Complete Genome Sequence of Microbulbifer sp. YPW1 from Mangrove Sediments in Yanpu Harbor, China

Wang Dingquan (regen8616@163.com)
Zhejiang Ocean University https://orcid.org/0000-0003-2055-6711
Jianxin Wang
Zhejiang Ocean University
Bonian Shui
Zhejiang Ocean University
Longqiang Zhu
Zhejiang Ocean University
Jiangqi Wang
Zhejiang Ocean University
Linxi Jin
Zhejiang Ocean University
Wu Qu
Zhejiang Ocean University

Research Article

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Abstract

In this work, a strain named YPW1 was isolated from the sediments of an artificial mangrove in Yanpu harbor, China. Whole genomic sequencing was conducted to reveal the potential ecological roles of strain YPW1 for the environment. The result of 16S rRNA gene alignment assigned strain YPW1 into Microbulbifer genus, and then, 10 representative genomes from Microbulbifer genus were selected to compare with YPW1. Results showed that the genome of strain YPW1 possessed more carbohydrate-active enzyme genes to transform various recalcitrant polysaccharides into bioavailable monosaccharides than those of the selected genomes. Furthermore, among the selected genomes, YPW1 was the only strain with nitrate, nitrite, and nitric oxide reductases which could appoint nitrous oxide, a powerful greenhouse gas, as the end-product of its denitrification process. Therefore, strain YPW1 was a member of Microbulbifer genus with special ecological roles that served as the center for the degradation and utilization of polysaccharides and the performer of denitrification with greenhouse gas as the end-product, and the abundance and biological activity of YPW1 could be an indicator to reflect the ecological function of this artificial mangrove in energy material providing and climate regulation.

1. Introduction

Mangroves occurred on the intertidal zones are among the world's most productive ecosystems and stored considerable carbon (C) in this ecosystem (Alongi 2014). As a typical blue C ecosystem (Alongi, Murdiyarso et al. 2016), mangroves are one of the centers of C-cycling in nature. On the other hand, mangroves are nitrogen (N)-deficiency environment (Lovelock, Feller et al. 2006), thus, the N-cycling process is also crucial for the living organisms in mangrove ecosystem. Previous works have demonstrated that the microorganisms play key roles for C- and N-cycling in mangroves (Maie, Pisani et al. 2008, Alongi 2014). Therefore, revealing the ecology functions of these microorganisms can give us a deeper understanding for researching and managing the mangrove resources.

Yanpu harbor is located on the coast of Wenzhou City, China. An artificial mangrove forest has been planted in this harbor for 5 years since 2015, and nowadays the area of this mangrove forest has reached ~50 km^2. As the results shown in the investigations, benthic environment of this area has been obviously improved after the mangrove planting (Zhang M M, Wang Y X et al. 2019). However, the ecology functions of the microorganisms in this artificial mangrove have rarely been reported for now.

Strains belonged to *Microbuibifer* genus were universally isolated from marine environments including coastal soil (Kämpfer, Arun et al. 2012), mangrove forests (Baba, Miyazaki et al. 2011), marine algae (Nishijima, Takadera et al. 2009), intertidal (Yoon, Kim et al. 2004) and deep-sea sediments (Miyazaki, Nogi et al. 2008), and marine pulp mill effluent (González, Mayer et al. 1997), and former studies have demonstrated that *Microbuibifer* genus possess the abilities for marine polysaccharide degradation (Ohta, Hatada et al. 2004, Kim, Jang et al. 2011, Vijayaraghavan and Rajendran 2012, Swift, Hudgens et al. 2014). In this study, a strain named YPW1 belonged to *Microbulbifer* genus was isolated from the sediments of Yanpu harbor mangrove and was analyzed by whole genome sequencing using Oxford

Nanopore platform. Through the comparisons with the genomic information derived from 10 other strains belonged to *Microbulbifer* genus, our work found some unusual characteristics laid in the genome of strain YPW1, especially for the functions related to the utilization of polysaccharides and the emission of greenhouse gas, indicating the significant ecological roles of strain YPW1 in energy material providing and climate change regulation.

2. Materials And Methods

2.1 Samples

The sediment samples were collected from the artificial mangrove located in Yanpu harbor, China (27.5°N and 120.3°E). Appropriate 50 g sediments were sampled into 50 mL sterile centrifuge tubes with sterile medicine spoons and were stored on ice. The isolation of strains had been finished within one week after the samples were taken back to the lab.

