3 Biotech (2018) 8:465
https://doi.org/10.1007/s13205-018-1490-x

GENOME REPORTS

Complete genome sequence of *Bacillus velezensis* ZY-1-1 reveals the genetic basis for its hemicellulosic/cellulosic substrate-inducible xylanase and cellulase activities

Zhen-yu Zhang[1] · Muhammad Fahim Raza[1] · Ziqiang Zheng[1] · Xuhao Zhang[1] · Xinxin Dong[1] · Hongyu Zhang[1]

Received: 2 June 2018 / Accepted: 23 October 2018 / Published online: 31 October 2018

Abstract

Bacillus velezensis ZY-1-1 was isolated from the larval gut of the lignocellulose-rich diet-fed scarab beetle, *Holotrichia parallela*, and confirmed to possess extremely high xylanase (48153.8 ± 412.1 U/L) and relatively moderate cellulase activity (610.1 ± 8.2 U/L). Notably, these xylanase and cellulase activities were enhanced by xylan (1.4 and 5.8-fold, respectively) and cellulose (1.1 and 3.5-fold, respectively), which indicated the hemicellulosic/cellulosic substrate-inducible lignocellulolytic activities of this strain. The complete genome of *B. velezensis* ZY-1-1 comprises of 3,899,251 bp in a circular chromosome with a G + C content of 46.6%. Among the predicted 3688 protein-coding genes, 24 genes are involved in the degradation of lignocellulose and other polysaccharides, including 8, 7 and 2 critical genes for the degradation of xylan, cellulose and lignin, respectively. This genome-based analysis will facilitate our understanding of the mechanism underlying the biodegradation of lignocellulose and the biotechnological application of this novel lignocellulolytic bacteria or related enzymes.

Keywords *Bacillus velezensis* · Complete genome · Xylanase · Cellulase · Substrate induction

Genome reports

The lignocellulosic biomass of plants is the most abundantly available raw material that is used for producing renewable biofuels and other high-value chemicals. However, these lignocellulosic materials are primarily composed of cellulose and hemicellulose strands, which are stably held together by lignin. Currently, lignocellulose degradation, the first key step of lignocellulosic biomass application, is still a great challenge (Glaser 2015; Mansour et al. 2016). In fact, there are many specific environments and niches (including the gut of insects living on lignocellulose-rich diets) that possess lignocellulolytic abilities, which have been partially attributed to the microbes and considered valuable treasures for screening lignocellulolytic microbes and related enzymes (Hongoh 2010; Sheng et al. 2012; de Gonzalo et al. 2016). This strategy of enzymatic hydrolysis of lignocellulose based on microbes and microbial sourced genes has received remarkable attention both in the industry and the academic communities worldwide.

In this study, we isolated a new lignocellulose-degrading bacterium, strain ZY-1-1, from the larval gut of the scarab beetle, *Holotrichia parallela* (coleoptera: scarabaeidae), which is fed on lignocellulose-rich diets (Zhang and Jackson 2008). Strain ZY-1-1 showed high extracellular lignocellulolytic activities, including hemicellulosic/cellulosic substrate-inducible, extremely high xylanase and relatively moderate cellulase activities. First, using Congo red staining method (Teather and Wood 1982), we found that strain ZY-1-1 grew well and formed a gradually increasing clear zone along with the strain's growth on a basic agar plate with xylan (for xylanase screening) or carboxymethylcellulose (CMC, for cellulase screening). Strain ZY-1-1, growing on the basic agar plate with xylan, formed larger clear zones and higher diameter ratios of clear zone to bacterial colony (highest ratio value of 9.70 ± 1.35 vs. 6.31 ± 0.47), compared to the growth on the

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s13205-018-1490-x) contains supplementary material, which is available to authorized users.

✉ Hongyu Zhang
hongyu.zhang@mail.hzau.edu.cn

[1] State Key Laboratory of Agricultural Microbiology, College of Plant Science and Technology, Huazhong Agricultural University, Wuhan 430070, Hubei, People's Republic of China

