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METAGENOMIC ANALYSIS OF MICROBIAL CONSORTIUM GF-20 IN CORN STOVER DEGRADATION AT LOW TEMPERATURE

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Highlights

- The characteristics degradation of corn stover at low temperatures by GF-20 were assessed.
- The dominant microbials, functional genes, metabolisms and species diversity of GF-20 were analyzed using Metagenomics.
- The systematic overview of lignocellulose degradation by GF-20 was analyzed.
- The findings serve as a reference for rational utilization of GF-20.

Abstract. In our previous work, a microbial consortium GF-20 (Qinggeer et al., 2016) was enriched from compost habitats and adapted to efficiently and stably degrade corn stover under low temperatures. While the main microorganism and degradation-related functions and degradation-related coding enzyme genes of GF-20 were not clear. Therefore, the current study used the metagenomic to decipher the systematic and functional contexts within such microbial consortium under low temperatures. The results showed that the dominant functional microbials in GF-20 consortium were bacteria. The dominant phylums in GF-20 consortium were *Proteobacteria* (62.84%) and *Bacteroidetes* (10.24%). The dominant genus was *Pseudomonas* (50.84%), followed by *Dysgonomonas* (5.86%), *Achromobacter* (4.94%), *Stenotrophomonas* (3.67%) and *Flavobacterium* (2.04%). The metabolism was mainly composed of carbohydrate metabolism and amino acid metabolism, and included signal transduction, cell transport and other metabolic modes. The functional genes encoded were mainly distributed in glycosidolytic enzyme genes, and the functional enzymes were β-glucosidase, acetyl-CoA, pyruvate dehydrogenase and galactosidase. The GF-20 microbial consortium degraded the cellulose in corn stover primarily by β-glucosidase and endoglucanase, which were produced by 12 genera of microorganisms. The hemicellulose synergistic effect was produced by 15 genera of microorganisms including xylanase, xyloglucanase, mannolanase and branching enzyme.

Keywords: metagenomic, microbial consortium, low temperature, corn stover degradation.

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Introduction

Crop residue is an abundant renewable resource that can be transformed into useable fertilizer for cultivated land in order to reduce the use of chemical fertilizer. According to the United Nations Environment Program, global annual yield of crop wastes is approximately 20 billion, of which 2–3 billion tons is from corn stover (Kubicek et al., 2009). Large amounts of straw are discarded or burned, which not only wastes resources but also pollutes the environment. Returning corn stover to the field is one effective way to improve the stover utilization rate. However, low-temperatures and dry climate conditions in northern regions limit the effectiveness of returning corn stover to the field. One way to promote the rapid and efficient decomposition of straw is through the application of a microbial agent.

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Due to limitations of pure culture technology, it is hard to obtain pure cultured microorganisms (Zinger et al., 2012; Fierer & Lennon, 2011). Therefore, the lignocellulose-degrading microorganisms screened from the culturable microorganisms are from a single species and exhibit high repetition rate, insufficient novelty and low degradation performance, which seriously hinder the application of microbial degradation of lignocellulosic (Gilbert & Dupont, 2011; Tringe & Rubin, 2005; Hugenholtz & Tyson, 2008). The recent development of metagenomics (Handelsman et al., 1998) avoids the traditional process of microbial isolation and culture, and instead, directly studies genomic DNA in specific environments and analyzes microbial community diversity with the help of new generation sequencing technology and bioinformatics platforms (Sim & Kim, 2015; Scholz et al., 2012). The application of metagenomic approaches has been proven to be very effective in unveiling biodegradative potentials of microbial consortia and recovering genes encoding novel enzymes with improved properties, as shown in a recent discovery of highly halotolerant and ionic liquid-resistant cellulases using a metagenomics guided strategy (Yang et al., 2016).

Microorganisms are the main producers of enzymes that decompose cellulose and hemicelluloses in soils, which makes them the most important players in plant biomass decomposition (Koeck et al., 2014; Himmel et al., 2010). Numerous studies about the large diversity in lignocellulolytic microbial communities from various growth environments have used metagenomics, such as those regarding compost (Allgaier et al., 2010), forest soil (Jiménez et al., 2014), poplar wood chips (Van der Lelie et al., 2012), sugarcane bagasse (Mhuantong et al., 2015), termite hindgut (Warnecke et al., 2007), microbial consortium (Zhu et al., 2016) and biogas reactor (Stolze et al., 2015). However, most of these studies focused on conditions under normal or high temperatures, and since microbial biological activity lowers or weakens under low temperature environments, it would be important and meaningful to study degradation acceleration effects of low temperature resistant microorganisms under low temperature condition in northern areas of corn stover.

1. Materials and methods

1.1. Sample information

The microbial consortium GF-20 used in this study was obtained through low-temperature acclimation in low-temperature in our lab in 2016. The microbial consortium GF-20 had stable corn stover decomposing activity, it could significantly promote corn stover decomposition-relating capacity at low temperature (4 °C–10 °C) (Qinggeer et al., 2016).

1.2. Sample collection

GF-20 (10% v/v) was inoculated in triplicate into corn stover cotaining medium (2 g $L^{-1}(NH_4)_2SO_4$, 1 g L^{-1} K_2HPO_4, 0.5 g L^{-1} $MgSO_4{\cdot}7H_2O$, 2 g L^{-1} $CaCO_3$, 2 g L^{-1} NaCl, 8 g L^{-1} corn stover) and incubated under static conditions at 10 °C for 15 d. At the end of the cultivation, 1 g thallus were collected by centrifugation at 20 000 r/min for 10 min at 4 °C under aseptic conditions. Three technical replications were used for experiment.

1.3. Analysis of FPA, Xylanase and Laccase activity in the microbial consortium GF-20

To estimate enzyme activities of FPA, Xylanase and Laccase activity, the microbial consortium (5%, v/v) inoculated on corn stover cotaining medium at 15 °C under shaking conditions cultivated 30 d. The activities of FPA, Xylanase and Laccase were assayed using the techniques as previous described (Qinggeer et al., 2016; Wolfenden & Willson, 1982).

