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Production of glycoprotein bioflocculant from untreated rice straw by a CAZyme-rich bacterium, *Pseudomonas* sp. HP2

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ABSTRACT

It has been reported that certain biomass-degrading bacteria can produce bioflocculant through directly utilizing untreated biomass as carbon source. However, little is known about the synthesis mechanism of bioflocculant in these bacteria. In this study, a biomass-degrading bacterium *Pseudomonas* sp. HP2 showing excellent production ability of bioflocculant was isolated from the forest soil. The HP2 strain secreted alkali-thermo-tolerant CMCase and xylanase, with the maximum activities of 0.06 and 1.07 U ml^{-1}, respectively, when the untreated rice straw was used as carbon source. The maximum flocculating efficiency with the value of 92.5% was produced from untreated rice straw by HP2 strain. Component analysis showed that this bioflocculant was abundant in the amino acids and monosaccharides with the total contents of 384.9 and 478.3 mg g^{-1} dry bioflocculant, respectively. The most amino acid and monosaccharide in this bioflocculant were proline and rhamnose, which accounted for 26.5% and 33.3% of total amino acids and total monosaccharides, respectively. To explore the synthesis mechanism of bioflocculant in HP2, the genome of HP2 strain was measured by Illumina HiSeq PE150 platform. The results showed that the genome of HP2 strain possessed abundant CAZy family related genes, which may play an important role in biomass degradation and bioflocculant synthesis.

1. Introduction

Bioflocculants are a class of extracellular polymeric substances (EPS), which are secreted by microorganisms during their growth. The EPS are mainly composed by one or more of the following compounds, including glycoproteins, polysaccharides, proteins, nucleic acids (Salehizadeh et al., 2018; Mohammed and Dagang, 2019). Recently, bioflocculants have been proposed as harmless and environment-friendly compounds in various applications including wastewater treatment, drinking water purification and microalgae harvest due to their nontoxic and biodegradation properties (Gao et al., 2009; Guo et al., 2017a). However, the high production cost of bioflocculants is the major bottleneck for large-scale application in industry. To solve this problem, two strategies have been proposed by microbiologists to either screen more efficient strains or seek low-cost substrates as growth medium (Liu et al., 2015a).

Cellulose and hemicellulose from lignocellulosic biomasses are the most abundantly sustainable carbon source for bacteria growth (Salas et al., 2010). In nature, the cellulose and hemicellulose cannot be used directly by microorganisms, but some biomass-degrading bacteria can decompose them to short oligo-/monosaccharides outside the cell through releasing a series of carbohydrate-active enzymes (CAZymes), including glycoside hydrolases (GHs), carbohydrate esterases (CEs), polysaccharide lyases (PLs), glycosyltransferases (GTs), auxiliary activities (AA) and carbohydrate-binding domains (CBM) (Nguyen et al., 2018; Vincent et al., 2014). Then these small molecular sugars can be absorbed by microorganisms as an important carbon source to release energy (Pennacchio et al., 2018). Therefore, if we can screen a biomass-degrading bacterium with simultaneously possessing the ability of bioflocculants production, which will make it possible to reduce the production cost of bioflocculants through directly using lignocellulosic biomasses as substrates for fermentation.

Recent research has found that certain biomass-degrading bacteria can indeed produce bioflocculants via directly utilizing the raw material of biomass as a carbon source (Guo et al., 2017a; Liu et al., 2017). A lignocellulosic enzyme-producing bacterium *Cellulosimicrobium*

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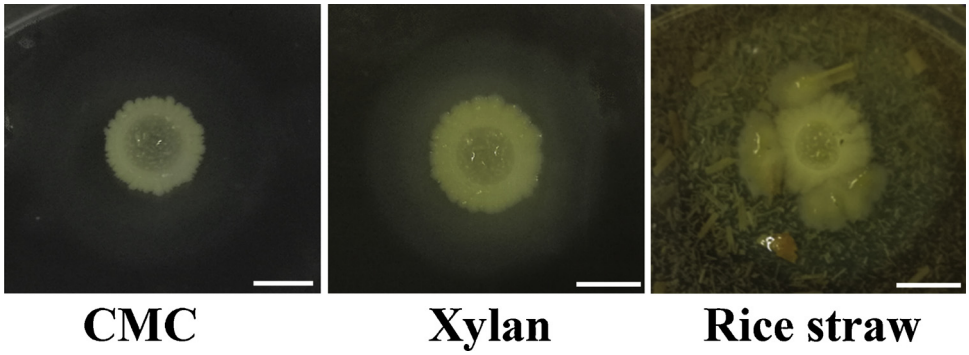

Fig. 1. Evaluation of the hydrolysis ability of *Pseudomonas* sp. HP2 using untreated rice straw, CMC, or xylan as carbon source by Gram's iodine staining. The plaques in the center of plate indicate the bacterial size, and the halo regions indicate that carbohydrate polymers are hydrolyzed. Bar = 1 cm.