basic agar plate with CMC (Fig. 1a–d). Then, the extracellular lignocellulolytic activities of strain ZY-1-1 were determined by the dinitrosalisylic acid (DNS) spectrophotometric method (Dutta et al. 2014). Strain ZY-1-1 was cultured in liquid Luria broth (LB) for 23 h, and the xylanase and cellulase activities of the supernatant were determined to be 48153.8 ± 412.1 and 610.1 ± 8.2 U/L, respectively (Fig. 1e). To evaluate the carbohydrate substrate-inducing effect on the lignocellulolytic activities, strain ZY-1-1 was cultured in the liquid basic medium with or without carbohydrate substrates (basic medium, basic + xylan medium and basic + CMC medium). The xylanase and cellulase activities were highest just before the early-stationary phase (22–23 h) during the growth, and then fluctuated in the stationary phase (Fig. 1f–h). After 23 h culturing, the xylanase activity of strain ZY-1-1 in the supernatant of the basic + xylan medium (35892.3 ± 234.3 U/L) was 1.3- and 1.4-fold higher than that of the basic + CMC medium (27988.2 ± 524.8 U/L) and the basic medium (26119.5 ± 111.1 U/L), respectively. Meanwhile, after culturing for 23 h, the cellulase activities in the supernatant of the basic + xylan medium, the basic + CMC medium and the basic medium were 2104.9 ± 65.7, 1274.0 ± 23.2 and 365.8.5 ± 0.5 U/L, respectively, which indicated a significant xylan and CMC-induced effect on cellulase activity of strain ZY-1-1 (5.8- and 3.5-fold, respectively) (Fig. 1i). Taken together, the strain ZY-1-1 exhibited a significant hemicellulosic/cellulosic substrate-induced, and novel pattern of lignocellulolytic activities, which indicated much higher xylanase activity than cellulose activity. Our results are quite different from previous reports of other lignocellulolytic *Bacillus* strains, such as *Bacillus* sp. 275, *Bacillus* sp. R2 and *B*. velezensis 157, which indicated much higher cellulase activities than xylanase activities (Khelil et al. 2016; Gong et al. 2017; Chen et al. 2018). This implies that the strain ZY-1-1 possess a varied regulation mechanism for novel genes encoding lignocellulolytic enzymes, that deserves further investigation.

Then, the genome of strain ZY-1-1 was sequenced using two sequencing techniques, including the PacBio RSII system (MenloPark, CA, USA) as the third-generation sequencing technology and Illumina HiSeq (151-bp paired-end) as the second-generation sequencing technology. The reads from the former system were assembled by Canu software (Koren et al. 2017) and the latter by A5-miseq (Tatusova et al. 2016). Then, to form the complete genome sequence, the assembled contigs from both sequencing systems were combined and rectified using pilon software (Walker et al. 2014). The complete genome sequence was then generated by combining the data followed by rectification using pilon software (Walker et al. 2014). The complete genome of strain ZY-1-1 consisted of one 3,899,251 bp chromosome with 3688 protein-coding genes, 87 tRNA genes and 27 rRNA genes, and an average G + C content of 46.57% (Table 1 and Fig. S2).

Afterward, strain ZY-1-1 was classified into *B. velezensis*, which was a re-classified *Bacillus* species including conspecific *B. velezensis, B. methylotrophicus* and *B. amyloliquefaciens* subsp. *Plantarum* (Dunlap et al. 2016; Fan et al. 2017), according to its morphological characteristic, 16S rRNA and complete genome sequences. Strain ZY-1-1 held the typical morphological characteristics of the *Bacillus* species with rod-shaped vegetative cells and endospores (Fig. S1). Based on the 16S rRNA gene phylogenetic analysis result, it was then affiliated with the *B. subtilis* group (Fig. 2). To identify the species information, the whole genome sequence of strain ZY-1-1 was analyzed by genome BLAST, and six strains (including *B. velezensis* JJ-D34, *B. velezensis* M75, *B. velezensis* CAU B946, *B. velezensis* NJN-6, *B. amyloliquefaciens* Y14 and *B. amyloliquefaciens* LM2303) were found to be extremely similar with strain ZY-1-1 (all identities = 99%), and all six strains belonged to the *B. velezensis* (Table 2). Correspondingly, high average nucleotide identity (ANI) values (> 97.5%) and a similar G + C content were observed when the genome sequence of strain ZY-1-1 was compared with 19 strains of *B. velezensis*, while all ANI values were below 94.5% when compared with strains of other *Bacillus* species (Table 2). Based on the above results, the strain ZY-1-1 was named *B. velezensis* ZY-1-1.