1.3.1. DNA extraction, library construction, and metagenomic sequencing

Total genomic DNA was extracted from 1g thallus of GF-20 using the MoBio PowerSoil DNA Isolation Kit (QIAGEN, U.S.) according to the method of Marmur (1961). Concentration and purity of extracted DNA was determined with NanoDrop2000 (Thermo Fisher Scientific). DNA extract quality was checked on 1% agarose gel.

DNA extract was fragmented to an average size of about 300 bp using Covaris M220 (Gene Company Limited, China) for paired-end library construction which using NEXTFLEX® Rapid DNA-Seq (Bioo Scientific, USA). Adapters containing the full complement of sequencing primer hybridization sites were ligated to the blunt-end of fragments. Paired-end sequencing was performed on Illumina Hiseq (Illumina Inc., USA) at Allwegene technologies Co., Ltd. (Beijing, China) using HiSeq 4000 Reagent Kits following the manufacturer's instructions (www.illumina.com).

1.3.2. Sequence quality control and genome assembly

Adapter sequence were stripped from the 3' and 5' end of paired end Illumina reads using SeqPrep (https://github.com/jstjohn/SeqPrep). Low-quality reads (length < 50 bp or with a quality value < 20 or having N bases) were removed by Sickle (https://github.com/najoshi/sickle).

1.3.3. Data collection and analysis

MetaGeneMark (Hyatt et al., 2012) software was used to predict the ORF (Open Reading Frame) of the assembled contig sequence. Functional annotation of predicted genes was performed by BLASTP search against the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (Kanehisa & Goto, 2000) and the evolutionary genealogy of genes: Non-supervised Orthologous Groups (eggNOG) database (Jensen et al., 2008) using an e-value cutoff of 1e-5. With the help of HMMER software, the non-redundant gene sequences were compared with the CAZymes in the db CAN database (Yin et al., 2012), and the unknown sequences were annotated.

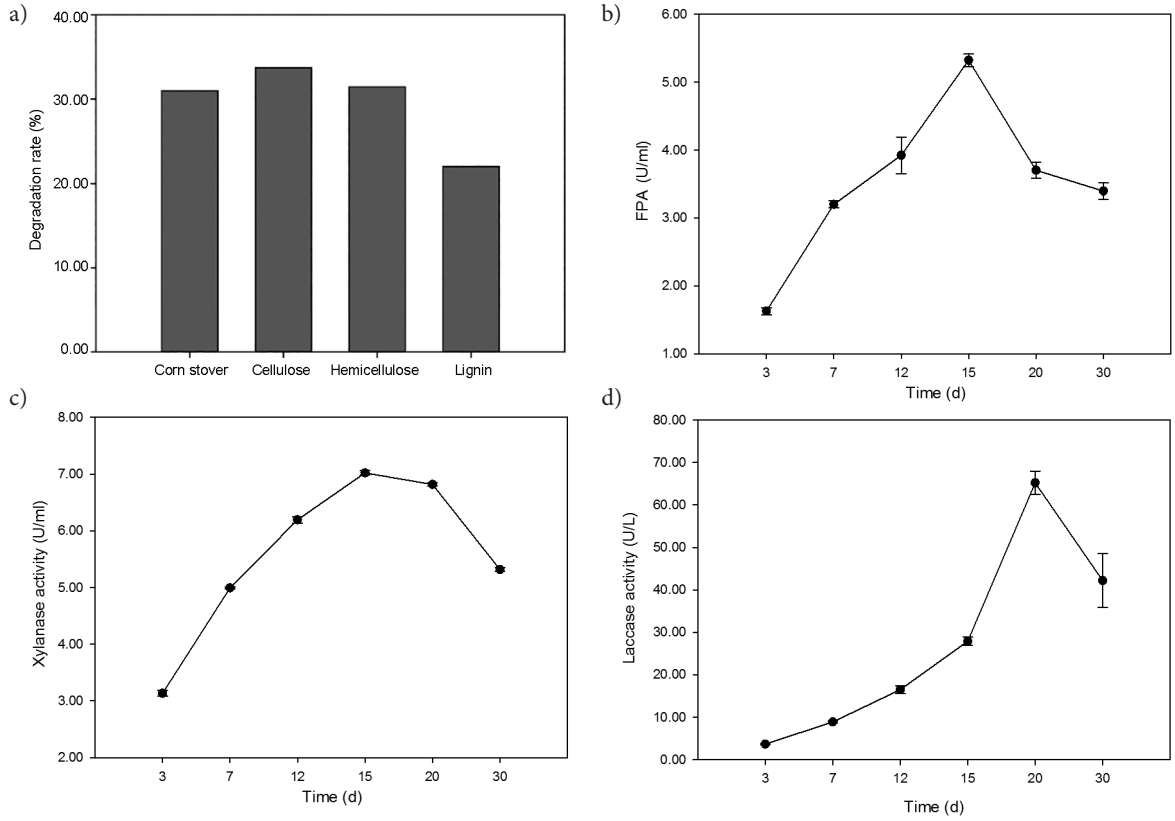


Figure 1. Degradation of characteristics of corn stover by GF-20

2. Results

2.1. Degradation of characteristics of corn stover by GF-20

The results indicated that the GF-20 degraded corn stover more efficiently within 30 d at 15 °C, the weight-loss ratio of corn stover by GF-20 reached 31.03%, the content of cellulose, hemicellulose and lignin decreased by 33.76%, 31.49%, 22.06% at 30 d, respectively (Figure 1a). The corn stover degradation rate is lower than that of the high temperature and medium temperature bacteria studied by predecessors, but GF-20 can realize the rapid degradation of corn stover in low temperature area. GF-20 secreted an extracellular enzyme as shown in Figure 1b, 1c and 1d. The FPA and Xylanase activity reached its maximum at 15 d, which were 5.32 U/mL and 7.02 U/mL. The highest laccase activity were at 20 d, which were 65.22 U/L.

