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GENOME REPORTS

The complete genome sequence of *Bacillus velezensis* LPL061, an exopolysaccharide-producing bacterium

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Abstract

Bacillus velezensis LPL061, which shows strong exopolysaccharide (EPS) producing capacity, was isolated from carnations in Beijing, China. The complete genome of LPL061 comprised a single circular chromosome (3,907,268 bp; G+C content of 46.7%) with 3,737 coding DNA sequences, 26 rRNA, and 89 tRNA. According to genome analysis, 12 protein-coding genes which related to polysaccharide biosynthesis in LPL061 were identified. Comparative genome analysis revealed that the EPS biosynthetic gene cluster was relatively conserved among *Bacillus* species. EPS showed approximately 60% inhibitory activity on the α-glucosidase at 100 μg/mL. The results of quantitative reverse transcription PCR further demonstrated that compared to insulin-resistant model with insulin (500 μg/mL) (without EPS treatment), the insulin-resistant HepG2 cells treated with EPS decreased the expression of phosphoenolpyruvate carboxykinase (*PEPCK*) from 4.425 to 0.1587, glucose-6-phosphatase (*G6Pase*) decreased from 4.272 to 0.1929, and glycogen synthase kinase3β (*GSK(3)β*) decreased from 2.451 to 0.993, respectively. Meanwhile, EPS treatment increased GS expression and resulted in intracellular glycogen concentration increased from 28.30% to 86.48%, which further supported that EPS form LPL061 could reduce the concentration of blood glucose effectively. These results could be beneficial for better understanding of the hypoglycemic mechanism of *B. velezensis* LPL061 EPS and developing an EPS-based anti-diabetic agent in the future.

Keywords *Bacillus velezensis* LPL061 · Complete genome sequence · Exopolysaccharide · Hypoglycemic

Introduction

Beneficial microorganisms can produce a variety of exopolysaccharides (EPS) that are safe for human beings. EPSs isolated from *Bifidobacterium* and *Lactobacillus* have immunomodulatory activity, thus protecting hosts against bacterial and viral infections (Emanuele et al. 2016; Liu et al. 2011; Yu et al. 2019). Hypoglycemic is one of the most important biological functions of polysaccharides produced by lactic acid bacteria (Bajpai et al. 2016). Diabetes mellitus is a chronic metabolic disease caused by insulin deficiency or receptor dysfunction. Nowadays, there are a few commercially available anti-diabetic drugs, including alpha-glucosidase inhibitors, biguanides, and sulfonylureas. Among those drugs, the anti-diabetic mechanisms are different (Guo et al. 2014; Rangika et al. 2015). Metformin, for example, can reduce blood glucose by promoting the sensitivity of peripheral tissues to glucose, inhibiting gluconeogenesis, inhibiting glycogen degradation, and delaying the glucose uptake in the intestine. Polysaccharides can decrease blood glucose concentration by enhancing the effect of insulin and improving insulin sensitivity and its mode of action is similar to that of metformin. Due to its safety, good hypoglycemic activity, and few side effects, EPSs have received researchers' attention in the diabetes field.

Strain LPL061 was originally isolated from carnations with a high yield of EPS production and phylogenetically

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characterized as *Bacillus velezensis* by 16S rRNA gene analysis. (Zhang et al. 2012). We also studied the physical and chemical properties, thermal stability, rheological properties, and emulsifying properties of the EPS produced by this strain (Han et al. 2015). However, the biological activity of *Bacillus* polysaccharide in regulating blood glucose concentration remains largely unknown. In this study, the complete genome of *B. velezensis* LPL061 was sequenced, putative EPS biosynthetic pathway was identified, and the hypoglycemic activity of EPS in vitro was determined. The genome information of *B. velezensis* LPL061 and hypoglycemic activity of EPS provided a theoretical foundation for its application as a potential hypoglycemic agent in the future.

Materials and methods

Bacterial strains and culture

Bacillus velezensis LPL061 with high EPS yield was isolated from the carnation, China (Zhang et al. 2012). *B. velezensis* LPL061 was grown in previously optimized EPS-producing medium containing 22-g/L sucrose (AoBoxing, Beijing, China) and 18.4-g/L yeast extract (AoBoxing, Beijing, China) (pH 7.0) at 28 °C for 24 h under shaking condition at 220 rpm (Li et al. 2013).

Genome sequencing, assembly, and annotation

Genomic DNA of LPL061 was isolated using the QIAGEN DNA extraction kit (Qiagen, CA, USA) according to manufacturer's instruction. Genome sequencing was performed by the Illumina Hiseq 2000 platform (GATC Biotech) (2 × 100 bp). After sequencing, the short reads were assembled by SOAPdenovo v2.04 (https://soap.genomics.org.cn). Gaps between scaffolds were closed by polymerase chain reaction (PCR) and Sanger sequencing. Annotation was carried out using Rapid Annotation Subsystem Technology (Arjan et al. 1991; Aziz et al. 2008). The Kyoto Encyclopedia of Genes and Genomes (KEGG) database (Kanehisa et al. 2016), Clusters of Orthologous Groups (COG) (Tatusov et al. 2003) and Pfam (https://pfam.xfam.org/) were used to predict functional genes. The genes related to the synthesis of exopolysaccharide were identified from the genome of LPL061 using BLAST of NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Production, isolation, and purification of the EPS

The EPSs were isolated and purified using our previous method with slight modifications (Li et al. 2013; Xu et al. 2011). In brief, the fermentation culture was centrifuged for 10 min at 10,000 rpm to remove the cell pellets. After that, the cell-free supernatant was mixed with three times volume of 95% ethanol at 4 °C for overnight and then centrifuged for 10 min at 10,000 rpm. The precipitation was collected and dissolved in deionized water (50%, w/v), and then dialyzed (Mw cut-off 8000–14,000 Da) at 4 °C for overnight. After that, water was removed by freeze-drying.