The *B. velezensis* ZY-1-1 genes were annotated using the NCBI Prokaryotic Genomes Annotation Pipeline (PGAP) (Tatusova et al. 2016). Then, the protein-coding genes were further annotated and were classified into 22, 74, and 43 functional classes based on Cluster of Orthologous Groups (COG), Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG), respectively. In addition, 243, 126, and 232 carbohydrate metabolism-related genes were classified into the classes of "Carbohydrate transport and metabolism", "Carbohydrate metabolic process" and "Carbohydrate metabolism" through the COG, GO and KEGG analyses, respectively (Table S1). The detailed classification information for the protein-coding genes could be found in Fig. S4–S6. Based on HMMER (version 3.0) software, which is based on the Carbohydrate active enzymes (CAZy) database, 125 genes were predicted into CAZy family, including 40 glycoside hydrolases (GHs) genes, 34 glycosyl transferases (GTs) genes, 2 polysaccharide lyases (PLs) genes, 31 carbohydrate esterases (CEs) genes and 6 auxiliary activities (AAs) genes. Moreover, 33, 22 and 3 genes were predicted for antibiotic resistance, antibiotic target and antibiotic biosynthesis, respectively, through the BLAST analysis against Comprehensive Antibiotic Resistance Database (CARD) (McArthur et al. 2013).

To identify the genes related to the lignocellulosic degradation in *B. velezensis* ZY-1-1, the genes encoding known lignicellulolytic enzymes, which were confirmed