2.2 Isolation and identification of YPW1

The sediment samples with 10^7-fold dilution using sterile sea water were spread on the 2216E plates (0.5% of peptone, 0.1% of yeast extracts, 0.001% of ferric phosphate, and 2% of agar powder in 1 L sea water), and the plates were cultured at 28°C for 5 days. A light-yellow strain with irregular edge and agar collapses around was named YPW1 and was selected for the future study. Taxonomic information of YPW1 was identified by 16S rRNA gene sequence analysis according to the previous study (Weisburg, Barns et al. 1991). Phylogenetic tree of 16S rRNA gene of YPW1 was conducted by using Mega 10.0 software with neighborhood-joining method.

2.3 Genomic DNA extraction and whole genomic sequencing of strain YPW1

The genomic DNA of strain YPW1 was extracted by using MiniBEST Bacteria Genomic DNA Extraction Kit Ver.3.0 (Takara, Japan). The purity and integrity of genomic DNA were tested by Nanodrop 2000 (Thermo, USA) and 1% agarose gel electrophoresis. High-quality DNA was used to construct the sequencing library with SQK-LSK109 Ligation Sequencing Kit (Oxford Nanopore Technologies, UK). Then, the sequencing was future performed using Oxford Nanopore GridION platform by Biomarker Technologies (Beijing, China) according to the standard protocols. Sequencing by Illumina Miseq platform was also performed to correct the data from GridION platform. The genomic sequences had been deposited in NCBI GenBank under the Accession No. CP055157.

2.4 Annotation and analysis of the genomes from *Microbulbifer* genus

Canu v1.5 (Koren, Walenz et al. 2017) and Pilon (Walker, Abeel et al. 2014) were used for the genome assembly and data correction, respectively. Coding genes in the genome was predicted by Prodigal (Hyatt, Chen et al. 2010). tRNA genes were annotated using tRNAscan-SE (Lowe and Eddy 1997). rRNA and other non-coding RNA (ncRNA) genes were predicted with Infernal 1.1 (Nawrocki and Eddy 2013) based on Rfam database (Nawrocki, Burge et al. 2015). The gene islands were analyzed using IslandPath-DIMOB software (Langille, Hsiao et al. 2008). The prediction of prophage in the genome was conducted using PhiSpy software (Akhter, Aziz et al. 2012). The gene functions were annotated by BLAST software against NCBI nr database. The glycometabolism functions were annotated using HMMER software against dbCAN database (http://bcb.unl.edu/dbCAN2/) to find the carbohydrate-active enzymes in the genome of strain YPW1, including glycoside hydrolases (GHs), glycosyltransferases (GTs), polysaccharide lyases (PLs), carbohydrate esterases (CEs), and auxiliary activities (AAs). To compare with other strains from *Microbulbifer* genus, all the 10 representative genomes with the definite taxonomic information on species level from *Microbulbifer* genus, including *M. agarilyticus* GP101 (NZ_CP019650.1), *M. variabilis* ATCC 700307 (NZ_AQYJ01000029.1), *M. hydrolyticus* IRE-31 (NZ_CP047491.1), *M. thermotolerans* DAU221 (NZ_CP014864.1), *M. pacificus* LD25 (NZ_PREV01000026.1), *M. aggregans* CCB-MM1 (NZ_CP014143.1), *M. mangrovi* DD-13 (NZ_LZDE01000347.1), *M. donghaiensis* CGMCC 1.7063 (NZ_FQVA01000001.1), *M. marinus* CGMCC 1.10657 (NZ_FNQO01000001.1), and *M. yueqingensis* CGMCC 1.10658 (NZ_FNFH01000001.1), were downloaded from NCBI genome database, and the gene functions in these genomes were annotated against NCBI nr and dbCAN databases using the same software. The locations of proteins were predicted by SignalP 4.0 (Petersen, Brunak et al. 2011) and tmhmm (Krogh, Larsson et al. 2001). The values of average nucleotide identity (ANI) and DNA-DNA hybridization (DDH) were calculated based on the genomes of *Microbulbifer* strains and ZHDP1 by using the websites of https://www.ezbiocloud.net/tools/ani and https://tygs.dsmz.de/user_requests/new, respectively.