The purification and collection conditions of EPS are in accordance with the methods reported in the previous laboratory (Han et al. 2015). After that, water was removed by freeze-drying, and the pure EPS product needed for the experiment was prepared. The carbohydrate content of the fractions was determined with phenol–sulfuric acid method using glucose as the standards (Dubois et al. 1956).

EPS was dissolved into a 0.1-mg/mL solution and deionized water was used as the blank control. UV–Vis spectrophotometer (Uv-1800, Meishida, Shanghai, China) was used to scan the whole wavelength of EPS in the range of 200–580 nm. Detect whether the extracted EPS contains protein (280 nm) and nucleic acid (260 nm).

α-Glycosidase inhibitory activity of EPS

The inhibition rate of α-glucosidase was determined according to Kim et al. (2011). Briefly, 60 µL of PBS, 20 µL of different concentrations (0, 10, 20, 40, 80,100 µg/mL) of EPS, and 20 µL of 0.2 IU/mL α-glucosidase solution (Sigma, MO, USA) was added into 96-well plate and incubated at 37 °C for 10 min. To start the reaction, 20 µL of PNPG solution (2.5 mmol/L) was added. After 10 min, 30 µL of Na_2CO_3 solution (0.1 mol/L) was added to terminate the reaction. Thereafter, absorbance at 405 nm (A_{405}) was measured by Microplate Reader (Multiscan SK3, Thermo Fisher Scientific, USA) to calculate the inhibition rate of α-glucosidase (R) according to the following formula.

$$R = \left(1 - \frac{A_{\text{sample}} - A_{\text{sample control}}}{A_{\text{blank}} - A_{\text{blank control}}}\right) \times 100,$$

where R represents α-glucosidase inhibitory activity, A_{blank} represents the absorbance of the control reaction (containing all reagents except the EPS), $A_{\text{blank control}}$ represents the absorbance of treatment without EPS and α-glucosidase, A_{sample} represents the absorbance of the EPS, and $A_{\text{sample control}}$ represents the absorbance of the EPS but without α-glucosidase.

Mammalian cell culture

Human hepatoma cell line HepG2 was purchased from the Cell Bank of Chinese Academy of Sciences (Shanghai, China) and cultured in DMEM medium (Gibco, New York, USA) supplemented with 10% of fetal bovine serum (FBS,

Every Green, Zhejiang, China) and 1% of penicillin–streptomycin (100 U/mL, Gibco, New York, USA) at 37 °C in a humidified 5% of CO_2 incubator (MCO-15AC, SANYO, Japan).

3-(4,5 Dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay

The MTT assay was performed according to the method developed by Mosmann (1983). Briefly, 5×10^3 of HepG2 cells/well were seeded in triplicate in 96-well flat bottom tissue culture plates in the Dulbecco's modified eagle (DMEM) medium for 12 h, and then cells were treated with 100 µL of EPS (10, 20, 40, 80, 100 µg/mL) for 24 h. After that, 15 µL of 3-(4,5 dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium Bromide (MTT, Beyotime, Shanghai, China) was added to each well and incubated at 37 °C for 4 h. Supernatants were then discarded and cells were washed by phosphate buffer saline (PBS) solution (Gibco, New York, USA) for once. After that, 100 µL of Dimethyl sulfoxide (DMSO, Solebo, Beijing, China) was added to each well to dissolve crystallization precipitation. Absorbance at 490 nm was measured.

Glucose consumption assay

Glucose consumption assay was carried out based on a previous published method with minor modifications (Teng et al. 2016). Specifically, HepG2 cells were seeded into 96-well plates in DMEM supplemented with 10% of FBS and 1% of penicillin–streptomycin for 12 h. Then, the medium was replaced with serum-free high-glucose DMEM with 500 µg/mL of recombinant human insulin (Beyotime, Shanghai, China) and the cells were incubated for 24 h to establish insulin-resistant HepG2 cells. Then different concentrations of EPS (0, 10, 20, 40, 80, 100 µg/mL) or metformin (10 µg/mL) were prepared in DMEM without FBS and 100 µL of each treatment was added into the insulin-resistant cells. After 24 h, the glucose concentrations in the culture supernatant were determined using the Glucose test kit (Beijing Sino-UK Institute of Biological Technology, Beijing, China). Glucose uptake was calculated by subtracting the glucose concentration of control groups from treatment groups. The number of living cells was calculated by MTT method (Zheng et al. 2011) and glucose uptake per cell was calculated.

Determination of intracellular glycogen content

Intracellular glycogen concentration was measured using a Glycogen Assay Kit (Solebo Biology Co., Ltd., Beijing, China), according to the manufacturer's instructions with some modifications. Cells were seeded in six-well plates (1×10^6 cells/well) in DMEM supplemented with 10% of FBS and 1% of penicillin–streptomycin for 12 h. After that, cells were resuspended in PBS buffer (1 mL/well) on ice, and then boiled for 15 min to inactivate enzymes, followed by centrifugation at 2000 rpm for 15 min. The supernatants were collected and assayed for glycogen concentration.