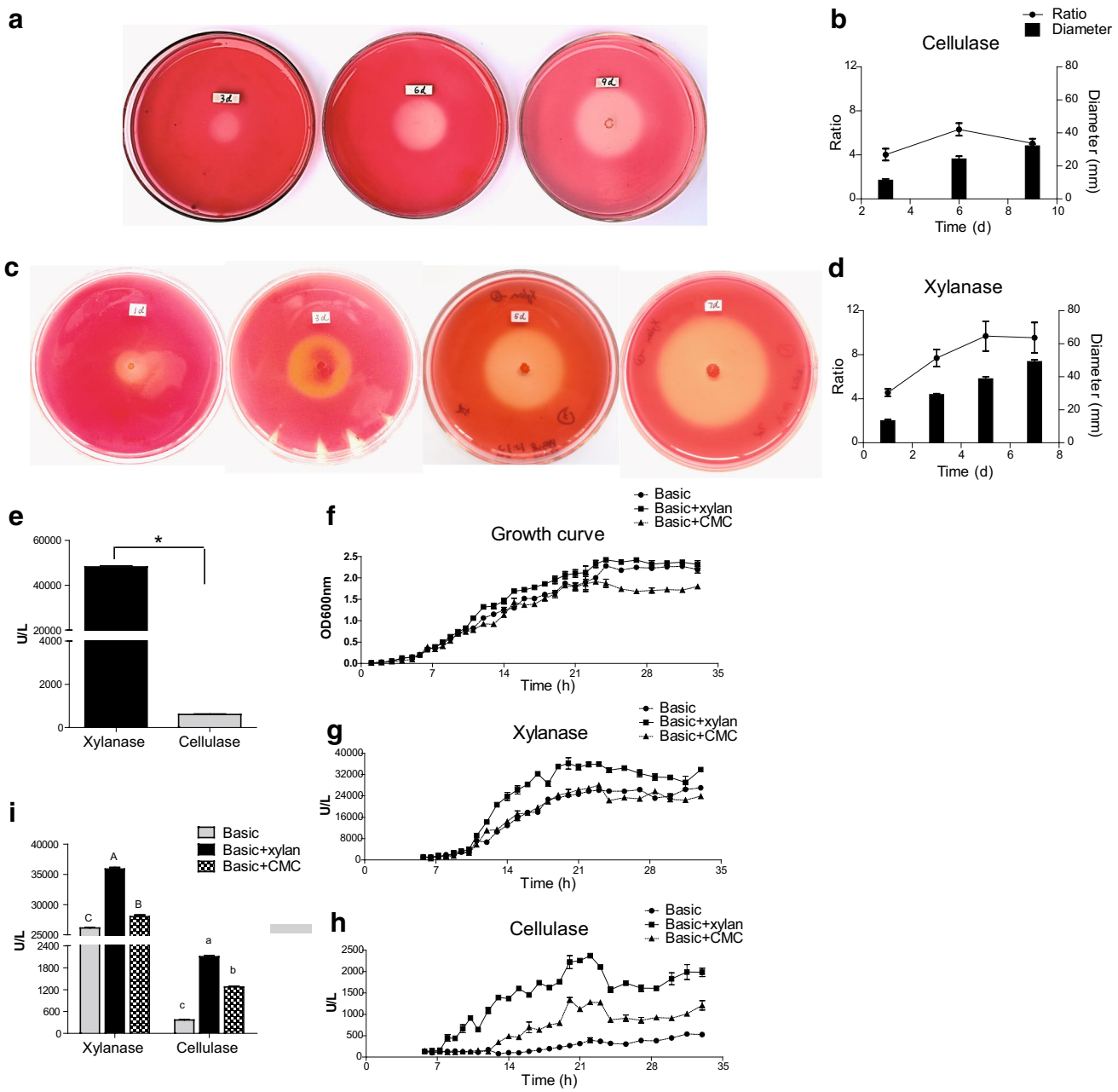

Fig. 1 Lignocellulolytic activities of *Bacillus velezensis* ZY-1-1. **a, b** Degradation of carboxymethylcellulose (CMC) during the cultivation of *Bacillus* ZY-1-1 for 3 days, 6 days and 9 days on the basic agar plate with CMC was determined by the Congo red staining (Teather and Wood 1982) (**a**); meanwhile, the clear zone diameter and the diameter ratio of clear zone to bacterial colony (**b**) were calculated. **c, d** The degradation of xylan during cultivation of *Bacillus* ZY-1-1 for 1 day, 3 days, 5 days and 7 days on the basic agar plate with xylan was determined by the Congo red staining (**c**); meanwhile, the clear zone diameter and the diameter ratio of clear zone to bacterial colony (**d**) were calculated. **e** The xylanase and cellulase activities after cultivation in liquid Luria broth (LB) for 23 h; "Asterisk" indicates a significant difference between xylanase and cellulase activities ($P < 0.05$, *t* test). **f–h** The growth curve (**f**) and the dynamics of xylanase (**g**) and cellulase (**h**) activities of *B. velezensis* ZY-1-1 during the cultivation in the liquid basic, basic + xylan and basic + CMC medium were determined by the method described in the Supplementary Methods. The optimal temperature and pH for the enzymatic reaction were confirmed as 50 °C and pH 5.0 for xylanase, and 50 °C and pH 4.0 for cellulase (Fig. S1). (i) The xylanase or cellulase activities of *B. velezensis* ZY-1-1 were compared between the culture supernatants of the liquid basic, basic + xylan and basic + CMC medium after 23 h cultivation. The different letters indicate significant differences in the activity ($P < 0.05$, Tukey's test following ANOVA analysis). The values are means ± SE. The repetition numbers (n) were 3 for (b) and (d), and 4 for (**e–i**). All medium formulas and statistical analyses mentioned above were described in the Supplementary Methods

Table 1 Genome features of *B. velezensis* ZY-1-1

Features	Chromosome
Genome size (bp)	3,899,251
G + C content (%)	46.6
Total genes	3919
Protein-coding genes (CDS)	3688
5 s rRNA	9
16 s rRNA	9
23 s rRNA	9
tRNA	87

in other bacterial species, were selected as the query sequences, and a BLASTp search of the *B. velezensis* ZY-1-1 genome was carried out (Table 3, S2). For xylan degradation, the genes encoding endo-1,4-β-xylanase (AVX15758.1), glucuronoxylanase (AVX17235.1) and 1,4-β-xylosidase (AVX17306.1) to hydrolyze the main chain of xylan were annotated, which cooperated with arabinosidase (AVX16491.1, AVX16510.1), arabinoxylan arabinofuranohydrolase (AVX17234.1), β-mannanase (AVX15568.1), and acetylxylan esterase (AVX18602.1) to hydrolyze the branched chain of xylan or other hemicelluloses (Sheng et al. 2014). For cellulose degradation, we observed the endo-1,4-β-glucanase (AVX17239.1) and endo-β-1,3-1,4 glucanase (AVX15545.1) for endo-form hydrolysis of (1-4)-beta-D-glucosidic linkages, and β-glucosidase (AVX18716.1, AVX15571.1, AVX15585.1, AVX16352.1, AVX17112.1) for hydrolysis of cellobiose or cellooligosaccharides (Wilson 2011). Meanwhile, for lignin degradation, the genes encoding laccase (AVX18327.1) and deferrochelatase (AVX15607.1) were also found. Furthermore, we observed some other glycosidases, including endo-1,5-α-L-arabinanase (AVX16483.1), galactanase (AVX17829.1), 6-phospho-β-galactosidase (AVX17823.1), oligo-1,6-glucosidase (AVX16273.1, AVX18210.1, AVX18633.1) and 6-phospho-α-D-glucosidase (AVX18175.1). These findings imply that this lignocellulolytic strain may have the potential ability to utilize other polysaccharides, such as arabinan, starch and galactoside.