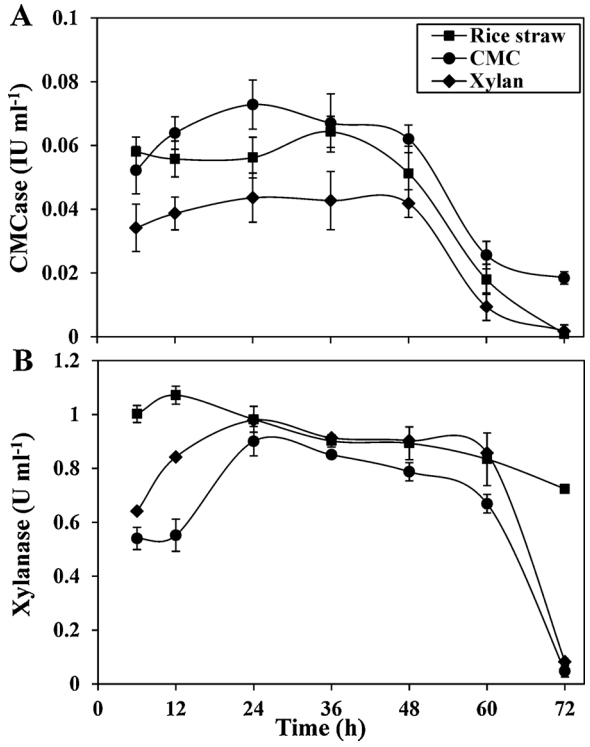

Fig. 2. CMCase and xylanase production by *Pseudomonas* sp. HP2 using 0.5% untreated rice straw, CMC and xylan as carbon source. Values represent mean ± SDs (n = 3).

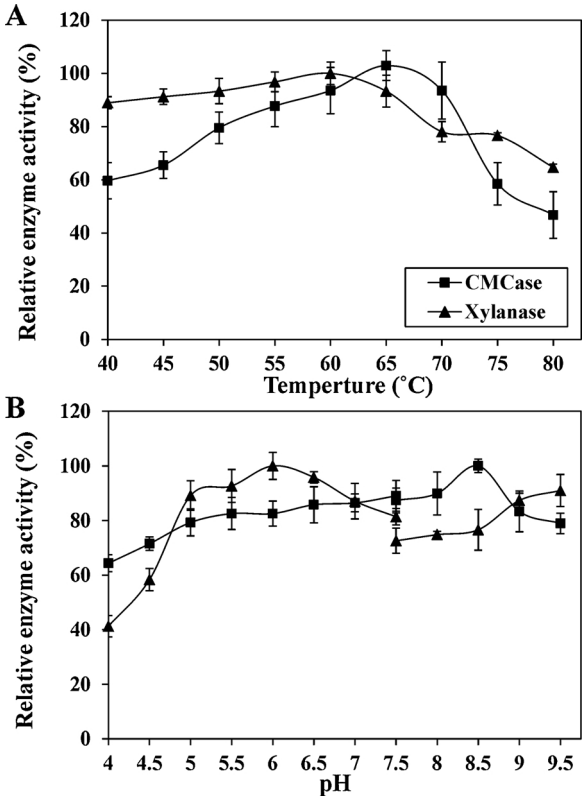

Fig. 3. Effects of different temperature (A) and pH values (B) on CMCase and xylanase activity in Pseudomonas sp. HP2. Values represent mean ± SDs (n = 3).

cellulans L804 isolated from corn farmland soil produce a polysaccharide bioflocculant through using 2.0% dry corn stover as carbon source (Liu et al., 2015b). An alkaliphilic strain *Bacillus agaradhaerens* C9 can efficiently convert untreated rice bran into bioflocculant through secreting alkali-tolerant xylanase and cellulase (Liu et al., 2017). Recently, we found certain bacteria belonging to *Pseudomonas* spp. can also efficiently convert various untreated biomasses into bioflocculants through secreting CAZymes (Guo et al., 2017a, 2018). However, the biosynthesis pathway and regulation mechanism of bioflocculant synthesis by these biomass-degrading bacteria are scarce due to the lack knowledge about the genome information of them. In this study, a CMCase and xylanase-producing bacterium *Pseudomonas* sp. HP2 with the potential ability to synthesis bioflocculant was isolated from forest soil. Then the enzymatic characteristics of CMCase and xylanse were analyzed and the bioflocculants' production and composition were measured. Subsequently, the genome sequences of *Pseudomonas* sp. HP2 was determined by using the high-throughput sequencing technology, and the CAZy family genes and the bioflocculant synthesis pathway were initially identified.