Real-time quantitative PCR (RT-qPCR) analysis

After treated with different concentrations of (0, 10, 40, 80 µg/mL) EPS for 24 h, insulin-resistant HepG2 cells were washed twice by PBS and then digested by trypsin (Gibco, New York, USA). Digested cells were centrifuged at 1000 rpm for 5 min to collect the pellets.

The total RNAs of different samples were isolated using the Trizol reagent (Invitrogen, New York, USA) and first-strand cDNA was reverse transcribed at 37 °C for 1 h, 95 °C for 5 min and 4 °C for 5 min using PrimeScript 1st strand cDNA Synthesis Kit (Takara, Kyoto, Japan). RT-qPCR was performed using the 7500 Fast Real-Time PCR system (Applied Biosystems) with SYBR FAST qPCR Kit (Kapa Biosystems, New York, USA). The *β-actin* was used as a reference gene and the qRT-PCR data were analyzed by the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen 2001). Table 1 listed the gene-specific primer sequences.

Statistical analysis

All biochemical and cell culture assays were performed with at least three independent repeats for each experiment. Statistical analysis of differences between groups was carried out by Student's *t* test or by one-way ANOVA followed by Duncan's test procedures using GraphPad Prism V 5.01 (GraphPad Software Inc, San Diego, CA, USA). $p < 0.05$ indicates statistically significant. Data were presented as means ± standard deviation of the independent repeats in each experiment.

Table 1 The primers used in real-time PCR

Genes	Gene-specific primer sequences (5′→3′)
β-actin	F: TCAGGTCATCACTATCGGCAAT
	R: AAAGAAAGGGTGTAAAACGCA
G6Pase	F: GTCCACAITGACACCACACC
	R: GAGCCACTTGCTGAGTTTCC
PEPCK	F: AACCCTGAGAACGGCTTCTT
	R: TGGTCTCAGCCACATTGGTA
GSK(3)β	F: AGGAGAACCCAATGTTTCGTAT
	R: ATCCCCTGGAAATATTGGTTGT
GS	F:AGACCCCAACAAACTGGTCC
	R: CAACCAAAAGGGTGCCCATC

Table 2 Features of *Bacillus velezensis* LPL061 genome

Attributes	Chromosome
Genome size (bp)	3,907,268
GC content (%)	46.7%
rRNAs (5S, 16S, 23S)	26
tRNAs	89
Coding proteins	3737

Results and discussion

Genome features of strain LPL061

The complete genome of LPL061 contained one gapless circular chromosome of 3,907,268 bp and no plasmid, with G+C content of 46.7%. 3737 protein-coding sequences, 26 rRNA, and 89 tRNA were found in the chromosome of LPL061 (Table 2; Fig. 1). Phylogenetic analysis of LPL061 genomes showed that strain LPL061 presented approximately 100% sequence similarity to *B. velezensis* (Fig. S1).

According to the Cluster of Orthologous Groups (COG) of protein designation (Tatusov et al. 2003), all the identified genes were classified into 19 functional categories (Table 3). In addition, 2.54% of the genome of LPL061 (class Q) was involved in the secondary metabolite synthesis, transportation, and decomposition which suggests that EPS biosynthetic pathways could be present in LPL061.

The biosynthesis of EPS in B. velezensis

The genome analysis results revealed that the genome of *B. velezensis* LPL061 contained the complete EPS biosynthetic gene cluster as shown in Fig. 2. 12 *eps* genes (*epsD*, *epsE*, *epsF*, *epsG*, *epsH*, *epsI*, *epsJ*, *epsK, epsL*, *epsN, epsM* and *epsO*) encode putative glycosyltransferases, presumably for EPS biosynthesis (Table 3).