In conclusion, *B. velezensis* ZY-1-1 displayed tremendous xylanolytic activity and relatively moderate cellulolytic activity. Both activities were significantly induced by hemicellulosic/cellulosic substrates. Based on the complete genome information, the lignocellulose degradation-related genes were annotated using a BLAST analysis by comparing them to reference genes with confirmed lignicellulolytic activities. This genome-based analysis facilitated the identification of novel functional genes and provided an insight into the regulation mechanism underlying the degradation of lignocellulose in bacteria, especially the genus *Bacillus*. These results shed light into the bacteria-sourced mechanism of lignocellulolytic degradation and enhanced the application potential of *B. velezensis* ZY-1-1 for the biomass energy industry.

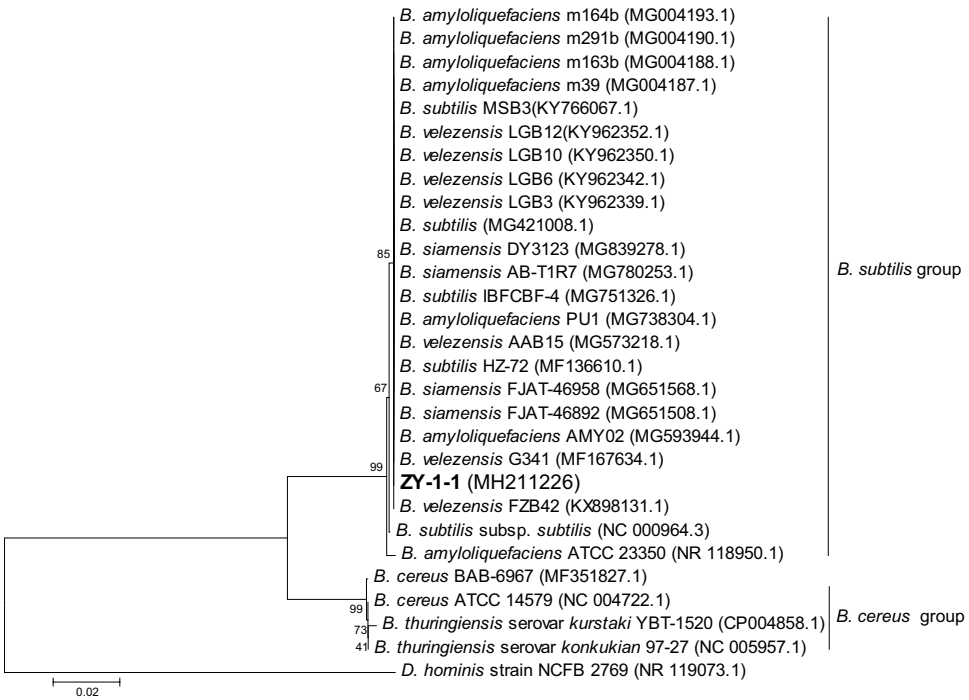

Fig. 2 Phylogenetic tree based on the partial 16S rRNA sequence of *B. velezensis* ZY-1-1 and other homologous *Bacillus* strains. The tree was constructed using the neighbor-joining method by MEGA7 (Kumar et al. 2016). The GenBank accession numbers of all the sequences are indicated in parentheses. The bar represents 0.02 substitutions per site. *Dermabacter hominis* (*D. hominis*) strain NCFB 2769 was used as the outgroup