2.2. Taxonomic annotation profiles

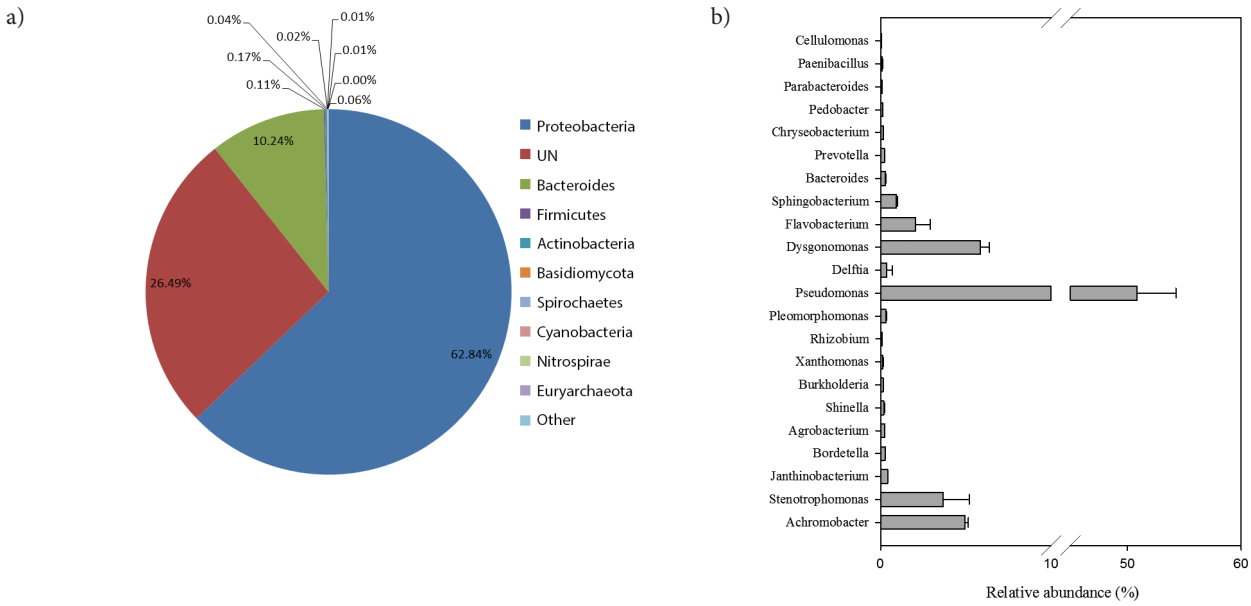
Metagenome sequencing of GF-20 generated a total of 864,196 high-quality reads. After de novo assembly, 102,400 contigs longer than 500 bp were obtained. The metagenome of GF-20 was predicted to contain 1,049,185 open reading frames (ORFs) with an average length of 608.47 bp, and the longest ORF was 22,749 bp.

The taxonomic analysis of all predicted genes in the metagenome showed that GF-20 was predominated by bacteria (72.83% of total sequences) (Additional file 1: Table S1), along with a small number of Eukarya (0.10%), Phage (0.0049%) and Archaea (0.0033%). About 26.5% of total sequences could not be assigned to a definite bacterial phylum using the NCBI NT database in combination with the MEGAN LCA algorithm, likely representing an uncharacterized bacteria. The results showed that the *Proteobacteria* (62.84%) was the predominant phylum among the bacteria in the GF-20, followed by *Bacteroidetes* (10.24%), *Firmicutes* (0.17%), *Actinobacteria* (0.11%), *Basidiomycota* (0.04%), *Spirochaetes* (0.019%), *Cyanobacteria* (0.012%), *Nitrospirae* (0.011%), *Euryarchaeota* (0.003%) and so forth (Figure 2a).

The taxonomic analysis of all predicted genes indicated that the predominant families were *Pseudomonas* (50.84%), *Dysgonomonas* (5.86%), *Achromobacter* (4.94%), *Stenotrophomonas* (3.67%), *Flavobacterium* (2.04%) and included a small number of *Janthinobacterium*, *Bordetella*, *Agrobacterium*, *Shinella*, *Burkholderia*, *Xanthomonas*, *Rhizobium*, *Pleomorphomonas*, *Delftia*, *Sphingobacterium*, *Bacteroides*, *Prevotella*, *Chryseobacterium*, *Pedobacter*, *Parabacteroides*, *Paenibacillus*, *Cellulomonas* and others (Figure 2b).

2.3. Functional annotation of the metagenomes

The predicted genes from the metagenomic data set were analyzed for abundant functions using KEGG and eggNOG databases. The KEGG annotation revealed that 16.9% of total proteins were grouped in membrane transport, 12.3% in carbohydrate metabolism and 9.9% in amino acid metabolism. Based on the functional categories of



Note: NA: No annotation information; Other: The sum of the Phylum level annotation information excluding the top 10; Total: The total number of databases.

Figure 2. Species distribution for Phylum (a) and Genus (b) of GF-20

COGs (Additional file 2: Figure S1), approximately 35.7% of all proteins in the metagenome were poorly characterized with general or unknown functions. Of the genes with an assigned function, most were associated with amino acid transport and metabolism (11.2%), carbohydrate transport and metabolism (10.3%) and transcription (9.2%) (Figure 3). The functional profiles demonstrate that GF-20 acquired an enhanced capacity for polysaccharide degradation and sugar uptake during processing of the lignocellulose. The study found that the metabolic pathways with significant metabolic activity during corn stover degradation of GF-20 were TCA cycle, Bacterial secretion system, Pyrimidine metabolism and Ribosome (Additional file 3: Table S2).