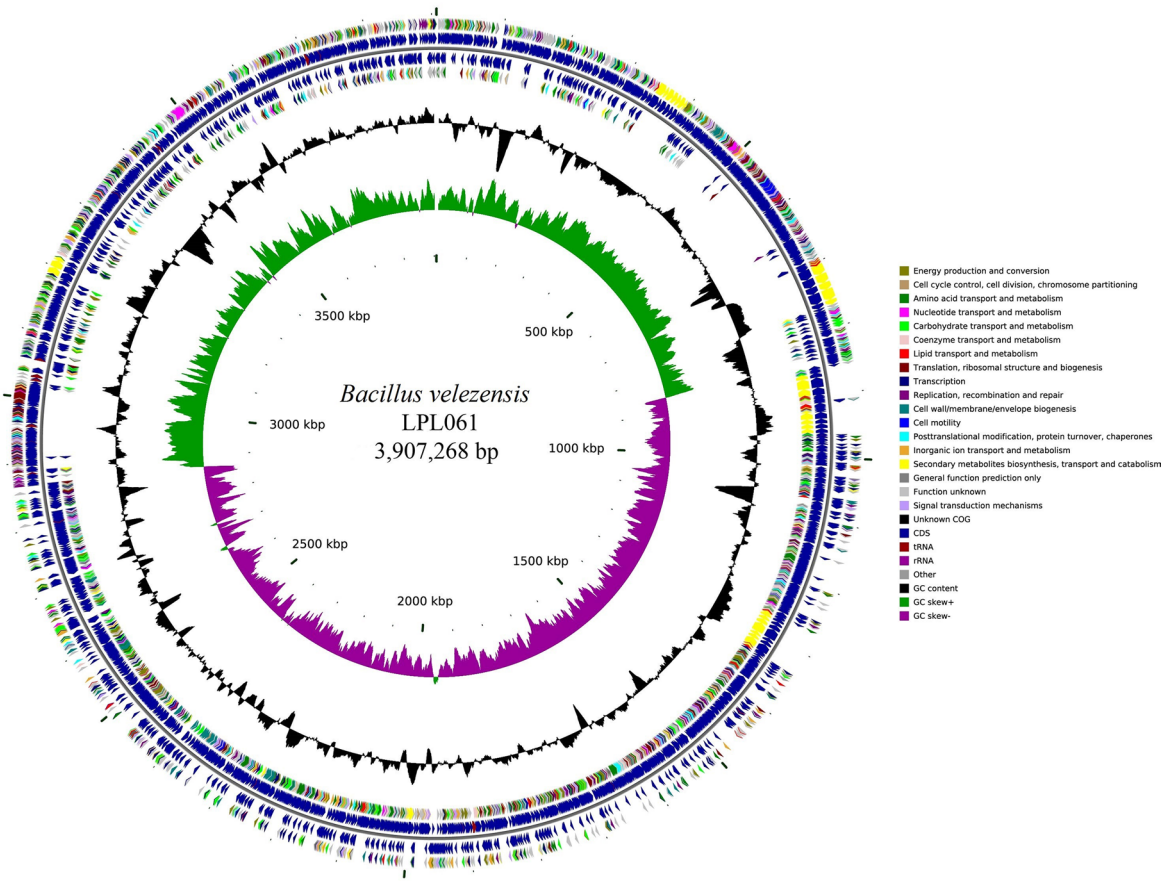

Fig. 1 Circular genome map of *Bacillus velezensis* LPL061. The orientation of circles is shown from the inner to outer. Ring 1 represents the GC skew (green, positive skew; purple, negative skew). Ring 2 represents the G+C content. Ring 4 represents reverse CDSs and ring 3 represents reverse COG Annotated coding sequences. Ring 5 represents forward CDSs. Ring 6 represents forward COG Annotated coding sequences. Very short features were enlarged to enhance visibility. Clustered genes, such as several rRNA genes, may appear as one line due to space limitation. The image was created using the software cgview

Among those genes, the *epsE* gene encodes a membrane-associated priming glycosyltransferase and it does not catalyze glycosidic linkage but transfers sugar-1-phosphate to the undecaprenyl-phosphate-lipid carrier on the cytoplasmic face of the membrane (Blair et al. 2008; Charnock and Davies 1999). *epsF*, *epsG*, *epsH*, *epsI*, *epsJ* and *epsK* may transfer a variety of nucleotide sugars. Moreover, the genes *epsC*, *epsI*, *epsO*, acyltransferase gene *epsM*, and aminotransferase gene *epsN* are all involved in the modification of polysaccharide repeating units (Chen et al. 2006).

The comparative analysis results of EPS biosynthesis gene clusters among different species revealed that the synthesis of EPS in *Bacillus* was relatively conserved. The whole genome of *B. amyloliquefaciens* LL3 strain (Geng et al. 2011) and *B. velezensis* L-S60 (Qin et al. 2015) has been sequenced and the clusters have been found related to

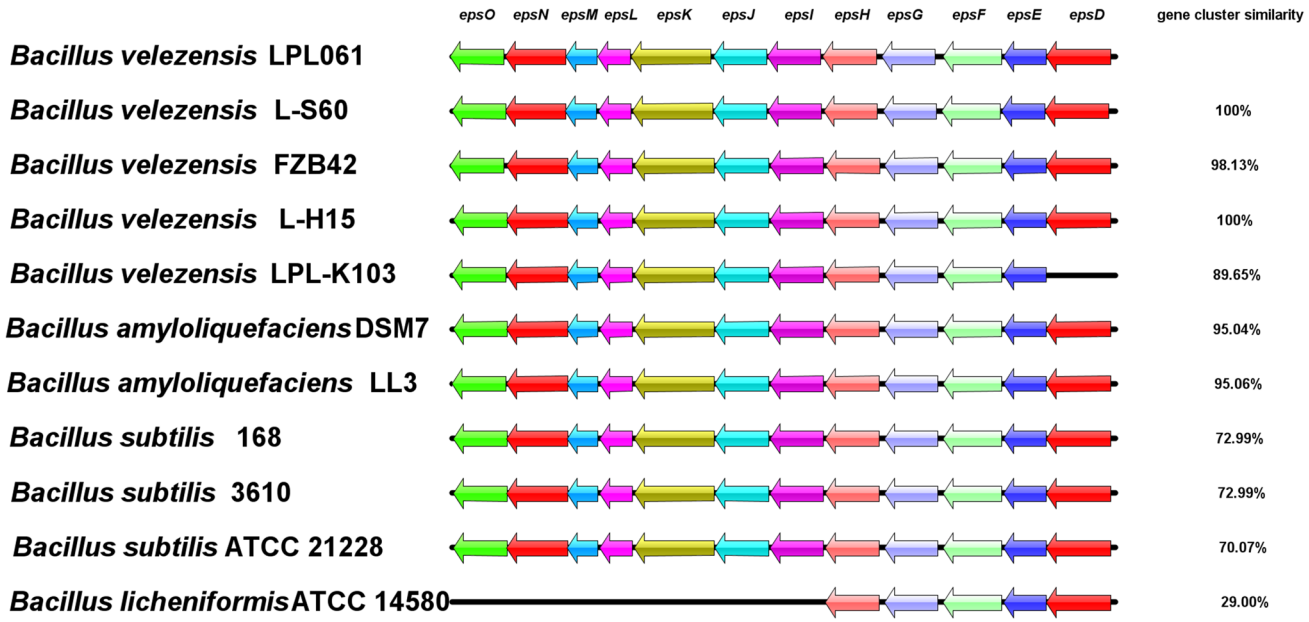

Fig. 2 Analysis and comparison of exopolysaccharide biosynthetic gene clusters among different *Bacillus* species