Table 2 Average nucleotide identity (ANI) analysis of *B. velezensis* ZY-1-1

Strain name	Accession number	Assembly level	G+C%	OrthoANIu value (%)[a]
Operational group *B. amyloliquefaciens*[b]				
B. velezensis (*B. velezensis* /*B. methylotrophicus*/*B. amyloliquefaciens* ssp. *Plantarum*)[c]				
B. velezensis KCTC 13012^T	GCA_001267695.1	Scaffold	46.3	97.8
B. velezensis JJ-D34[d]	CP011346.1	Complete	46.4	99.5
B. velezensis M75[d]	CP016395.1	Complete	46.4	99.4
B. velezensis AS43.3	NC_019842.1	Complete	46.6	97.7
B. velezensis CAU B946[d]	NC_016784.1	Complete	46.5	99.4
B. velezensis NAU-B3	NC_022530.1	Complete	46.0	97.7
B. velezensis NJN-6[d]	NZ_CP007165.1	Complete	46.6	99.5
B. velezensis SQR9	NZ_CP006890.1	Complete	46.1	97.7
B. velezensis TrigoCor1448	NZ_CP007244.1	Complete	46.5	97.7
B. amyloliquefaciens ssp. *plantarum* FZB42^T	NC_009725.1	Complete	46.5	97.7
B. amyloliquefaciens ssp. *plantarum* CAU B946	HE617159.1	Complete	46.4	99.4
B. amyloliquefaciens CC178	NC_022653.1	Complete	46.5	97.7
B. amyloliquefaciens IT-45	NC_020272.1	Complete	46.6	99.5
B. amyloliquefaciens KHG19	NZ_CP007242.1	Complete	46.6	97.7
B. amyloliquefaciens Y14[d]	CP017953.1	Complete	46.4	99.5
B. amyloliquefaciens LM2303[d]	CP018152.1	Complete	46.4	99.5
B. amyloliquefaciens LFB112	NC_023073.1	Complete	46.7	99.4
B. amyloliquefaciens UMAF6639	NZ_CP006058.1	Complete	46.3	97.6
B. methylotrophicus KACC 13105^T	GCA_000960265.2	Contig	46.4	97.8
B. amyloliquefaciens				
B. amyloliquefaciens DSM 7^T	NC_014551.1	Complete	46.1	94.0
B. amyloliquefaciens TA208	NC_017188.1	Complete	45.8	93.9
B. amyloliquefaciens XH7	NC_017191.1	Complete	45.8	94.0
B. siamensis				
B. siamensis KCTC 13613^T	AJVF00000000	Contig	46.3	94.3
B. siamensis XY18	LAGT01000000	Contig	46.3	94.3
B. siamensis 7551	NPCI01000000	Contig	46.4	94.3
Other *Bacillus* species				
B. vallismortis				
B. vallismortis DV1-F-3 ^{T}	AFSH01000000	Scaffold	43.8	76.8
B. vallismortis TD3	NXEM01000000	Scaffold	43.9	77.1
B. vallismortis B4144_201601	LQYR01000000	Scaffold	43.0	77.1
B. subtilis				
B. subtilis subsp. *subtilis* 168	NC_000964.3	Complete	43.5	77.2
B. cereus				
B. cereus ATCC 14579^T	NC_004722.1	Complete	35.3	68.1
B. thuringiensis				
B. thuringiensis serovar konkukian 97 − 27	NC_005957.1	Complete	34.9	68.3

[a]The ANI values were calculated using OrthoANIu, an improved algorithm and software for calculating ANI (Yoon et al. 2017), through online tools of EZBioCloud (https://www.ezbiocloud.net/tools/ani)

[b]Operational group *B. amyloliquefaciens* is composed of *B. velezensis, B. amyloliquefaciens* and *B. siamensis* (Dunlap et al. 2016; Fan et al. 2017)

[c]*B. velezensis* is a re-classified *Bacillus* species including conspecific *B. velezensis, B. methylotrophicus* and *B. amyloliquefaciens* subsp. *Plantarum* (Dunlap et al. 2016)

[d]This strain was extremely similar (identity = 99%) to *B. velezensis* ZY-1-1 based on genome BLAST analysis

Table 3 Annotated genes encoding lignocellulose-degrading enzymes in *B. velezensis* ZY-1-1