2. Materials and methods

2.1. Isolation of a bioflocculant-producing bacterium

The bioflocculant-producing bacteria were isolated from forest soil (Ningbo, Zhejiang, China) according to the method of our previous study (Guo et al., 2017a, 2018). Briefly, about 1.0 g soil samples were inoculated in 10 ml of sterile phosphate buffer saline (0.27 g L^{-1} KH_2PO_4, 1.42 g L^{-1} Na_2HPO_4, 8.0 g L^{-1} NaCl and 0.2 g L^{-1} KCl, pH 7.4) and boiled for 5 min. The mixture was shaken at 30 °C and 180 rpm for 10 min. Then the 1 ml of above culture was added into 50 ml mineral salt medium (0.1 g L^{-1} $FeC_6H_5O_7$, 3.24 g L^{-1} $MgSO_4$, 1.8 g L^{-1} $CaCl_2$, 0.55 g L^{-1} KCl, 0.16 g L^{-1} $NaHCO_3$, 0.08 g L^{-1} KBr, 0.5 g L^{-1} yeast extract, 1.5 g L^{-1} peptone, pH 7.4) containing 0.5% rice straw biomass at 30 °C

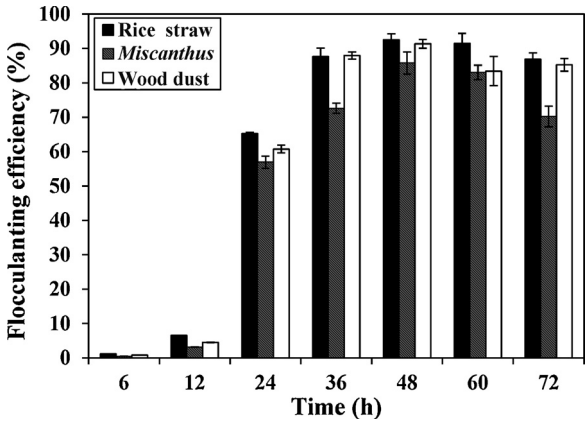

Fig. 4. Biofocculants' production along with time by *Pseudomonas* sp. HP2 using 0.5% untreated rice straw, *Miscanthus* and wood dust biomass as carbon source. Values represent mean ± SDs (n = 3).

with shaking at 180 rpm for 2 d. After 3 days of incubation, the fermentation broths became transparent and viscous, and the rice straw biomass was compact and settled at the bottom of flask. To isolate the bioflocculant-producing bacteria, the supernatant was removed carefully and the solid residues were separated and resuspended in phosphate buffer saline by vortex for 30 s. Then the solution was spread on the mineral salt medium containing 0.5% rice straw biomass and 1.5% agar by the standard serial dilution plate method. After 3 days of incubation at 30 °C, all colonies were purified and cultured in 50 ml of the mineral salt medium containing 0.5% rice straw. The fermentation broths were collected and measure their biomass degradation ability and flocculating efficiency. Finally, a biomass-degrading strain HP2 with simultaneously showing high flocculating activity was selected for further study.

2.2. Biomass degradation and enzyme activity assay

The biomass degradation ability was measured on an agar plate using rice straw biomass, carboxymethyl cellulose (CMC) or beechwood xylan as the sole carbon source according to our previous study (Guo et al., 2017b). Briefly, 5 µL overnight-grown bacterial cultures were dropped on an agar plate containing above mineral salt medium, 1.5% agar, and 0.5% rice straw, CMC or xylan. After 3 days of incubation at 30 °C, the biomass degradation abilities were visualized according to the size of halos by staining the plates with Gram's iodine solution for 3–5 min.

Table 2
Summary of sequences analysis of HP2 genome.

Description	Number
Before trimming	
Raw data (Mb)	929
After trimming	
Clean data (Mb)	800
GC content (%)	68.62
Q20 percentage (%)	97.87
After assembly	
Gene number	3878
Gene length (bp)	3,946,071
Gene average length (bp)	1018
NR annotated (%)	95.9
KEGG annotated (%)	94.5
COG annotated (%)	80.0
GO annotated (%)	66.3
SwissProt annotated (%)	43.0
Annotated in all databases (%)	29.3
Annotated in at least one databases (%)	96.2
Secretory proteins	490
CAZymes	172

The strain HP2 was cultured in above mineral salt medium containing rice straw biomass, carboxymethyl cellulose (CMC) or beechwood xylan as the sole carbon source at 30 °C with agitation at 180 rpm. The fermentation broths for each medium were harvested at 6, 12, 24, 36, 48, 60 and 72 h by centrifuging at 12,000 g for 3 min. The supernatants were used to measure enzyme activity. The activities of CMCase and xylanase were determined by detecting the release of reducing sugars from CMC and beechwood xylan using a microplate spectrophotometer (Epoch, BioTek Instruments, Inc., Vermont, USA) according to the description of Guo et al (2017b). The reducing sugar content was measured by using the 3,5-dinitrosalicylic acid (DNS) method.

2.3. Effects of pH and temperature on CMCase and xylanase activity

The fermentation broths harvested at 12 h were taken to test the effects of temperature and pH on CMCase and xylanase activities. The temperature was set in the range of 40 °C to 80 °C at 5 °C intervals, and the pH was surveyed ranging from 4.0 to 9.5 at intervals of 0.5. The buffer solutions used were as follows: pH 4.0 to 7.5 in 0.05 M citrate buffer and pH 8.5 to 9.5 in 0.05 M Tris − HCl buffer.