Table 3 COG categories of *Bacillus velezensis* LPL061

COG class	Name	Count	Proportion (%)
C	Energy production and conversion	180	5.45
D	Cell cycle control, cell division, chromosome partitioning	32	0.97
E	Amino acid transport and metabolism	268	8.11
F	Nucleotide transport and metabolism	78	2.36
G	Carbohydrate transport and metabolism	226	6.84
H	Coenzyme transport and metabolism	114	3.45
I	Lipid transport and metabolism	90	2.72
J	Translation, ribosomal structure and biogenesis	165	5.00
K	Transcription	261	7.90
L	Replication, recombination and repair	135	4.09
M	Cell wall/membrane/envelope biogenesis	203	6.15
N	Cell motility	34	1.03
O	Posttranslational modification, protein turnover, chaperones	97	2.94
P	Inorganic ion transport and metabolism	181	5.48
Q	Secondary metabolites biosynthesis, transport and catabolism	84	2.54
S	Function unknown	921	27.89
T	Signal transduction mechanisms	136	4.12
U	Intracellular trafficking, secretion, and vesicular transport	33	1.00
V	Defense mechanisms	65	1.97

the synthesis of polysaccharide. Meanwhile, EPS biosynthetic gene cluster of LPL061 was compared with that of the other four bacteria. The analysis revealed that the EPS biosynthetic gene cluster in LPL061 was 100% and 95% similar to that of *B. velezensis* L-S60 and *B. amyloliquefaciens* LL3, respectively. The similarity of the gene cluster with other strains such as *B. velezensis* FZB42, *B. velezensis* L-H15, *B. velezensis* LPL-K103, *B. amyloliquefaciens* DSM7, *B. subtilis* 168, *B. subtilis* 3610, and *B. subtilis* ATCC 21228 was 98.13%, 100%, 89.65%, 95.04%, 72.99%, 72.99%, and 70.07%, respectively. The similarity of EPS biosynthesis gene clusters between LPL061 and *B. licheniformis* ATCC 14580 (Rey et al. 2004; Veith et al. 2004) was poor, only 29.00% and this is consistent with the fact that ATCC 14,580 does not secrete polysaccharide bioflocculant into EPS-producing medium (Fig. 2).

Isolation, purification, and quantification of EPS

The yield of EPS before and after purification was determined by phenol–sulfuric acid method. The results showed that the yield of EPS was 4.51 g/L before purification and 3.22 g/L after purification (Fig. 3a).

To further prove whether the prepared EPS component contains nucleic acid and protein, it is scanned with full-wavelength UV, and the scanning results are shown in Fig. 3b. The results of UV full-wavelength scanning showed that EPS had no absorption peak at 260 nm and 280 nm, indicating that purified EPS did not contain nucleic acid or protein.

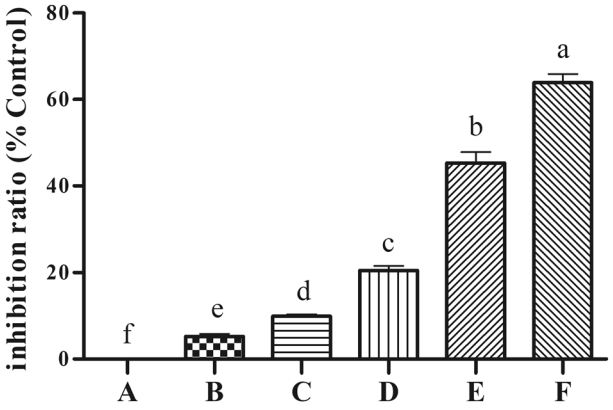

Fig. 4 Inhibitory activities of exopolysaccharides (EPS) of *Bacillus velezensis* LPL061 against α-amylase. Values were expressed as the mean ± SD. $n = 3$ for each group. $P < 0.05$ compared with the model group. Mean values with different superscript letters are significantly different ($P < 0.05$). A: blank control without insulin and EPS treatment; B–F: cells treated with insulin and different concentrations of EPS (10, 20, 40, 80, 100 μg/mL) treatment

Inhibition of α-glucosidase by polysaccharides

The inhibitory activity of polysaccharides on α-glucosidase is shown in Fig. 4. EPS showed a dose-dependent effect on inhibiting a-glucosidase. The highest concentration of EPS showed more than 60% of inhibition rate.