Gene of *B. velezensis* ZY-1-1		Reference gene		BLASTp identity (%)	BLASTp coverage (%)	
Annotation	Accession no. (NCBI)	Accession no. (UniProtKB)	Species		Annotated Gene	Reference Gene
Hemicellulose-related						
Endo-1,4-β-xylanase	AVX15758.1	P18429	*B. subtilis* 168	95.00	100.00	100.00
Glucuronoxylanase	AVX17235.1	Q45070	*B. subtilis* 168	90.00	100.00	100.00
1,4-β-Xylosidase	AVX17306.1	P94489	*B. subtilis* 168	94.90	100.00	88.37
Arabinosidase	AVX16491.1	P94531	*B. subtilis* 168	87.10	99.40	99.20
Arabinosidase	AVX16510.1	P94552	*B. subtilis* 168	80.73	99.40	99.60
Arabinoxylan arabinofuranohydrolase	AVX17234.1	Q45071	*B. subtilis* 168	91.56	100.00	87.72
β-Mannanase	AVX15568.1	O05512	*B. subtilis* 168	74.31	100.00	100.00
Acetylxylan esterase	AVX18602.1	P94388	*B. subtilis* 168	83.96	100.00	100.00
Endo-1,5-α-L-arabinanase	AVX16483.1	Q93HT9	*Geobacillus thermodenitrificans*	51.00	89.41	94.25
Galactanase	AVX17829.1	Q65CX5	*B. licheniformis* ATCC 14580	30.48	79.19	70.52
6-Phospho-β-galactosidase	AVX17823.1	C7N8L9	*Leptotrichia buccalis* ATCC 14201	61.00	99.36	99.36
Cellulose-related						
Endo-1,4-β-glucanase	AVX17239.1	P07983	*B. subtilis DLG*	96.79	100.00	100.00
β-Glucanase/Endo-β-1,3 – 1,4 glucanase	AVX15545.1	P07980	*B. amyloliquefaciens*	92.47	98.35	100.00
β-Glucosidase	AVX18716.1	Q7WUL3	*Cellulomonas fimi*	29.12	65.78	68.44
6-Phospho-β-glucosidase	AVX15571.1	O05508	*B. subtilis* 168	81.96	98.71	98.92
6-Phospho-β-glucosidase	AVX15585.1	P46320	*B. subtilis* 168	94.57	100.00	100.00
6-Phospho-β-glucosidase	AVX16352.1	P46320	*B. subtilis* 168	29.50	99.31	97.51
Aryl-phospho-β-D-glucosidase	AVX17112.1	P42973	*B. subtilis* 168	88.66	99.17	99.37
Oligo-1,6-glucosidase	AVX16273.1	P29093	*Bacillus* sp. F5	47.56	90.40	99.61
Oligo-1,6-glucosidase	AVX18210.1	O06994	*B. subtilis* 168	50.27	98.04	99.11
Oligo-1,6-glucosidase	AVX18633.1	O06994	*B. subtilis* 168	49.19	98.57	98.75
6-Phospho-α-D-glucosidase	AVX18175.1	P54716	*B. subtilis* 168	92.43	100.00	100.00
Lignin-related						
Laccase	AVX18327.1	D4GPK6	*Haloferax volcanii* ATCC 29605	38.92	98.83	85.32
Deferrochelatase	AVX15607.1	Q8XAS4	*Escherichia coli* O157:H7	39.04	84.93	86.76

The references which confirmed the specific enzymatic activities of the reference genes are listed in Table S2

Accession numbers

The genome sequence of *B. velezensis* ZY-1-1 was deposited into the GenBank under the accession number CP027061. The strain is available from the China Center for Type Culture Collection (CCTCC) with the deposition number M2018180.

Acknowledgements This study was supported by National Natural Science Foundation of China (31501634 and 30671404) and National Key R&D Program of China (2017YFD0202000).

Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

References

Chen L, Gu W, Xu H, Yang G, Shan X, Chen G, Wang CF, Qian AD (2018) Complete genome sequence of *Bacillus velezensis* 157 isolated from *Eucommia ulmoides* with pathogenic bacteria

inhibiting and lignocellulolytic enzymes production by SSF. 3 Biotech 8(2):114. https://doi.org/10.1007/s13205-018-1326-8

de Gonzalo G, Colpa DI, Habib MH, Fraaije MW (2016) Bacterial enzymes involved in lignin degradation. J Biotechnol 236:110–119. https://doi.org/10.1016/j.jbiotec.2016.08.011

Dunlap CA, Kim S, Kwon S, Rooney AP (2016) *Bacillus velezensis* is not a later heterotypic synonym of *Bacillus amyloliquefaciens; Bacillus methylotrophicus, Bacillus amyloliquefaciens* subsp. *plantarum* and '*Bacillus oryzicola*' are later heterotypic synonyms of *Bacillus velezensis* based on phylogenomics. Int J Syst Evol Micr 66(3):1212–1217. https://doi.org/10.1099/ijsem.0.000858

Dutta N, Mukhopadhyay A, Dasgupta AK, Chakrabarti K (2014) Improved production of reducing sugars from rice husk and rice straw using bacterial cellulase and xylanase activated with hydroxyapatite nanoparticles. Bioresour Technol 153:269–277. https://doi.org/10.1016/j.biortech.2013.12.016

Fan B, Blom J, Klenk HP, Borriss R (2017) *Bacillus amyloliquefaciens, Bacillus velezensis*, and *Bacillus siamensis* form an "Operational group *B. amyloliquefaciens*" within the *B. subtilis* species complex. Front Microbiol 8:22. https://doi.org/10.3389/fmicb.2017.00022