Table 1
The amino acid and monosaccharide composition of bioflocculant produced by HP2.

Amino acids	Content (mg/g bioflocculant)	% of total amino acids	Monosaccharides	Content (mg/g bioflocculant)	% of total monosaccharides
Proline	101.88	26.5	Rhamnose	159.05	33.3
Aspartate	41.96	10.9	Glucose	118.73	24.8
Glutamate	30.32	7.9	Mannose	96.75	20.2
Glycine	24.90	6.5	Glucuronic acid	49.85	10.4
Alanine	24.60	6.4	Galactose	29.45	6.2
Arginine	24.14	6.3	Ribose	14.13	3.0
Leucine	23.06	6.0	Fucose	4.10	0.9
Valine	17.40	4.5	Xylose	3.85	0.8
Serine	15.84	4.1	Arabinose	1.80	0.4
Threonine	15.44	4.0	Galacturonic acid	0.63	0.1
Phenylalanine	15.30	4.0	Total	478.33	
Lysine	14.12	3.7			
Tyrosine	13.22	3.4			
Isoleucine	12.90	3.4			
Cystine	4.60	1.2			
Methionine	4.44	1.2			
Histidine	0.78	0.2			
Total	384.9				

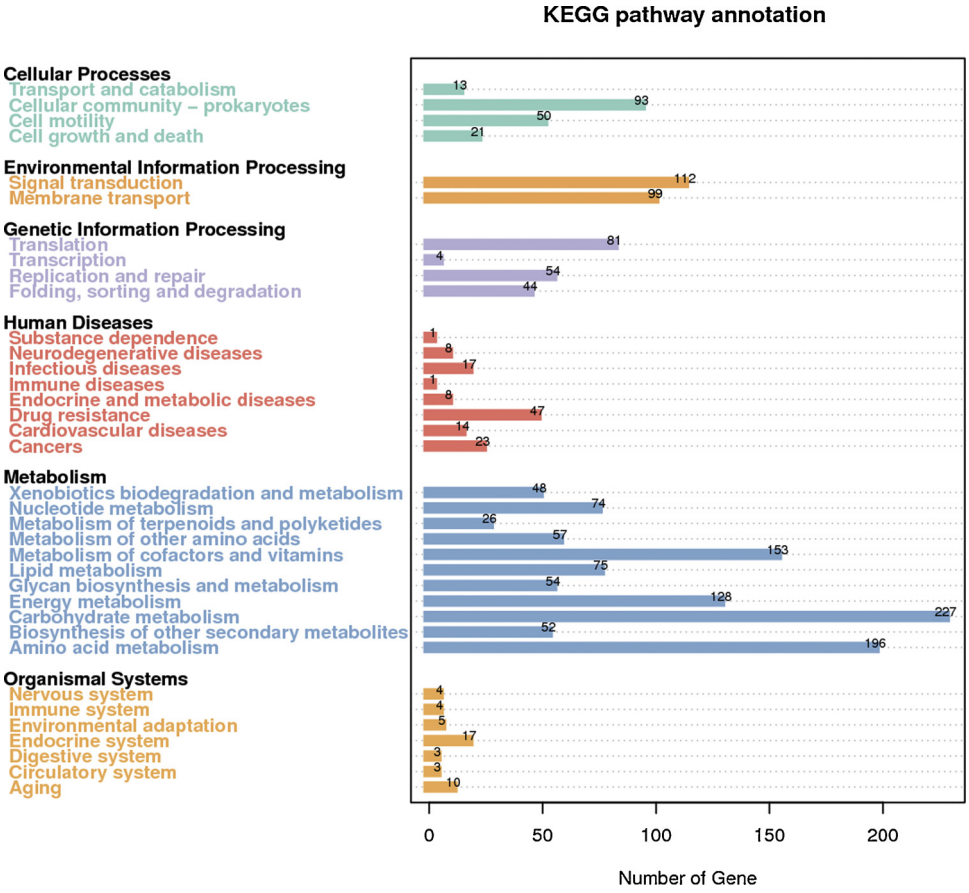

Fig. 5. KEGG pathway annotation of assembled genes.

2.4. Determination of flocculating efficiency

The flocculating efficiency was determined by using kaolin clay as substrate according to the description of Guo et al. (2017a). Briefly, 100 µl of supernatant and 1.0 ml of 1.0% (w/v) $CaCl_2$ were mixed with 40 ml of 0.4% (w/v) kaolin clay suspension in a 50 ml glass-beaker, and the solution was left to stand for 1 min after agitating at 100 rpm for 2 min at room temperature. The optical density (OD) of the upper phase was measured at 550 nm using a microplate spectrophotometer (Epoch, Bio Tek Instruments, Inc., Vermont, USA). The flocculating efficiency was calculated as follows: Flocculating efficiency (%) = (A − B)/A × 100, where A and B are the optical densities of the control and the samples at 550 nm, respectively.