3. Results

3.1 Descriptions of the morphological, taxonomic, and genomics characteristics

A strain named YPW1 was isolated from mangrove sediments in Yanpu harbor. Strain YPW1 can be cultured on 2216E solid plates with yellow color and irregular circle. Agar collapse was observed around the strain, indicating the agar degradation by YPW1. Subsequently, the sequence of the 16S rRNA gene of strain YPW1 (accession no. MZ311580) showed 99.44% of similarity with that of *M. mangrovi* DD-13 (NR_109105.1), and strain YPW1 was also closest to *M. mangrovi* DD-13 according to the phylogenetic tree of 16S rRNA genes constructed by neighborhood-joining method (Fig. 1A). Therefore, strain YPW1 was assigned into *Microbulbifer* genus. The whole genome of strain YPW1 without any gap was obtained

(Fig. 1B and Table 1). The genome size of strain YPW1 was 4,578,595 bp, and no plasmid was found. In addition, 12 rRNA genes, 52 tRNA genes, and 13 other ncRNA genes were annotated in the genome. Six genome islands with a mean length of 2,589,666 bp were also found. Two prophages were predicted in the genome of YPW1. The maximum values of ANI and DDH of ZHDP1 genome were respectively 90.36 and 68.1 and were lower than the thresholds (ANI < 95%-96%; DDH < 70%), indicating that YPW1 was a potential new species in genus *Microbulbifer*.

3.2 Polysaccharide utilization abilities of strain YPW1

Strain YPW1 possessed the abilities for the degradation and utilization of different polysaccharides, including agarose, alginate, xylan, starch, pullulan, cellulose, chitin, and pectate. one agarase, one alginate lyase, two xylanases, one amylase, two cellulases, one chitinase, and one pectate lyase were located in cytoplasm. In detail, five agarases, one alginate lyase, one xylanase, two amylases, one pullulanase, one cellulase, two chitinases, and two pectate lyases were secretory proteins. One agarase, one alginate lyase, one pullulanase, and two chitinases distributed on the cell membrane of strain YPW1. These results demonstrated that strain YPW1 can degrade various polysaccharides into oligosaccharide with low polymerization degrees and further produced monosaccharides (Fig. 2A).

Ten genomes from other strains belonged to *Microbulbifer* genus were analyzed and compared with strain YPW1. The gene numbers of AA, GH, GT, CE, PL, and CBM of YPW1 were obviously higher than those of other *Microbulbifer* strains, even higher than that of *M. mangrovi* DD-13, the most closet strain to YPW1 (Fig. 2B). Therefore, strain YPW1 possessed more versatile abilities for polysaccharide utilization than other representative strains belonged to *Microbulbifer* genus.

3.3 Denitrification ability of stain YPW1

According to the genomes from *Microbulbifer* genus, we found that most selected genomes had the denitrification ability (Table 2). In 9/11 of the selected genomes, including *M. agarilyticus* GP101, *M. donghaiensis* CGMCC 1.7063, *M. hydrolyticus* IRE-31, *M. mangrovi* DD-13, *M. marinus* CGMCC 1.10657, *M. pacificus* LD25, *M. variabilis* ATCC 700307, *M. yueqingensis* CGMCC 1.10658, and strain YPW1, possessed the genes of nitrate and nitrite reductases that can reduce nitrate to nitrite and then to nitric oxide. *M. thermotolerans* DAU221 only possessed nitrate reductases that can only reduce nitrate to nitrite. In addition, *M. aggregans* CCB-MM1 was not able to denitrify without any nitrate or nitrite reductase. However, strain YPW1 had not only nitrate and nitrite reductases but also nitric oxide (NO) reductase that can further reduce NO to nitrous oxide (N_2O). No N_2O reductase was found in the genome of YPW1. Therefore, N_2O served as the end-product of the denitrification process of strain YPW1 (Fig. 2A and Table 2).

4. Discussion

In this work, strain YPW1 assigned into *Microbuibifer* genus was isolated from the sediments in an artificial mangrove. Although strain YPW1 was not a novel species based on the high similarity (99.44%) of 16S rRNA gene with *M. mangrovi* DD-13, this strain still possessed new genomics characteristics compared with other reported genomes belonged to *Microbuibifer* genus, indicating the special ecological role of strain YPW1.