Influence of EPS on cell viability in HepG2 cells

As shown in Fig. 5a, the cell viability showed no significant difference ($P > 0.05$) between normal HepG2 cells and cells treated with EPS at 10, 20, 40 or 80 μg/mL, but

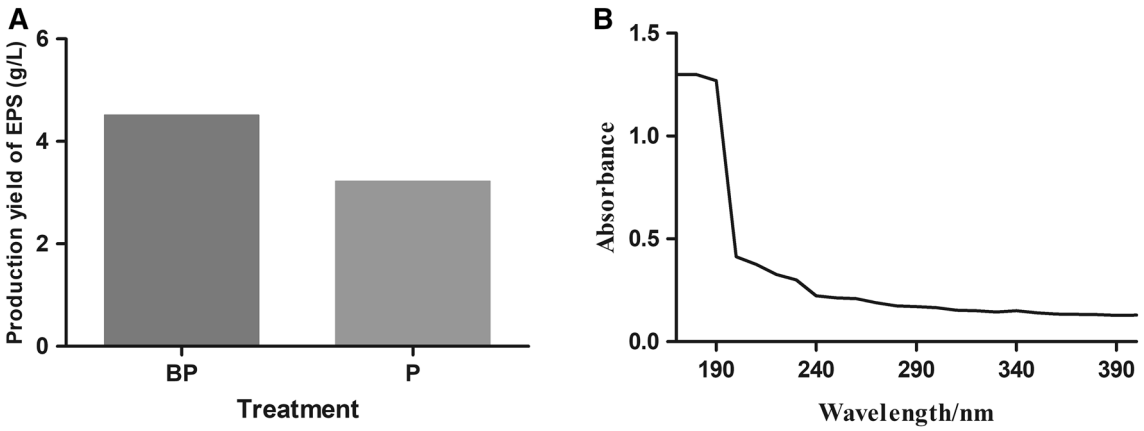

Fig. 3 Purification, and quantification of exopolysaccharides (EPS) of *Bacillus velezensis* LPL061. **a** The yield of EPS before and after purification was determined by phenol–sulfuric acid method. **b** Ultraviolet spectra of exopolysaccharides (EPS) of *Bacillus velezensis* LPL061. EPS was prepared at 0.1 mg/mL in deionized water and deionized water was used as the blank control. UV–Vis spectrophotometer was used to scan the whole wavelength of EPS in the range of 200–580 nm. The absorption peaks of protein (280 nm) and nucleic acid (260 nm) were measured. *BP* the yield of EPS before purification, *P* the yield of EPS after purification

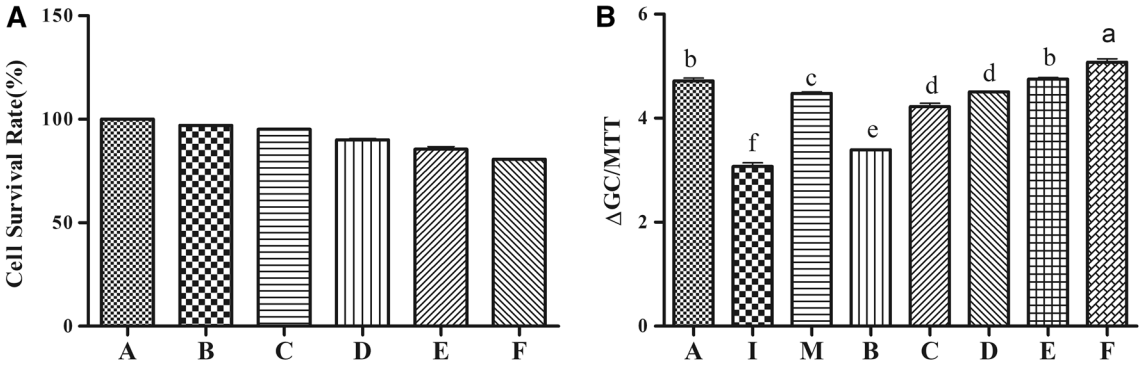

Fig. 5 Effect of EPS on glucose uptake in insulin-resistance HepG2 cells. **a** Effect of EPS on the cell viability in HepG2 cells, **b** effects of EPS on glucose consumption in Insulin-resistant HepG2 cells. Values were expressed as the mean ± SD. $n = 3$ for each group. $P < 0.05$ indicates significant different when compared with the model group. *I* insulin-resistant model with insulin (500 μg/mL), without EPS treatment; *M* insulin-resistant model with insulin and metformin (10 μg/mL) without EPS treatment; A: blank control without insulin and EPS treatment; B–F: cells treated with insulin and different concentrations of EPS (10, 20, 40, 80, 100 μg/mL)

the viability of HepG2 cells treated with 100 μg/mL EPS was significantly decreased by 19.3% ($P < 0.05$) which indicates that EPS concentrations lower than 100 μg/mL were not toxic to HepG2 cells and can be used for following experiments.

EPS increased glucose uptake of insulin-resistant HepG2 cells

To establish the insulin resistance model, HepG2 cells were treated with a wide range of insulin (5 to 5×10^4 μg/mL) for 24 h. To understand the potential roles of EPS on glucose uptake in high glucose-induced insulin-resistant HepG2 cells, the cellular glucose uptake was determined using the Glucose test kit. As shown in Fig. 5b, compared with the control group, high glucose stimulation significantly decreased the cellular glucose uptake ($P < 0.05$). This reduced glucose uptake was reversed by EPS treatment. Moreover, the MTT assay showed that EPS concentrations used in this assay were not toxic (Fig. 5a). These results indicated that EPS could increase glucose uptake in insulin-resistant HepG2 cells. The stimulatory effect of 100 μg/mL of EPS (5.078/cells) was significantly higher than that of Met controls (4.478/cells). Previously published studies have reported that probiotics and their metabolites could effectively ameliorate glucose and lipid metabolism disorders (Park et al. 2015; Yadav et al. 2008). Our results indicate that EPS from Bacillus also can effectively regulate glucose metabolism.

