidase, *endo*-1,4-β-galactanase, 6-phospho-β-galactosidase, arabinan *endo*-1,5-α-L-arabinosidase and *endo*-α-(1- > 5)-L-arabinanase. The existence of these genes implies that strain 275 has the potential for utilizing or degrading starch, mannan, galactoside and arabinan.

Most of the genes related to the degradation of lignocellulose were highly observed in all strains (Table 4). However, in the genome of *B. amyloliquefaciens* DSM 7^T, endoglucanase, α-amylase and 1,4-β-xylosidase were not found. The distribution of lignocellulose degradation-related genes between *Bacillus* sp. 275 and *B. siamensis* SDLI1 were also somewhat different. In our study, all lignocellulose-degrading genes in *Bacillus* sp. 275 were found in *B. velezensis* FZB42. *B. velezensis* has been mainly studied for its relationship with plant and fungi, because *B. velezensis* produces antifungal secondary metabolites (Kim et al., 2017).

In conclusion, *Bacillus* sp. 275 has the cellulolytic, xylanolytic and ligninolytic enzyme activities and also has genes encoding lignocellulolytic enzymes. The complete genome information of *Bacillus* sp. 275 would enhance a better understanding on the degradation of lignocellulose in the genus *Bacillus*. These results would provide insight