2.5. Effects of different biomass substrates on bioflocculant production

To evaluate the effects of different biomass substrates on bioflocculants production of HP2 strain, the strain was cultured in the mineral salt medium including 0.5% rice straw, *Miscanthus* and wood dust, respectively. The samples were collected by centrifuging at 12,000 rpm for 3 min after incubation of 12, 24, 36, 48, 60 and 72 h, and the supernatant was used to test the bioflocculant production.

2.6. Bioflocculant extraction and composition determination

The bioflocculants produced by HP2 were extracted according to the description of Guo et al. (2017a), with some minor modification. Briefly, HP2 strain was cultured using 0.5% rice straw biomass as carbon source at 30 °C with shaking at 180 rpm. After 2 days of fermentation, the culture was stored at 4 °C for 12 h to settle the suspended solids. The supernatants were carefully collected and mixed with two volumes of pre-cooling ethanol. Then the solids were harvested by centrifugation at 5500 g for 8 min, washed with 75% pre-cooling ethanol for three times and finally lyophilized to get dry bioflocculants. The extracted bioflocculants were frozen at -80 °C prior to analyze the composition. The monosaccharide and amino acid compositions were analyzed by using HPLC according to the methods of Dai et al. (2010) and Chaisorn et al. (2016), respectively.

2.7. Genome sequencing and analysis

Genomic DNA was extracted with a Bacteria DNA kit following the manufacturer's instructions. The harvested DNA was quantified by a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) and the DNA quality was assayed with Agilent Bioanalyzer (Agilent, Santa Clara, CA, USA). Whole-genome sequencing was performed on the Illumina HiSeq PE150 platform. A-tailed, ligated to paired-end adaptors and PCR amplified with a 350 bp insert was used for the library construction at the Beijing Novogene Bioinformatics Technology Co., Ltd. Raw data produced by Illumina HiSeq PE150 platform were initially processed to get clean data via filtering adapter reads and low-quality reads. Then, all good quality paired reads were assembled by using the SOAP denovo into a number of scaffolds (112). All assembled transcripts were subjected to seven public databases, including GO (Gene Ontology), KEGG (Kyoto Encyclopedia of Genes and Genomes), COG (Clusters of Orthologous Groups), NR (Non-Redundant Protein Database databases), TCDB (Transporter Classification Database), and Swiss-Prot. A whole genome Blast search (E-value less than 1e-5, minimal alignment length percentage larger than 40%) was performed against above seven databases. Carbohydrate-Active enzymes (CAZymes) were predicted by the Carbohydrate-Active Enzymes Database (Levasseur et al., 2013).

Table 3
Statistics of putative plant cell wall degradation related enzymes from the genome sequence of HP2.

CAZy family	Known activity	Gene No.	CAZy family	Known activity	Gene No.
Cellulases family					
GH3	β-glucosidase/β-D-xylosidase,	7	GH31	glucosidase	2
GH5	endoglucanase	1	GH94	cellobiose phosphorylase,	1
GH9	endoglucanase	1			
Hemicellulases family					
Xylose related enzymes					
GH10	endo-1,4-beta-xylanase	1	CE1	acetyl xylan esterase	1
GH11	endo-1,4-beta-xylanase	1	CE4	acetyl xylan esterase	2
GH43	β-xylosidase	13	CBM13	binding to xylan	2
GH67	alpha-glucuronidase	1	CBM35	binding to xylan	1
Mannose related enzymes					
GH2	β-mannosidase	7	GH92	α-mannosidase	3
GH47	α-mannosidase	1	GH125	exo-α-1,6-mannosidase	1
Galactose related enzymes					
GH27	α-galactosidase	3	GH53	endo-β-1,4-galactanase	2
GH35	β-galactosidas	3	GH125	α-1,6-mannosidase	1
GH36	α-galactosidase	2	CMB32	binding to galactose	5
GH42	β-galactosidase	1			
Arabinose related enzymes					
GH51	α-L-arabinofuranosidase	1	GH127	β-L-arabinofuranosidase	1
GH121	β-L-arabinobiosidase	1			
Pectinases family					
PL1	pectate lyase	2	GH115	xylan α-1,2-glucuronidase	1
PL10	pectate lyase	1	CE8	pectin methylesterase	1
GH28	polygalacturonase	1	CE12	pectin acetylesterase	2
Auxiliary activity					
AA6	1,4-benzoquinone reductase	1			