EPS down-regulates expression of key gluconeogenic genes in insulin-resistant HepG2 cells

In recent years, the study of liver glycometabolism key genes as a new target of diabetes treatment has attracted much attention (Kurukulasuriya et al. 2003; Bartlett et al. 2014); liver is the most important organ of glucose metabolism, which can regulate the balance of blood glucose through glycolysis, gluconeogenesis, and glycogen synthesis (Postic et al. 2004). Gluconeogenesis refers to the synthesis of glucose from the precursor of non-sugar substances such as oxaloacetic acid. Phosphoenolpyruvate carboxykinase (*PEPCK*) and 6-phosphoglucokinase (*G6Pase*) are the rate-limiting enzymes in the process of gluconeogenesis. Their expression levels determine the speed of gluconeogenesis which is key to the pathogenesis of diabetes (Lochhead et al. 2000).

To investigate the impacts of EPS on gluconeogenesis in insulin-resistant HepG2 cells, the expression of genes encoding key enzymes involved in gluconeogenesis was assayed on the transcriptional level. As shown in Fig. 6a, b, compared to insulin-resistant model with insulin (500 μg/mL), without EPS treatment group (I), when cells were treated with high concentration of EPS (100 μg/mL), the expression level of glucose-6-phosphatase (*G6Pase*) was decreased from 4.272 to 0.1929, and phosphoenolpyruvate carboxykinase (*PEPCK*) was decreased from 4.425 to 0.1587 ($P < 0.05$). It indicates that EPS inhibit gluconeogenesis.

EPS regulates the transcription level of key genes in glycogen synthesis and increases intracellular glycogen in insulin-resistant HepG2 cells

The expression of glycogen synthase kinase gene *GSK*(3)β and glycogen synthetic gene (*GS*) was measured by qRT-PCR in EPS-treated insulin-resistant HepG2 cells. As shown in Fig. 6c, d, compared with control group (A), the expression of *GSK*(3)β gene was 2.45-fold higher in the high glucose-induced insulin resistance HepG2 cells model, suggesting that enhanced *GSK*(3)β activity could inhibit glycogen