Glaser R (2015) Enzyme-based lignocellulose hydrolyzation—Sauter mean diameter of raw materials as a basis for cellulase performance characterization and yield prediction. J Biotechnol 214:9–16. https://doi.org/10.1016/j.jbiotec.2015.08.024

Gong G, Kim S, Lee SM, Woo HM, Park TH, Um Y (2017) Complete genome sequence of *Bacillus* sp. 275, producing extracellular cellulolytic, xylanolytic and ligninolytic enzymes. J Biotechnol 254:59–62. https://doi.org/10.1016/j.jbiotec.2017.05.021

Hongoh Y (2010) Diversity and genomes of uncultured microbial symbionts in the termite gut. Biosci Biotechnol Biochem 74(6):1145–1151. https://doi.org/10.1271/bbb.100094

Khelil O, Choubane S, Cheba BA (2016) Polyphenols content of spent coffee grounds subjected to physico-chemical pretreatments influences lignocellulolytic enzymes production by *Bacillus* sp. R2. Bioresour Technol 211:769–773. https://doi.org/10.1016/j.biortech.2016.03.112

Koren S, Walenz BP, Berlin K, Miller JR, Bergman NH, Phillippy AM (2017) Canu: scalable and accurate long-read assembly via adaptive k-mer weighting and repeat separation. Genome Res 27(5):722–736. https://doi.org/10.1101/gr.215087.116

Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 33(7):1870–1874. https://doi.org/10.1093/molbev/msw054

Mansour AA, Da CA, Arnaud T, Lu-Chau TA, Fdz-Polanco M, Moreira MT, Cacho RJ (2016) Review of lignocellulolytic enzyme activity analyses and scale-down to microplate-based assays. Talanta 150:629–637. https://doi.org/10.1016/j.talanta.2015.12.073

McArthur AG, Waglechner N, Nizam F et al (2013) The comprehensive antibiotic resistance database. Antimicrob Agents Chemother 57(7):3348–3357. https://doi.org/10.1128/AAC.00419-13

Sheng P, Huang S, Wang Q, Wang A, Zhang H (2012) Isolation, screening, and optimization of the fermentation conditions of highly cellulolytic bacteria from the hindgut of *Holotrichia parallela* larvae (Coleoptera: Scarabaeidae). Appl Biochem Biotechnol 167(2):270–284. https://doi.org/10.1007/s12010-012-9670-3

Sheng P, Xu J, Saccone G, Li K, Zhang H (2014) Discovery and characterization of endo-xylanase and beta-xylosidase from a highly xylanolytic bacterium in the hindgut of *Holotrichia parallela* larvae. J Mol Catal B-Enzym 105:33–40. https://doi.org/10.1016/j.molcatb.2014.03.019

Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Pruitt KD, Borodovsky M, Ostell J (2016) NCBI prokaryotic genome annotation pipeline. Nucleic Acids Res 44(14):6614–6624. https://doi.org/10.1093/nar/gkw569

Teather RM, Wood PJ (1982) Use of Congo red-polysaccharide interactions in enumeration and characterization of cellulolytic bacteria from the bovine rumen. Appl Environ Microbiol 43(4):777–780

Walker BJ, Abeel T, Shea T, Priest M, Abouelliel A, Sakthikumar S, Cuomo CA, Zeng Q, Wortman J, Young SK, Earl AM (2014) Pilon: an integrated tool for comprehensive microbial variant detection and genome assembly improvement. Plos One 9(11):e112963. https://doi.org/10.1371/journal.pone.0112963

Wilson DB (2011) Microbial diversity of cellulose hydrolysis. Curr Opin Microbiol 14(3):259–263. https://doi.org/10.1016/j.mib.2011.04.004

Yoon SH, Ha SM, Lim J, Kwon S, Chun J (2017) A large-scale evaluation of algorithms to calculate average nucleotide identity. Antonie Van Leeuwenhoek 110(10):1281–1286. https://doi.org/10.1007/s10482-017-0844-4

Zhang H, Jackson TA (2008) Autochthonous bacterial flora indicated by PCR-DGGE of 16S rRNA gene fragments from the alimentary tract of *Costelytra zealandica* (Coleoptera: Scarabaeidae). J Appl Microbiol 105(5):1277–1285. https://doi.org/10.1111/j.1365-2672.2008.03867.x