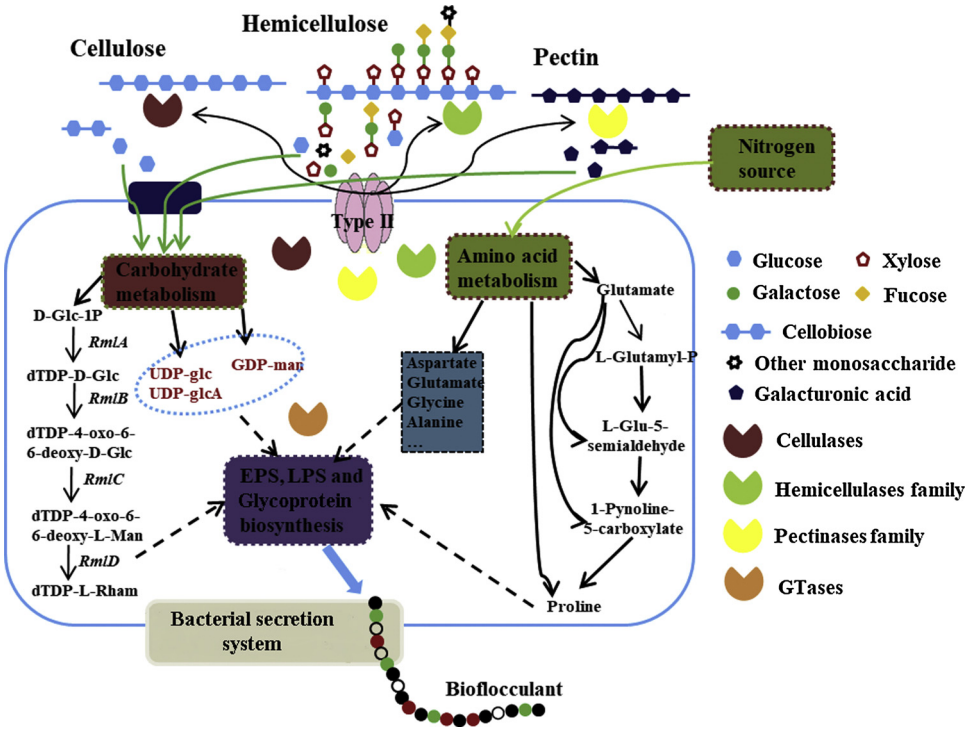

Fig. 6. A hypothetical model for the bioflocculant synthesis by *Pseudomonas* sp. HP2.

3. Results and discussion

3.1. Biomass degradation ability and lignocellulolytic enzyme activity of HP2

In total 15 different bacterial strains were isolated according to the bacterial color and size from the forest soils. Then the 15 strains were further cultured in 50 ml mineral salt medium containing 0.5% rice straw biomass, and one isolate (HP2) simultaneously showing the production of lignocellulolytic enzyme and flocculant efficiency was screened to perform the next experiments. The 16S rRNA gene sequence of HP2 had 99.9% similarity with that of *Pseudomonas* sp., and thus this strain was finally identified as *Pseudomonas* sp. HP2 according to the morphological and phylogenetic characteristics and the 16S rRNA gene sequence of this strain. To evaluate its biomass degradation ability, the strain HP2 was grown in the plate containing 0.5% CMC, xylan and rice straw biomass. After staining using Gram's iodine, the strain showed large halos in CMC, xylan and rice straw biomass plates, indicating that the strain HP2 had a good biomass degradation ability (Fig. 1). Gram's iodine has been reported to form a bluish-black complex with carbohydrates but not with their hydrolysates (Ramesh Chand et al., 2008). The enzyme ability assay found that the HP2 can simultaneously secret CMCase and xylanase with the maximum abilities of 0.06 and 1.07 U ml^{-1}, respectively, when the rice straw was used as the sole carbon source (Fig. 2), indicating the strain HP2 can degrade the biomass though secreting CMCase and xylanase (Woo et al., 2014; Guo et al., 2018). In addition, the optimal temperatures of CMCase and xylanase were 65 °C and 60 °C, respectively, and correspondingly the optimal pH of them were 8.5 and 6.0, respectively (Fig. 3). The optimal temperature and pH of CMCase were much higher than most bacterial CMCases, which have an optimal temperature and pH range of 40–55 °C and 4.8–7.2, respectively (Farinas et al., 2010; Sadhu and Maiti, 2013). These results indicated that HP2 is a thermo-alkaliphilic biomass degradation strain.