2.4. Glycoside hydrolases and CAZymes by GF-20

Since plant biomass-degrading capacities of microbial consortia are closely related to genes encoding carbohydrate active enzymes (CAZymes), an analysis of the CAZymes that catalyze carbohydrate-related substrates was conducted. The glycoside hydrolase (GH), carbohydrate-binding

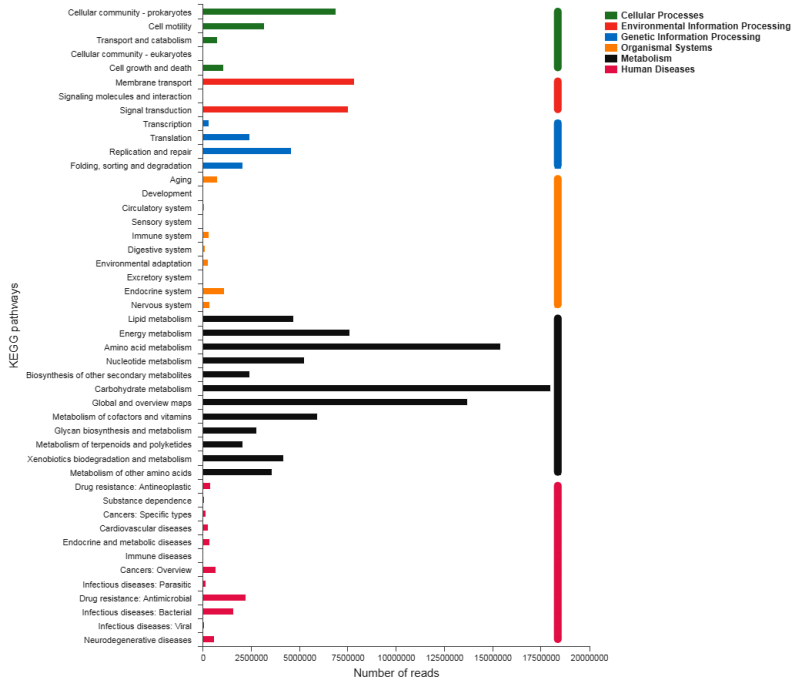


Figure 3. The annotation of the KEGG pathway of GF-20

module (CBM), glycosyl transferases (GT), carbohydrate esterases (CEs) and auxiliary activity (AA) were the main enzyme classes belonging to the CAZymes. The GF-20 metagenome harbored a total of 4465 presumed CAZyme-encoding genes, and among them, 1666 (37.3%), 1091 (24.4%), 215 (4.8%), 862 (19.3%), 532 (11.9%) and 99 (2.2%) were assigned to GH, GTs, AA, CEs, CBM and PL (Additional file 4: Table S3).

The Figure 4 showed the genes of GH, GT, CE, AA, CBM and PL encoded microbial by GF-20. The GH in GF-20 were encoded by *Pseudomonas* (32.32%), *Dysgonomonas* (28.11%), *Sphingobacterium* (16.26%), *Flavobacterium* (6.13%), *Stenotrophomonas* (3.70%), *Achromobacter* (2.30%) and *Cellulomonas* (1.53%), which belong to 111 different families and 75 sub-groups. 1091 glycosyl transferases (GTs) were from 54 families, and the GTs genes were mainly encoded by *Pseudomonas* (51.59%), *Dysgonomonas* (14.40%), *Sphingobacterium* (7.16%), *Flavobacterium* (6.22%), *Stenotrophomonas* (5.94%), *Achromobacter* (4.79%), *Cellulomonas* (0.82%), *Janthinobacterium* (0.49%) and *Delftia* (0.30%). 862 carbohydrate esterases (CEs) came from 15 families and the CEs genes were mainly encoded by *Pseudomonas* (51.58%), *Dysgonomonas* (12.1%), *Sphingobacterium* (9.6%), *Flavobacterium* (5.89%), *Stenotrophomonas* (8.79%), *Achromobacter* (3.82%), *Cellulomonas* (0.85%), *Janthinobacterium* (0.26%) and *Delftia* (0.25%). 215 auxiliary activities (AAs) genes derived from 11 families were mainly encoded by *Pseudomonas* (76.95%), *Sphingobacterium* (3.61%), *Dysgonomonas* (1.62%), *Flavobacterium* (0.96%), *Stenotrophomonas* (2.45%), *Achromobacter* (5.81%), *Cellulomonas* (0.78%) and *Delftia* (0.49%). 532 Carbohydrate binding module (CBM) genes from 54 families were mainly encoded by *Pseudomonas* (31.27%), *Dysgonomonas* (25.07%), *Sphingobacterium* (14.12%), *Flavobacterium* (11.05%), *Stenotrophomonas* (5.02%), *Achromobacter* (3.13%), *Cellulomonas* (2.54%)

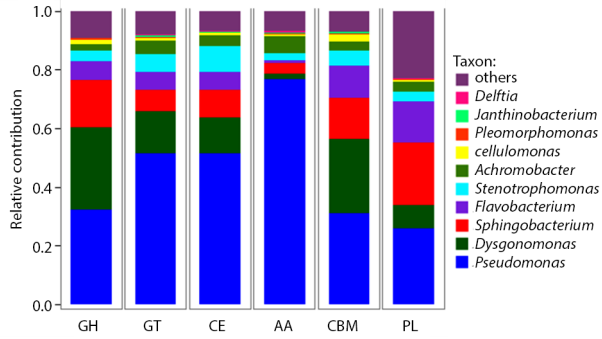


Figure 4. Bar plot of species and functional contribution analysis of GF-20

and *Pleomorphomonas* (0.28%). In addition, 99 PLs genes from 17 families were encoded by *Pseudomonas* (25.94%), *Dysgonomonas* (7.89%), *Sphingobacterium* (21.47%), *Flavobacterium* (14.03%), *Stenotrophomonas* (3.35%), *Achromobacter* (3.14%), *Cellulomonas* (0.70%) and *Delftia* (0.17%).