Considerable carbons (C) are stored in mangroves, and the polysaccharides are the important storage form (Alongi 2014). However, many polysaccharides, including cellulose, agarose, alginate, chitin, carrageenan and others, are extremely recalcitrant to be utilized by most organisms. Previous studies (Ohta, Hatada et al. 2004, Kim, Jang et al. 2011, Vijayaraghavan and Rajendran 2012, Swift, Hudgens et al. 2014) demonstrated that *Microbuibifer* genus possessed the degradation abilities of marine polysaccharides. Furthermore, our study showed that strain YPW1 had more versatile abilities to degrade various kinds of polysaccharides than other strains selected in this work (Fig. 2B). This result demonstrated that strain YPW1 could transform various polysaccharides into bioavailable monosaccharides and further provide energy sources to other microorganisms and organisms in mangrove sediments. Therefore, strain YPW1 is one of the C-cycling centers for mangrove ecosystems and the energy providers for other livings in Yanpu habor mangroves.

Besides, YPW1 was the only strain that can denitrify nitrate to N_2O among the 11 genomes analyzed in our work (Fig. 2A and Table 2). As the results shown, strain YPW1 was a special member in *Microbuibifer* genus that can produce N_2O, a greenhouse gas more powerful that CO_2 (Kampschreur, Temmink et al. 2009). Undoubtedly the community imbalance of strain YPW1 will have some resistance to the mangrove functions for the mitigation of global warming. Therefore, research on controlling and balancing the abundance and biological activity of strain YPW1 in Yanpu harbor artificial mangrove could be the potential guarantee for this artificial mangrove to give full play to its ecological function in climate regulation.

5. Conclusions

In this study, a potential new species belonged to *Microbuibifer* genus named strain YPW1 was isolated from an artificial mangrove in Yanpu harbor, China. This strain could metabolize various polysaccharides to provide bioavailable sugar for other organisms. Furthermore, among the 11 genomes from *Microbuibifer* genus, YPW1 was the only strain that could reduce nitrate into N_2O, a powerful greenhouse gas, as the end-product of its denitrification process. Therefore, strain YPW1 played a special ecological role in energy source providing and greenhouse gas emission, and the abundance and biological activity of this strain could have impacts on the livings in this mangrove and climate warming. Strain YPW1 could serve as the potential indicator for supervising the function of this artificial mangrove in material providing and climate regulation.

Declarations

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Conflicts of interest

Authors have no conflict of interest to declare.

Availability of data and material

The genome sequence has been deposited in Genbank database under the accession no. CP055157.

Code availability

Not applicable

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Tables

Table 1
General features of the genome of strain YPW1 and MIGS mandatory information

Item	Description
General features	
Classification	Family: Microbulbiferacea; Genus: Microbulbifer
Gram stain	Negative
Cell shape	Rod
Pigmentation	Light yellow
Temperature	15–40 °C (optimum at 25 °C)
Salinity	0.1-2.0% (optimum at 1.0%)
pH	4.0–9.0 (optimum at 6.5)
Motility	No
MIGS data	
Investigation type	Bacteria
Project name	Genome of Microbulbifer sp. YPW1
BioProject	PRJNA639395
BioSample	SAMN15234871
Latitude and longitude	27.5°N, 120.3°E
Depth	Depth 0 m
Geographic location	Yanpu harbor, China
Collected by	Dingquan Wang and Wu Qu
Collection date	06-25-2019
Environment (biome)	Mangrove biome (ENVO_01000181)
Environment (feature)	Marine benthic feature (ENVO_01000105)
Environment (material)	Environmental material (ENVO_00010483)
Biotic relationship	Free-living
Trophic level	Heterotrophic
Relate to oxygen	Aerobe
Isolation growth condition	2216E medium
Annotation source	RAST/NCBI blastx

Estimated size	4-5 M bp
Genome attribute	
Sequencing method	Illumina Miseq and Oxford Nanopore GridION
Assembly	Canu v1.5
Finishing strategy	Whole genome
Genome size	4,578,595 bp
GC content	57.64 mol%
Number of contigs	1
Largest contig	4,578,595 bp
Protein coding genes	3,680
tRNAs	52
rRNAs	12
Number of prophages	2
Number of gene islands	6
Number of CAZyme	281
Enzymes for denitrification	Nitrate, nitrite, and nitric oxide reductases

Table 2
Denitrifying enzymes in the genomes of the representative strains and YPW1.