3.2. Bioflocculant production and its composition characteristics

Bioflocculants produced by bacteria have been widely reported to apply in various wastewater treatment, heavy metal removal, drinking water purification and microalgae harvest (More et al., 2014; Zhao et al., 2017; Guo et al., 2018). However, the high production cost of them severely hinders their industrial application on scale-up. Recently, it has been reported that some bacteria like *Pseudomonas* sp. GO2 (Guo et al., 2017a), *Bacillus agaradhaerens* C9 (Liu et al., 2017) and *Cellulosimicrobium cellulans* L804 (Liu et al., 2015b) can produce polysaccharide bioflocculants via directly using various biomass as sole carbon source, and thus reducing the production cost of bioflocculants. In this study, a biomass degrading-bacterium HP2 was used to produce bioflocculants by using three kinds of lignocellulosic biomasses (rice straw, *Miscanthus* and wood dust) as sole carbon source. The flocculating efficiencies were rapidly increased after 24 h of incubation in the presence of three biomasses and all reached to the peak after 48 h of incubation (Fig. 4). The highest flocculating efficiency of HP2 was 92.5% when 0.5% rice straw was used as the sole carbon source, and the maximum value was higher than that of *Bacillus agaradhaerens* C9 (87.2%) and *Cellulosimicrobium cellulans* L804 (90.9%) when the 2.0% rice bran and corn stover were used as the sole carbon source, respectively (Liu et al., 2015b; 2017). In addition, the bioflocculant production of HP2 strain was 1.75 g L^{-1} when 0.5% untreated rice straw was used as carbon source. The yield of bioflocculant was lower than those in some other bacterial strains, such as 2.41 g L^{-1} in *Serratia ficaria*, 3.5 g L^{-1} in *Bacillus velezensis*, and 5.0 g L^{-1} in *Achromobacter xylosoxidans*, while it was higher than those in *Bacillus amyloliquefaciens, Bacillus licheniformis* and *Enterobacter aerogenes*, with the yields of 0.08, 0.2 and 1.3 g L^{-1}, respectively (Mohammed and Dagang, 2019).

To understand the chemical composition of the bioflocculant produced by HP2, the purified bioflocculant was used to determine the amino acid and monosaccharide compositions. The results showed that this bioflocculant was rich in the amino acids and monosaccharides with the total contents of 384.9 and 478.3 mg g^{-1} dry bioflocculant (Table 1). The amino acids were mainly composed of proline, aspartate, glutamate, glycine, alanine, arginine and leucine with the contents of 101.9, 42.0, 30.3, 24.9, 24.6 and 24.1 mg g^{-1} dry bioflocculant, respectively, and the monosaccharides mainly consisted of rhamnose, glucose, mannose, glucuronic acid and galactose, with the contents of 159.1, 118.7, 96.8, 49.9 and 29.5 mg g^{-1} dry bioflocculant, respectively (Table 1). This is similar with that the amino acids of protein in bioflocculant mainly included glutamic acid, alanine acid and aspartic acid, the total proportion of which was ranged from 25 to 42% (Chaisorn et al., 2016; Guo et al., 2013); while the main backbone of the polysaccharides was consisted of rhamnose, glucose, and galactose and with uronic acids, acetyl amino sugars, and proteins as the side chains (Yuan et al., 2010). These side chains provide abundant carboxyl, hydroxyl and amine groups, and thus increasing the number of effective sites for particles' adsorption (Maliehe et al., 2016). In addition, the proline and glutamate were reported to closely associated with various biotic and abiotic stresses (Liang et al., 2013; Mahipant et al., 2017), and the amino acids of proline and glutamate produced by HP2 strain accounted for 34.4% of total amino acids, indicating that HP2 strain may have a strong ability to growth under stress conditions.

3.3. The genome properties of HP2 and CAZy family genes

To investigate the metabolic pathway of bioflocculant produced by the biomass-degrading bacterium, the whole genome of HP2 strain was sequenced on an on the Illumina HiSeq PE150 platform. The genome sequence was deposited to the NCBI and the accession number was given as MK646023. According to the Illumina's sequencing, a total of 929 Mb raw data were generated. After trimming the adaptor sequences and removing the low-quality reads, 800 Mb clean data were obtained with a 68.6% GC content and 97.8% Q20 percentage. Then the clean data were assembled 3878 genes with the total length of 3,946,071 bp and the average gene length of 1018 bp. The gene length distribution is shown in Fig. S1. For validation of assembled genes, all gene sequences were annotated using BLAST based on sequence similarity searches against public databases, including the NR, KEGG, COG, GO and SwissProt databases. The results showed that the percentages of annotated genes by NR, KEGG, COG, GO and SwissProt databases were 95.9, 94.5, 80.0, 66.3 and 43.0%, respectively. A total of 96.2% genes were annotated in at least one database, and 29.3% genes were annotated in all databases (Table 2). According to the KEGG database, the genes were grouped into six specific pathways, including cellular processes, environmental information processing, genetic information processing, human diseases, metabolism and organismal systems. The most genes were found in the metabolic pathways, which were mainly involved in carbohydrate metabolism (5.9%) and amino acid metabolism (5.1%) (Fig. 5). The abundant genes of carbohydrate metabolism and amino acid metabolism may be responsible for the production of polysaccharide and protein (Wu et al., 2015; Fu et al., 2016).