Statistical analysis of GHs genes in GF-20 (Figure 5) showed that 73 GHs genes involved in cellulose decomposition accounted for 4.38% of the total gene number. The β-glucosidase was distributed in GH1 (0.42%) and GH3 (3.24%) and primarily encoded by the *Pleomorphomonas* and *Flavobacterium*; the Endoglucanase was distributed in GH5 (0.72%) and mainly encoded by the *Dysgonomonas* and *Flavobacterium*. Compared with the cellulase genes, there were more types and numbers of glycosidolytic enzymes involved in hemicellulose degradation, including 111 genes from 18 families. During hemicellulose degradation, the, β-mannosidase, β-galactosidase, α-galactosidase, oligo-1,6-glucosidase, α-L-fucosidase, Dextranase, α-glucosidase, α-D-xyloside xylohydrolase, xylosidase and endo-1,4-β-xylanase work on different parts of the hemicellulose. Genes encoded for these

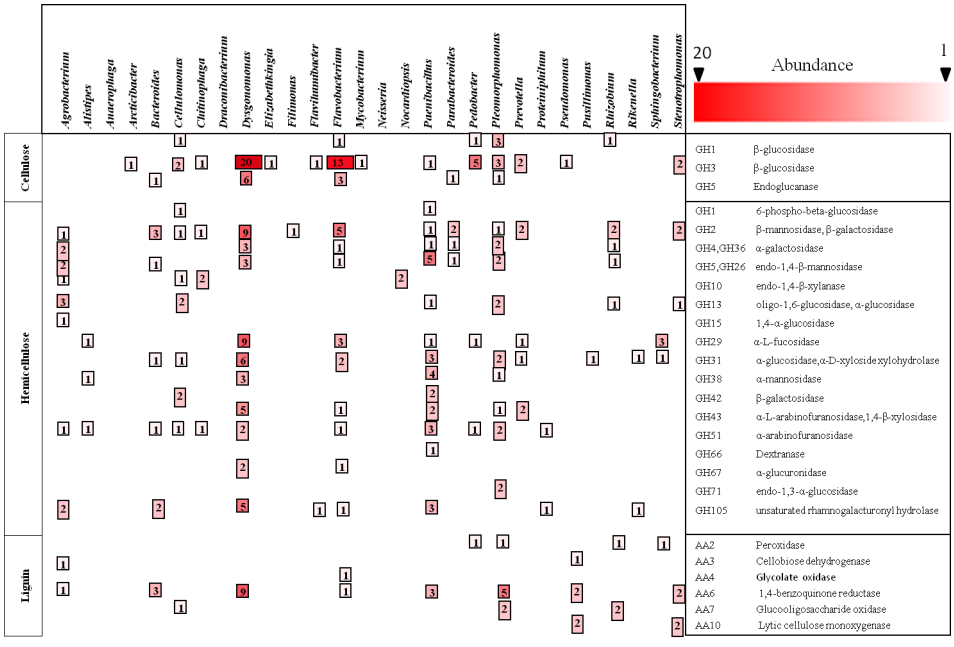


Figure 5. Degradation-related gene of corn stover of GF-20

predicted enzymes in the GF-20 were GH1, GH2, GH4, GH5, GH10, GH13, GH15, GH26, GH29, GH31, GH36, GH38, GH42, GH43, GH51, GH66, GH67 and GH71. *Pleomorphomonas*, *Dysgonomonas*, *Flavobacterium* and *Paenibacillus* were predominantly responsible for encoding the genes for the hemicellulose degradation. There were 19 genes from 6 families (AA2, AA3, AA4, AA6, AA7, AA10) associated with lignin degradation. Peroxidase, Cellobiose dehydrogenase, Glycolate oxidase, 1, 4-benzoquinone reductase, Glucooligosaccharide oxidase, and Lytic cellulose monoxygenase were related to lignin degradation in the GF-20 and were encoded primarily by *Pleurotus* and *Achromobacter*. Therefore, these results demonstrate that GF-20 contains abundant cellulose-degrading microorganisms for corn stover degradation. *Pleomorphomonas* and *Dysgonomonas* were the main microorganisms distributed to degrade complex polysaccharides such as cellulose and hemicellulose, while *Pleurotus* and *Achromobacter* were the main microorganisms to degrade lignin.

2.5. A systematic overview of lignocellulose degradation by GF-20

2.5.1. Degradation of cellulose by GF-20

Endoglucanase hydrolyzes the glycoside bonds of cellulose chains from inside the amorphous region and is mainly derived from *Dysgonomonas*, *Sphingobacterium*, *Flavobacterium, Parabacteroides*, *Pleomorphomonas*, *Neisseria* and *Cellulomonas*. The glucan chains produced by endoglucanase degradation can be degraded by β-glucosidase, and the multiformity of the strains producing β-glucosidase is the most abundant. The β-glucosidase was produced by *Dysgonomonas*, *Flavobacterium*, *Arcticibacter*, *Elizabethkingia*, *Cellulomonas*, *Mycobacterium*, *Stenotrophomonas, Firmicutes* and *Paenibacillus* (Figure 6a). Peptidoglycan in *Firmicutes* have a high mass concentration, and most can produce spores to resist dehydration and extreme environments. *Firmicutes* can assist Bacteroides to degrade cellulose macromolecules in corn stover.

2.5.2. Degradation of hemicellulose and lignin by GF-20

Since hemicellulose is more complex than cellulose, the functional microorganisms responsible for hemicellulose degradation are more diverse than those for cellulose degradation. Hemicellulose is composed of glucan, xylan, mannan, galactosan, polyarabinose, polygalactoic acid and various polysaccharides.

Dysgonomonas, *Prevotella* and *Flavobacteriumthe* were the main producers of xylosidase. Xylosidase can degrade D-xylose, which is attached to the xyloglucan skeleton. *Proteiniphilum* produced α-Arabinfuranosidease degrade the L-arabinose on the xyloglucan side chain. *Dysgonomonas*, *Flavobacterium*, *Parabacteroides* and *Filimonas* produced β-galactosidase and *Dysgonomonas, Sphingobacterium* produced α-fucosidase degrade the β-galactose and L-fucose on the xyloglucan side chain (Figure 6b).