Representative genome	No. of nitrate reductases	No. of nitrite reductases	No. of nitric oxide reductases	No. of nitrous oxide reductases
M. agarilyticus GP101	4 (WP_077400991.1; WP_077401614.1; WP_077401620.1; WP_077401623.1;)	2 (WP_077400994.1; WP_077400997.1)	0	0
M. donghaiensis CGMCC 1.7063	7 (WP_073272945.1; WP_073272950.1; WP_073272952.1; WP_073273034.1; WP_073276855.1; WP_073276859.1; WP_073276861.1)	3 (WP_073275713.1; WP_073273030.1; WP_073273032.1)	0	0
M. hydrolyticus IRE-31	1 (WP_161859362.1)	2 (WP_161859360.1; WP_161859361.1)	0	0
M. mangrovi DD-13	3 (WP_078085222.1; WP_078085224.1; WP_078082720.1)	2 (WP_078082721.1; WP_078082768.1)	0	0
M. marinus CGMCC 1.10657	7 (WP_091386641.1; WP_091386645.1; WP_091386647.1; WP_091386716.1; WP_091391223.1; WP_091391224.1; WP_091391226.1)	2 (WP_091386712.1; WP_091386714.1)	0	0
M. pacificus LD25	1 (WP_105103395.1)	2 (WP_105103396.1; WP_105103397.1)	0	0
M. thermotolerans DAU221	3 (WP_067151776.1; WP_067151773.1; WP_067151782.1)	0	0	0
M. variabilis ATCC 700307	4 (WP_020413756.1; WP_020413758.1; WP_020413759.1; WP_020414665.1)	2 (WP_020414666.1; WP_020414667.1)	1	0
M. yueqingensis CGMCC 1.10658	4 (WP_091509784.1; WP_091509790.1; WP_091509794.1; WP_091509898.1)	2 (WP_091509891.1; WP_091509894.1)	0	0
M. aggregans CCB-MM1	0	0	0	0
Microbulbifer	5	2	1	0