The CAZy family genes in genome are directly related to the number, function and activity of CAZymes (Cantarel et al., 2009). CAZymes mainly including GHs, GTs, CEs, PLs, AAs and CBMs, have been known to play important roles in synthesizing, degrading and modifying all the carbohydrates on Earth (Yin et al., 2012). The GHs, CEs, PLs and AAs are mainly involved in the degradation of various lignocellulosic biomass, like cellulose, hemicellulose and starch (Terrapon et al., 2017; Park et al., 2018), and CBMs can increase their catalysis rate of carbohydrate complexes by bringing the biocatalyst into intimate and prolonged association with its recalcitrant substrate (Shoseyov et al., 2006). GTs are related to the biosynthesis of oligosaccharides, polysaccharides and glycoconjugates through catalyzing the formation of glycosidic linkages to form glycosides (Park et al.,

2018). In this study, the genome sequence of *Pseudomonas* sp. HP2 possessed 172 CAZy family genes, which mainly consisted of 127 polysaccharide degradation related genes (96 GHs, 22 CBMs, 9 CEs, 3 PLs and 1 AA) and 45 polysaccharide synthesis related genes (Table 2 and Table S1). Among the 127 polysaccharide degradation related genes, 75 genes were directly related to the degradation of plant cell wall, which can be were further divided into four groups according to their degradation function (Table 3). The first group including twelve GHs genes, like *GH3*, *GH5*, *GH9*, *GH31* and *GH94*, were closely related to cellulose degradation. Forty-nine genes involved in hemicellulose degradation, including 22 xylose related genes, 12 mannose related genes, 12 galactose related genes and 3 arabinose related genes, were classified as the second group. The third group included eight genes for coding pectinases, such as *PL1*, *PL10*, *GH28*, *GH115*, *CE8* and *CE12*. The last group including 1 gene (AA6) was associated with auxiliary activity, which is an essential component for lignin breakdown (Levasseur et al., 2013). The abundant GHs, CEs and PLs in the genome of HP2 strain were also corresponding to the better biomass degradation, and CMCase and xylanase activities (Figs. 1 and 2). The remaining 52 polysaccharide degradation related genes were mainly associated with lysozyme, α-amylase, chitinase. In addition, the genome sequence of *Pseudomonas* sp. HP2 contained 490 secretory proteins, which are responsible for secreting the lignocellulosic enzymes and proteins out of the cells (Tuveng et al., 2019). These results indicated that the HP2 strain can secret various lignocellulosic enzymes to hydrolyze biomass, and the hydrolysates can be utilized by HP2 as carbon source for growth.

3.4. Glycoprotein bioflocculant synthesis prediction

Based on the composition characteristics of bioflocculant and genome properties of HP2 strain, the possible bioflocculant synthesis pathway of HP2 using biomass as carbon source was concluded as follows (Fig. 6): first, the plant cell wall components of biomass like cellulose, hemicellulose and pectin were hydrolyzed to monosaccharides by the CAZmes secreted by HP2. Second, the monosaccharides like glucose, xylose, mannose, galactose, etc. were used as substrates for the synthesis of various nucleotide sugar precursors, such as UDP-glucose, UDP-glucuronic acid and GDP-mannose, etc. through carbohydrate metabolic pathway (Ruffing and Chen, 2006). A whole L-Rhamnose biosynthetic pathway, which is ubiquitous and highly conserved in both Gram-positive and Gram-negative bacteria (Giraud and Naismith, 2000), was found in the genome of HP2 (Fig. 6 and Fig.S2), indicating that the high rhamnose content in the bioflocculant was highly dependent on this pathway. In addition, the nitrogen source of culture medium was used as a substrate for synthesizing a variety of amino acids, such as proline, aspartate, glutamate, and glycine, etc., through amino acid metabolic pathway (Wu, 2009). Third, the sugar precursors were used to form the repeat units under the action of various glycosyltransferases, such as UDP-glycosyltransferases (GT1, GT5, GT26, GT41 and GT70), GDP glycosyltransferase (GT94), and lipopolysaccharide synthesis related enzymes (GT8, GT19 and GT83) (Table S1). Then the repeated units were assembled to form exopolysaccharides or were polymerized with related proteins or lipids to produce glycoproteins or lipopolysaccharides. Finally, these exopolysaccharides, glycoproteins and lipopolysaccharides were transported from the cytoplasm to the extracellular environment as a bioflocculant.

4. Conclusions

Pseudomonas sp. HP2 strain showing excellent biomass degradation ability can simultaneously produce glycoprotein bioflocculant by directly using biomass as carbon source. The alkali-thermo- tolerant CMCase and xylanase with the maximum activities of 0.06 and 1.07 U ml^{-1}, respectively, were secreted by the HP2 strain, when the untreated rice straw was used as sole carbon source. Then the hydrolysates of untreated rice straw were used by HP2 strain to produce glycoprotein bioflocculant, which was mainly composed by 38.5% amino acids and 47.8% monosaccharides. To investigate the molecular mechanism of bioflocculant synthesis from biomass in HP2 strain, we analyzed the genome of HP2 and predicted the biosynthesis pathway of bioflocculant. The results showed that the genome of HP2 consisted of 172 CAZy family genes including 127 polysaccharide degradation related genes and 45 polysaccharide synthesis related genes, further indicating that the HP2 strain can secret various lignocellulolytic enzymes to degrade biomass, and then use the hydrolysates as nutrition to produce bioflocculant.

The author(s) have no conflicts of interest to declare.

Declaration of Competing Interest

The author(s) have no conflicts of interest to declare.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jbiotec.2019.10.011.

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