Dysgonomonas, Flavobacterium and *Pleomorphomonas* secreted β-1,4-xylosidase hydrolyzes the xylan skeleton structure from the inside. The xylooligosaccharides produced by β-1,4-xylosidase are further hydrolyzed by xylosidase. *Dysgonomonas, Flavobacterium* and *Chryseobacterium* secreted glucuronidase degraded the D-glucuronic acid residue of the xylan side chain structure. *Agrobacterium*, *Pusillimonas* and *Delftia* secreted ferulic acid esterase degrades the ferulic acid on the xylan side chain (Figure 6c).

Endotracheal manganese produced by *Dysgonomonas, Flavobacterium* and *Anaerophaga* can degrade mannan skeleton. The mannobiose and manninotriose were degraded by the β-1,4-mannose glycosidase produced by *Dysgonomonas*, *Stenotrophomonas* and *Agrobacterium*. The side chain glycoside galactosyl group attached to the mannan degraded by α-galactosidase produced by *Dysgonomonas, Flavobacterium, Parabacteroides, Parabacteroides*, *Pleomorphomonas* and *Rhizobium* (Figure 6d).

From the above analysis, it can be concluded that the dominant gate of cellulose and hemicellulose degradation in GF-20 were *Bacteroidetes*, *Proteobacteria*, *Firmicutes* and *Actinobacteria*; and the dominant genera were

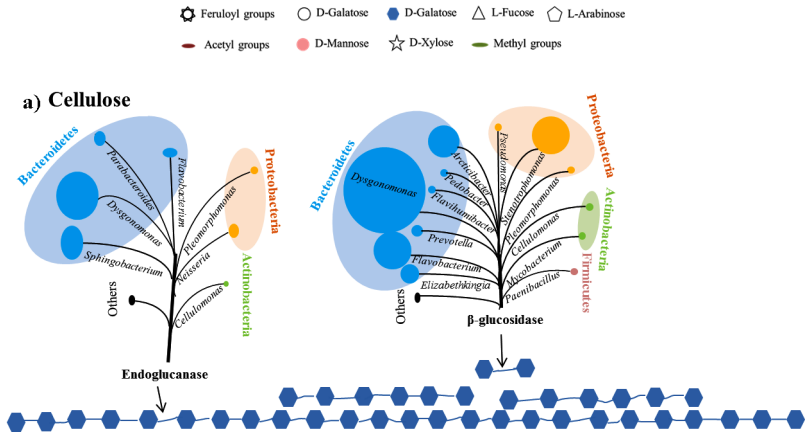
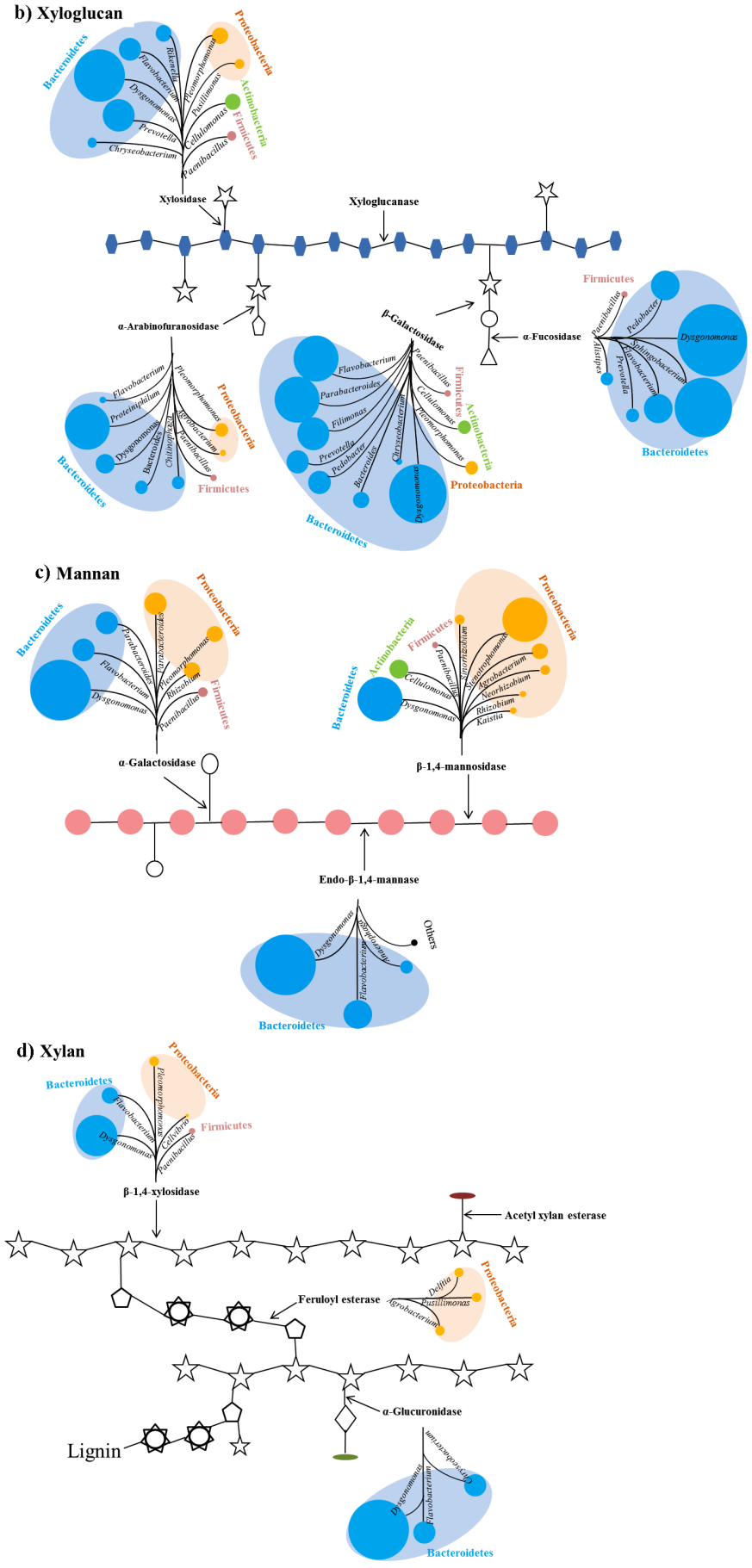


Figure 6. *To be continued*



Note: a)–d) represents dominant microorganism degradation for cellulose, Xyloglucan, Mannan and Xylan.