sp. YPW1

Figures

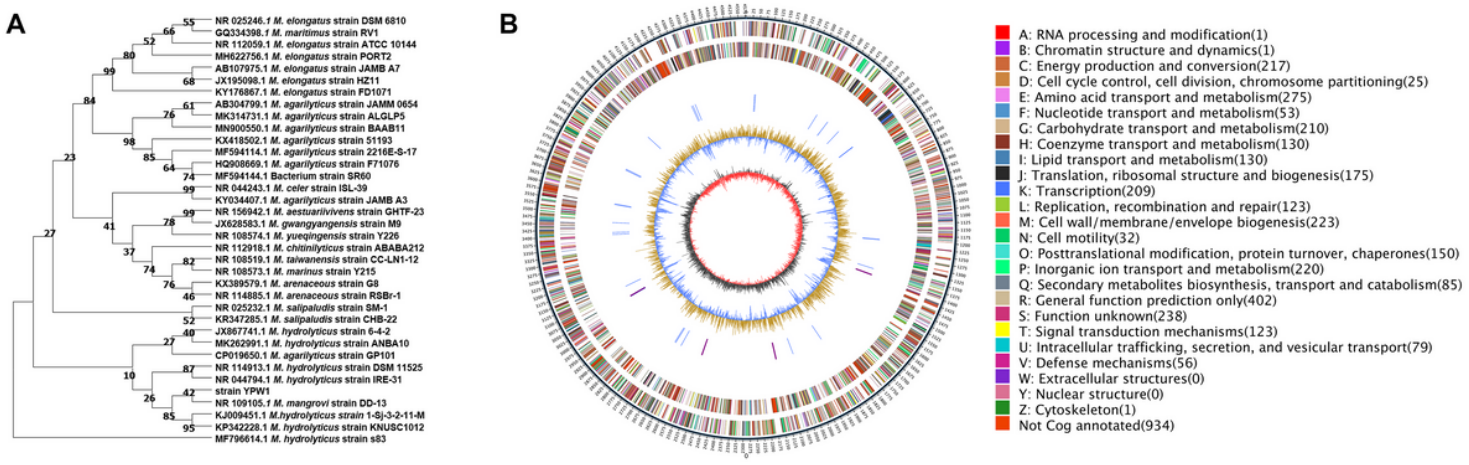
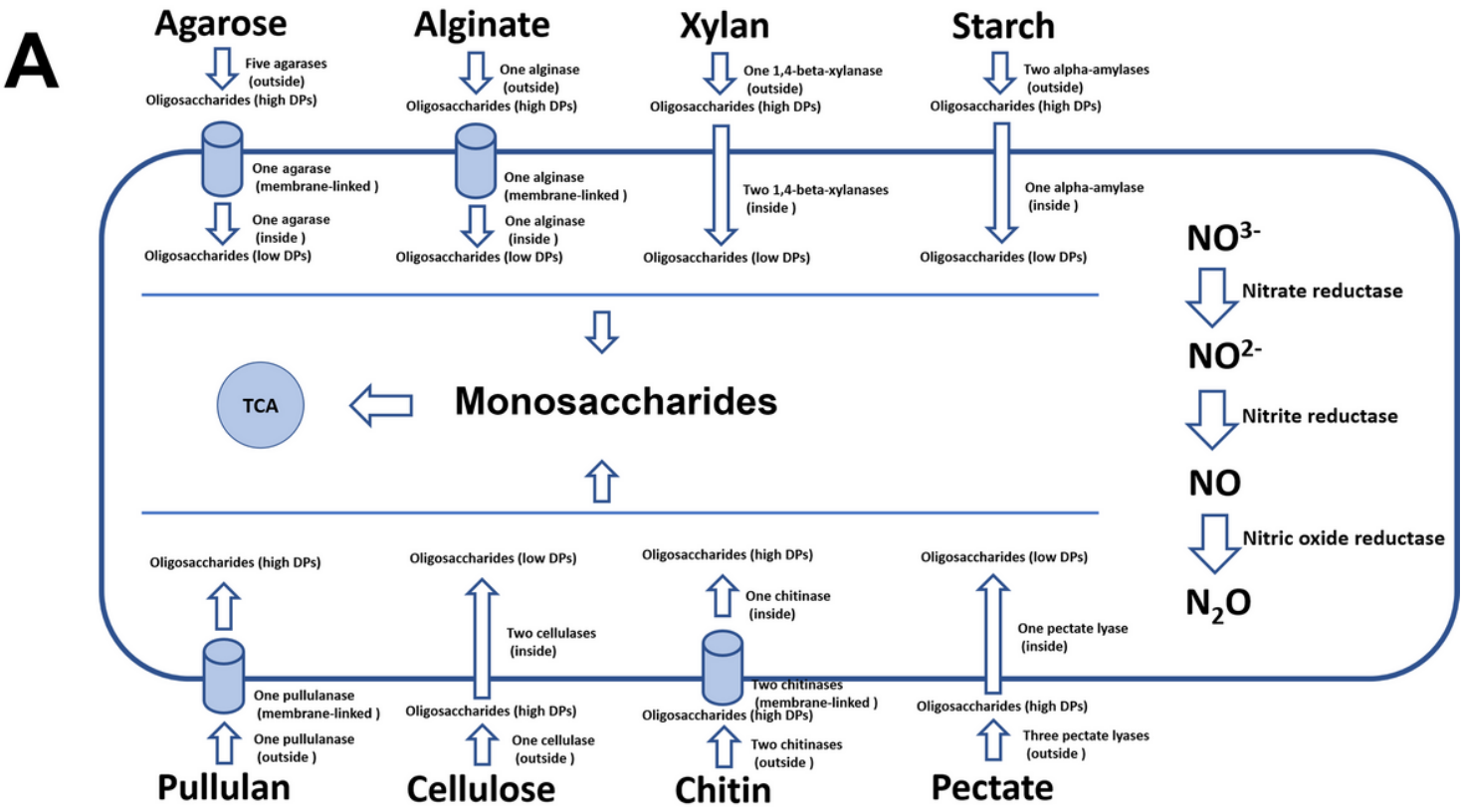
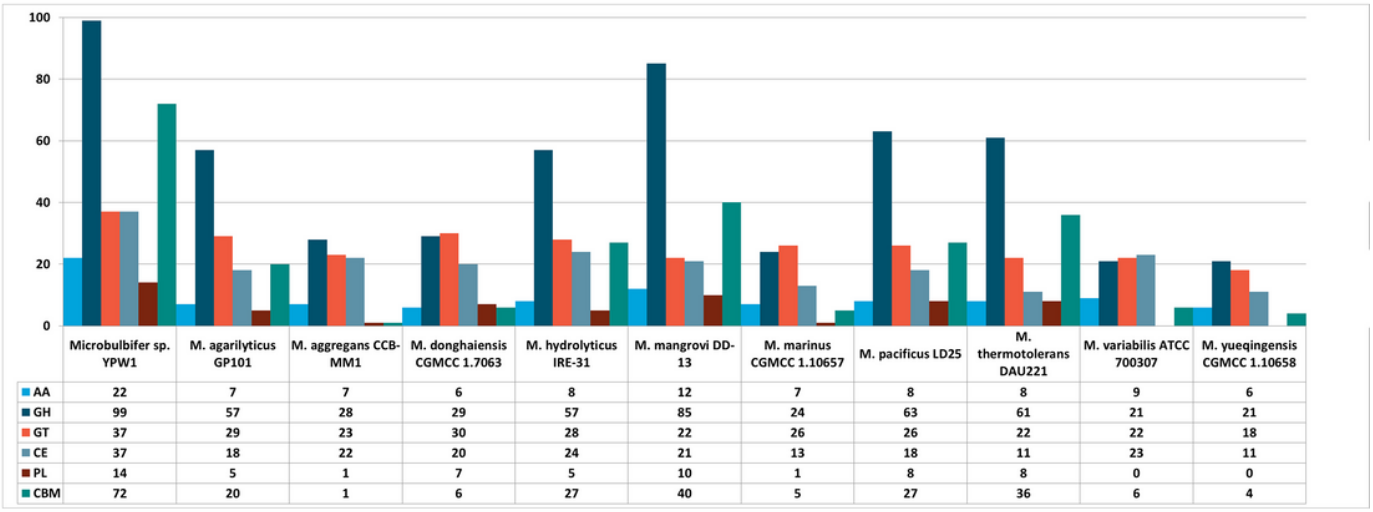