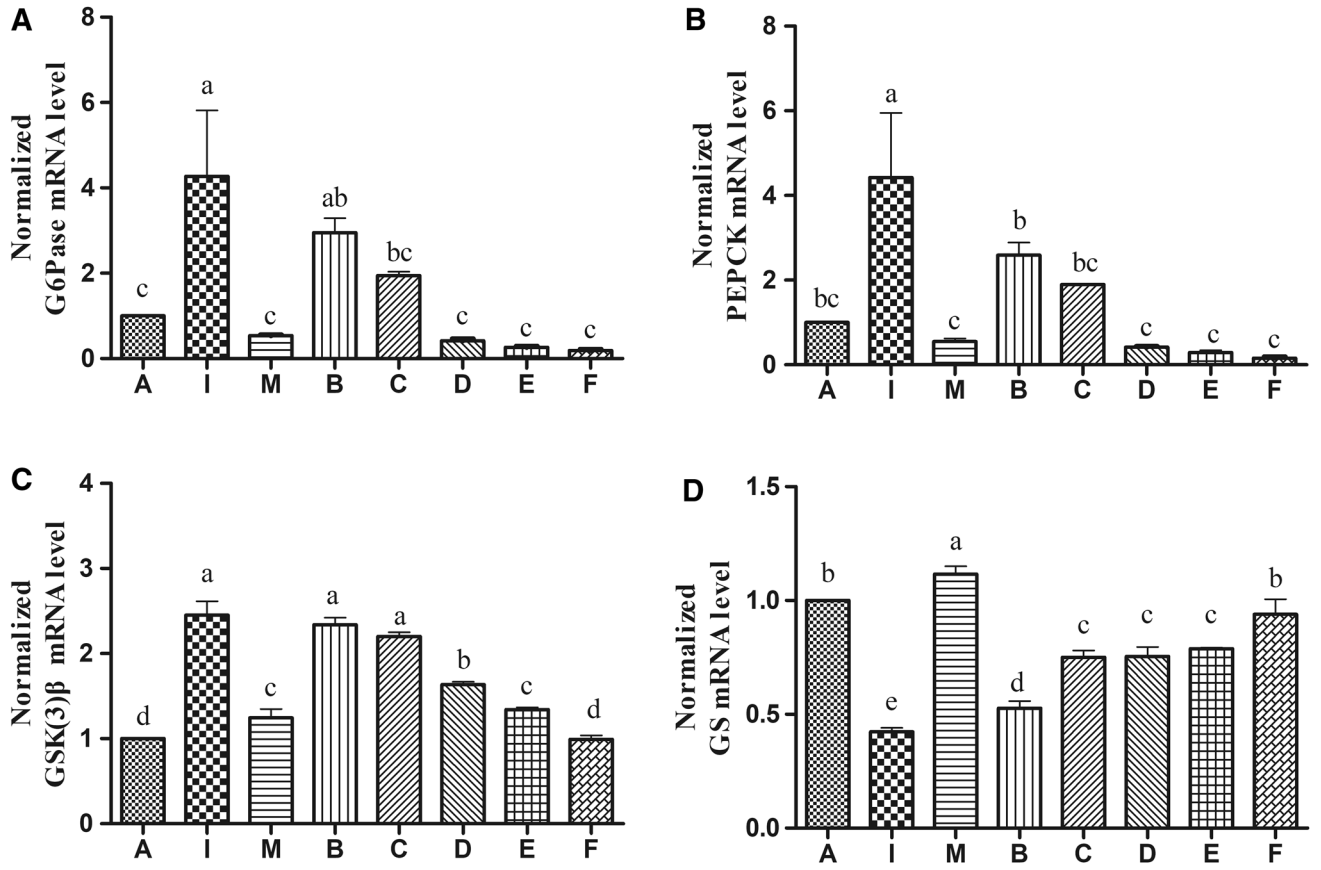

Fig. 6 Effect of EPS treatment on expressions of *G6Pase*, *PEPCK*, *GSK3*, and *GS* in insulin-resistant HepG2 cells. Cells were cultured in DMEM medium with 500 μg/mL insulin for 24 h and subsequently treated with different concentrations of EPS (0, 10, 20, 40, 80 and 100 μg/mL) for 24 h. The expressions of *G6Pase* (**a**), *PEPCK* (**b**), *GSK*(*3*)*β* (**c**) and *GS* (**d**) were determined by qRT-PCR. Values are means ± SD from three biological replicates. $P < 0.05$ indicates significant different when compared with model group. *I* insulin-resistant model with insulin (500 μg/mL), without EPS treatment; *M* insulin-resistant model with insulin and metformin (10 μg/mL) without EPS treatment; A: blank control without insulin and EPS treatment; B–F: cells with insulin and different concentrations of EPS (10, 20, 40, 80, 100 μg/mL)

synthesis. After treatment with high concentration of EPS (100 μg/mL), the expression of *GSK*(3)*β* decreased significantly (0.993), which was close to the level found in the control group. On the other hand, the expression of GS was increased by the EPS treatment in a concentration-dependent manner.

Measurement of the intracellular glycogen concentration. The results showed that the EPS increased intracellular glycogen concentration from 28.30 to 86.48% (Fig. 7) in insulin-resistant HepG2 cells after EPS treatment. Previous studies have found that hepatocytes in insulin-resistant states show impaired glucose utilization (Yin et al. 2008), which is related to changes in glucose metabolism, including glucose uptake, glycogen synthesis, and gluconeogenesis (Yang et al. 2019). Increased expression of key genes *PEPCK* and *G6Pase* on the transcription level can upregulate gluconeogenesis (Rui 2014) when insulin resistance occurs. In addition, in the insulin resistance model, *GSK*(3)*β*, the key enzyme that inhibits glycogen synthesis, is activated in the liver of insulin resistance, and *GSK*(3)*β* gene expression is up-regulated, which leads to the loss of *GS* activity and down-regulation of gene expression level; then, it led to the obstruction of glycogen synthesis. Therefore, inhibition of *GSK*(3)*β* expression has been proposed as a novel therapeutic target for type 2 diabetes by improving insulin resistance in muscle and/or liver (Kim et al. 2015). Taken together, down-regulation of *GSK*(3)*β* gene expression can lead to the increase of *GS* transcription level, thus promoting glycogen synthesis.

In this study, the treatment of EPS improved the glucose uptake of insulin-resistant HepG2 cells, and inhibited the expression of *PEPCK* and *G6Pase*, which indicated that EPS produced by LPL061 can inhibit gluconeogenesis of insulin-resistant hepatocytes. Meanwhile, EPS could promote glycogen production by decreasing the expression level of *GSK*(3)*β*. These results showed that EPS can

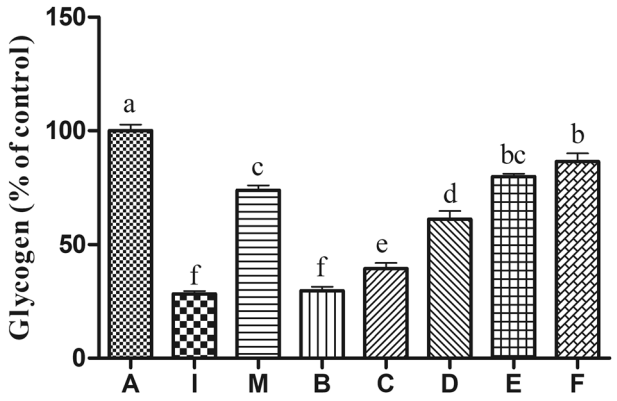

Fig. 7 Effect of EPS treatment on the levels of intracellular glycogen in insulin-resistant HepG2 cells. Values are expressed as means ± SD from three biological replicates. $P < 0.05$ indicates significant different when compared with model group. *I* insulin-resistant model with insulin (500 μg/mL), without EPS treatment; *M* insulin-resistant model with insulin and metformin (10 μg/mL) without EPS treatment; A: blank control without insulin and EPS treatment; B–F: cells with insulin and different concentrations of EPS (10, 20, 40, 80, 100 μg/mL)

improve abnormal glucose metabolism in insulin-resistant HepG2 cells by promoting glycogen synthesis and inhibiting gluconeogenesis.

Conclusions

The complete genome sequence of LPL061 facilitates our understanding of the *Bacillus* EPS biosynthesis. Further bioactivity experiments showed that EPS could reduce the glucose level by inhibiting the activity of α-glycosidase and promoting glucose consumption. Overall, this study provides new insights into the potential of secondary metabolites biosynthesis in *B. velezensis*, the physiological activity of EPS, and its hypoglycemic mechanism.

Author contributions RYW and PLL designed the experiments. RYW performed the experiments. RYW, YXQ and QS analyzed the results and wrote the manuscript. All authors read and approved the final manuscript.

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Data availability The complete genome sequence of *B. velezensis* LPL061 was deposited at GenBank under the accession number of CP042271. This strain has been deposited in China Center of Industrial Culture Collection under the accession number of CICC NO. 24192. The community metadata standards the "Minimal Information about any (X) Sequence" (MixS) was shown in Table S1.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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