Figure 6. Degradation of lignocellulose-related enzymes in GF-20

Dysgonomonas, *Flavobacterium*, *Prevotella*, *Pleomorphomonas*, *Agrobacterium*.

Figure 7 shows the common and exclusive genera that worked on the cellulose, hemicellulose, lignin and hemicellulose branched chain. There was only one genera of *Flavobacterium* distributed among the cellulose, hemicellulose, lignin and hemicellulose branched chain. This genera, which includes *Pedobacter*, *Pleomophomonas*, *Cellulomonas*, *Prevotella*, *Dysgonomonas* and *Parabacteroides*, exhibited high degradation levels of cellulose, hemicellulose and the hemicellulose branched chain. A variety of genera including *Pseudomonas*, *Mycobacterium*, *Flavihumibacter*, *Elizabethkingia*, *Arcticibacter*, *Anaerophaga* and *Neisseria* can degrade cellulose. Genera including *Pusillimonas*, *Nocardiopsis*, *Rhizobium*, *Chitinophaga*, *Agrobacterium*, *Rikenella*, *Alistipes*, *Draconibacterium* and *Proteiniphilun* were identified as degraders of the hemicellulose branched chain. It has widely known that corn stover is intricate, and thus, to better degrade corn stover, the synergistic action of microorganisms is needed.

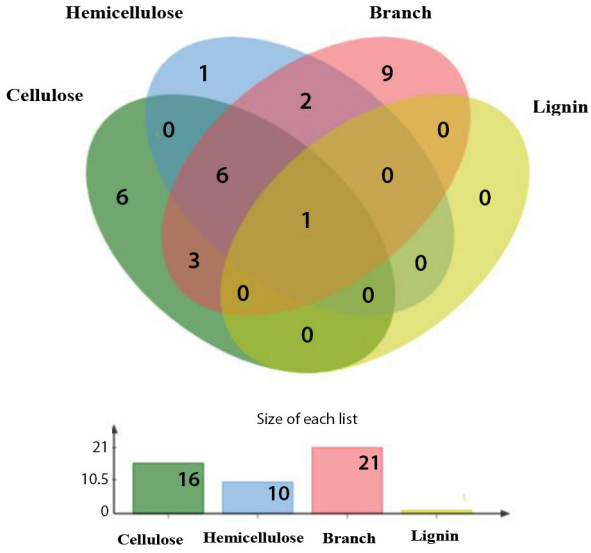


Figure 7. Corn stover degradation venn diagram of common and unique microorganisms in GF-20

2.6. Encoding related genes of GF-20 of corn stover-degrading enzymes

The results of the metagenomic analysis showed that the GF-20 had complex and diverse metabolic functions. The GF-20 encoding the key genes such as Amy, Pyg, Agl, bglX and endoglucanase which degrade complex compounds such as starch, sucrose, cellulose, galactose, and fructose to glucose (Additional file 5: Table S4). A corn stover degradation pathway by the GF-20 was predicted based on the various CAZymes in the GF-20. Within the predicted pathway, cellulose, hemicellulose and other components such as starch, mannan, fructose, glycogen, galactan and so forth degraded to monomers or oligomers by the key CAZymes encoded by the GF-20 (Additional file 5: Table S4, Figure 8).

3. Discussion

Proteobacteria as one of the largest phylum in nature, has a more perfect metabolic mechanism and a strong ability to resist adversity (Ciccarelli et al., 2006). *Firmicutes* can survive at extremely low temperature environments (Ming, 2011) and plays an important role in cellulose degradation (Zhang et al., 2015). *Bacteriodetes* can absorb materials in the surrounding environment for growth (Zhang et al., 2011). *Actinobacteria* promote decomposition of animal and plant remains in the environment and can utilize complex carbohydrates (Székely et al., 2009). *Pseudomonas* can degrade lignocellulose and plays a role in signal transduction (Marqués & Ramos, 1993). *Dysgonomonas* is capable of degrading complex polysaccharides such as cellulose and hemicellulose. *Achromobacter* is able to oxidize xylose and degrade hemicellulose (Ridderberg et al., 2012). *Stenotrophomonas* aerobic microorganisms can utilize organic acids (Heylen et al., 2007). *Flavobacterium* is strictly aerobic and can ferment glucose, fructose and maltose under optimal temperatures of less than 30 °C (Liu et al., 2008); at higher temperatures it can inhibit growth. *Flavihumibacter* contains catalase and oxidase activities (Lee et al., 2014). *Mycobacterium* can degrade complex organic matter (Heitkamp et al., 1988). *Arcticibacter* maintains activity at low temperatures (Shen et al., 2017) and ferments a variety of sugars from corn straw degradation intermediates (Shivaji et al., 2013). *Pusilmonas* cryophilic microorganisms exhibit degradation activity at 0 °C (Li et al., 2013). *Rikenella* secretes alkaline glycosidase (Ghanem et al., 2005). *Draconibacterium* degrade complex polysaccharides (Li et al., 2016). *Rhizobium* has a nitrogen fixation effect (Liu et al., 2020). *Chitinophaga* secretes arabidosidase (Kong et al., 2019; Sly et al., 1999). *Prevotella* can effectively degrade hemicellulose (Bekele et al., 2011), utilize plant non-fibrinoglycan and protein (Purushe et al., 2010) and play an important role in the degradation of starch, xylan and pectin. *Nocardiopsis* (Yan et al., 2011) and