Figure 1

(A) Phylogenetic analysis of the 16S rRNA gene of strain YPW1 constructed by using the neighbor-joining method. The bootstrap value was 1000. The evolutionary distances were computed using the maximum composite likelihood method and are in the units of the number of base substitutions per site. The 16S rRNA gene of strain YPW1 was most closet to that of M. mangrovi DD-13, another strain isolated from mangroves; (B) Circos map of the genome of strain YPW1. This map was divided into 6 circles from outside to inside, namely, markers of genome size (5 kb per scale), genes on the positive strand, genes on the negative strand, repetitive sequences, genes of tRNA (blue) and rRNA (purple), GC content, and GC-skew. The colors in the right legend represented the COG classification of the genes on the positive and negative strands. Light-yellow and blue represented the GC content higher and lower than the mean genomic GC content, respectively. In the circle of GC-skew, dark-grey and red represented that the G content was higher and lower than the C content, respectively.



B



	Microbulbifer sp. YPW1	M. agarilyticus GP101	M. aggregans CCB-MM1	M. donghaiensis CGMCC 1.7063	M. hydrolyticus IRE-31	M. mangrovi DD-13	M. marinus CGMCC 1.10657	M. pacificus LD25	M. thermotolerans DAU221	M. variabilis ATCC 700307	M. yueqingensis CGMCC 1.10658
AA	22	7	7	6	8	12	7	8	8	9	6
GH	99	57	28	29	57	85	24	63	61	21	21
GT	37	29	23	30	28	22	26	26	22	22	18
CE	37	18	22	20	24	21	13	18	11	23	11
PL	14	5	1	7	5	10	1	8	8	0	0
CBM	72	20	1	6	27	40	5	27	36	6	4

Figure 2

(A) The amounts and locations of polysaccharide-degrading enzymes along with the denitrification process and related enzymes in the genome of strain YPW1. According to the genomic information, one agarase, one alginate lyase, two xylanases, one amylase, two cellulases, one chitinase, and one pectate lyase were located in cytoplasm. Five agarases, one alginate lyase, one xylanase, two amylases, one pullulanase, one cellulase, two chitinases, and two pectate lyases were secretory proteins. One agarase, one alginate lyase, one pullulanase, and two chitinases distributed on the cell membrane of strain YPW1.

Besides, nitrate, nitrite, and nitrous oxide (N2O) reductases but no N2O reductase were found in the genome, indicating that N2O served as the end-product of the denitrification process of strain YPW1; (B) Carbohydrate-active enzyme genes in strain YPW1 and other 10 representative genomes from Microbulbifer genus, indicating that strain YPW1 possessed more versatile genes for polysaccharide degradation and glycometabolism than other strains selected in this study.