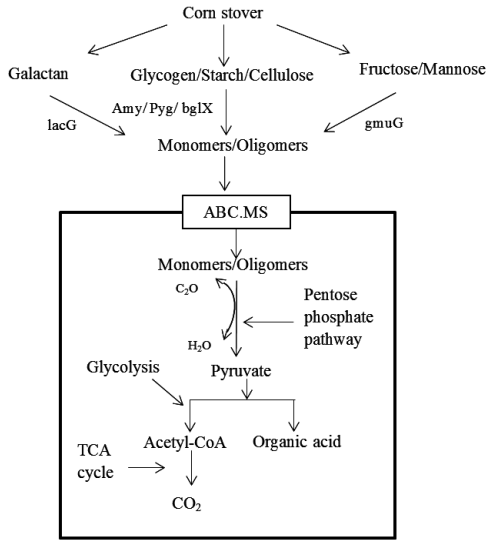


Figure 8. The metabolism model of GF-20

Agrobacterium belong to the phylum *Firmicutes* and use carbohydrates, organic acids and amino acids as carbon sources. *Pleurotus* use sawdust as raw material for growth and reproduction (Poidevin et al., 2014) and play a role in lignocellulose degradation in corn stover. The genera *Flavobacterium* and *Dysgonomonas,* with a high abundance of information in the flora, played a role in the degradation of cellulose, hemicellulose, lignin and the hemicellulose branch chain, and demonstrated a cooperative degradation relationship.

The GF-20 encoded complete pentose phosphate and glycolysis pathways, which can further utilize the products obtained from complex compounds, especially carbohydrates. Pyruvate is produced by glycolysis and is the end product of the following: the pentose-phophate pathway, the pyruvic acid salt metabolic pathway to glycolysis, and the degradation of the intermediate product of pyruvate pentose-phophate pathway for final metabolism, energy and water or CO_2 or small molecule volatile acid (Gosalbes et al., 2011). In the GF-20, 85.04% *Pseudomonas* encode the key metabolic enzymes of pyruvic acid salt pyruvate dehydrogenase in higher abundance. Meanwhile, many kinds of ABC.MS genes were annotated in the GF-20. The monomers or oligomers were further degraded through glycolysis, the pentose phosphate pathway and the TCA cycle to H_2O, CO_2 and organic acid. The GF-20 degraded the cellulose, mannose, xylose and xyloglucan of the corn stover to carbohydrates that can be used by glycolysis and the pentose phosphate pathway through sugar and starch metabolism and fructose and mannose metabolism. It was determined that the *Pseudomonas* as the dominant microbe in the GF-20 encoded complete glycolysis, pentose phosphate pathway and TCA cycle. The abundance information of the GF-20 carbohydrate-related metabolic pathways and enzyme annotation is relatively high, so it can be concluded that the GF-20 mainly performs carbohydrate metabolism and can completely degrade cellulose and hemicellulose.

Due to its special structure of lignin, lignocellulose was unable to be degraded directly and efficiently by the microbial. Meanwhile, the process of lignocellulose decomposition was completed due to the effect of series enzymes in the process of microbial metabolism, which needs participation from the synergism of enzymes. Hemicellulose and lignin intertwine to wrap cellulose, which limits the degradation of cellulose (Papa et al., 2015). Laccase, lignin peroxidase and manganese peroxidase are the main biodegradable enzymes of lignin. In this study, the metagenome annotation information of GF-20 showed that, there was no annotation information about laccase, only the oxidase information produced by *Pleurotus*, *Achromobacter* and *Flavobacterium*. β-glucosidase is an important hydrolase for cellulose microbial degradation (Berlemont & Martiny, 2013), and its activity is often stable and highly efficient in most habitats (Sinsabaugh et al., 2008). The degradation of side chain can release more enzymes from xylan, xyglucan and mannan on main chain (Zhang et al., 2011). There were 6 genes in GF-20 annotated that worked on the degradation of cellulose skeleton and hemicellulose. The degradation of cellulose was firstly induced by β-endoglucanase produced by *Dysgonomonas*, *Sphingobacterium*, *Flavobacterium*, *Parabacteroides*, *Pleomorphomonas*, *Neisseria* and *Cellulomonas* that worked on the 1,4-glycosidic bonds. The short chain sugar molecules degraded by β-glucosidase produced by *Dysgonomonas*, *Flavobacterium*, *Arcticibacter*, *Elizabethkingia*, *Cellulomonas*, *Mycobacterium*, *Stenotrophomonas*, and *Paenibaci*.

Conclusions

GF-20 microbial consortium is mainly composed of *Proteobacteria*, *Bacteroidetes*, *Firmicutes* and *Actinobacteria*, and at the genera level, it consists of *Pseudomonas, Dysromobacter, Stenotrophomonas and Flavobacterium*. Its metabolism is mainly carbohydrate metabolism and amino acid metabolism, and the functional genes encoded are mainly distributed in the glycoside hydrolase genes. Corn stover degradation by GF-20 was mainly achieved by β-glucosidase and endoglucanase produced by *Dysgonomonas*, *Sphingobacterium*, *Flavobacterium*, *Parabacteroides*, *Pleomorphomonas*, *Arcticibacter*, *Elizabethkingia*, *Neisseria*, *Mycobacterium*, *Stenotrophomonas*, *Paenibacillus* and *Cellulomonas*. Hemicellulose degradation was achieved through the synergistic degradation of xylanase, xyloglucanase, mannolanase and branched enzymes mainly produced by *Pedobacter*, *Pleomophomonas*, *Cellulomonas*, *Prevotella*, *Dysgonomonas*, *Parabacteroides*, *Pusillimonas*, *Nocardiopsis*, *Rhizobium*, *Chitinophaga*, *Agrobacterium*, *Rikenella*, *Alistipes*, *Draconibacterium* and *Proteiniphilun*.

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Author contributions

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