Complete genome sequence of *Microbulbifer* sp. YPW1 from mangrove sediments in Yanpu harbor, China

Dingquan Wang¹ · Jianxin Wang¹ · Bonian Shui² · Longqiang Zhu¹ · Jiangqi Wang¹ · Linxi Jin¹ · Wu Qu¹

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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. A complete genome of YPW1 was sequenced and assembled. The 16S rRNA gene assigned strain YPW1 into genus *Microbulbifer*, and the maximum values of average nucleotide identity and digital DNA–DNA hybridization of ZHDP1 genome were 90.36 and 68.1, respectively, indicating that YPW1 was a potential new species in genus *Microbulbifer*. A total of 10 representative genomes from genus *Microbulbifer* were selected to compare with YPW1. The 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 potential novel member of genus *Microbulbifer* with special ecological roles in the cycles of carbon and nitrogen in mangrove ecosystems.

Keywords *Microbulbifer* sp. YPW1 · Whole genome analysis · Artificial mangrove · Polysaccharide degradation · Greenhouse gas

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 et al. 2016), mangroves are one of the centers of C-cycling in nature. On the other hand, mangroves are nitrogen (N)-deficiency environment (Love-lock 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 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 km². As the results shown in the investigations, benthic environment of this area has been obviously improved after the mangrove planting (Zhang et al. 2019). However, the ecology functions of the...
microorganisms in this artificial mangrove have rarely been reported for now.

Strains belonged to genus Microbulbifer were universally isolated from marine environments including coastal soil (Kämpfer et al. 2012), mangrove forests (Baba et al. 2011), marine algae (Nishijima et al. 2009), intertidal (Yoon et al. 2004) and deep-sea sediments (Miyazaki et al. 2008), and marine pulp mill effluent (González et al. 1997), and former studies have demonstrated that genus Microbulbifer possesses the abilities for marine polysaccharide degradation (Ohta et al. 2004; Kim et al. 2011; Vijayaraghavan and Rajendran 2012; Swift et al. 2014). In this study, a strain named YPW1 belonged to genus Microbulbifer 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 genus Microbulbifer, 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.

Materials and methods

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.

Isolation and identification of YPW1

The sediment samples with 10⁷-fold dilution using sterile sea water were spread on the 2216E plates (0.5% of peptone, 0.1% of yeast extract, 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 et al. 1991). Phylogenetic tree of 16S rRNA gene of YPW1 was conducted by using Mega 10.0 software with neighborhood-joining method.

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.

Annotation and analysis of the genomes from genus Microbulbifer

Canu v1.5 (Koren et al. 2017) and Pilon (Walker et al. 2014) were used for the genome assembly and data correction, respectively. Coding genes in the genome was predicted by Prodigal (Hyatt et al. 2010). tRNA genes were annotated using tRNAscan-SE (Lowe and Eddy 1997). rRNA and other noncoding RNA (ncRNA) genes were predicted with Infernal 1.1 (Nawrocki and Eddy 1997) based on the Rfam database (Nawrocki et al. 2015). The gene islands were analyzed using IslandPath-DIMOB software (Langille et al. 2008). The prediction of prophage in the genome was conducted using PhiSpy software (Akhter et al. 2012). The gene functions were annotated by BLAST software against NCBI nr database. The glycometabolism functions were annotated using HHMDER 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 genus Microbulbifer, all the 10 representative genomes with the definite taxonomic information on species level from genus Microbulbifer, including M. agarlyticus 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. mangrovii DD-13 (NZ_LZDE01000347.1), M. donghaiensis CGMCC 1.7063 (NZ_FQVA01000001.1), M. marinus CGMCC 1.10657
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Fig. 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 closest 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 gray and red represented that the G content was higher and lower than the C content, respectively.

The determination of polysaccharide degradation activities

Strain YPW1 was cultured at 28 °C for 24 h with an inoculation size of 1% (v/v), and the supernatant was collected by centrifugation at 5000 rpm for 15 min. A total of 1 mL supernatant of the fermentation broth was added into the polysaccharide solution (1%; m/v) including agarose, alginate, xylan, starch, pullulan, carboxymethyl cellulose, chitin, and pectate, respectively. The reaction solutions were incubated at 28 °C for 24 h, and the reducing sugar was detected by using 3,5-dinitrosalicylic acid (DNS) method with a spectrophotometer at the wave length of 550 nm (OD550). The control groups were set as the same conditions except that the fermentation broth was boiled for 10 min.

Results

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 genus Microbulbifer. 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 dDDH of ZHDP1 genome were, respectively, 90.36 and 68.1 and were lower than the thresholds (ANI < 95%–96%; DHD < 70%), indicating that YPW1 was a potential new species in genus Microbulbifer.
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

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                  | illumina Miseq and Oxford Nanopore GridION                                  |
| Assembly                          | Canu v1.5                                                                   |
| Finishing strategy                | Whole genome                                                                |
| Genome size                       | 4,578,595 bp                                                                |
| GC content                        | 57.64%                                                                      |
| 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                              |
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 oligosaccharides with low polymerization degrees and further produced monosaccharides (Fig. 2A). The degradation activities in the fermentation broth of strain YPW1 for the above-mentioned polysaccharides were all detected based on the added values of OD550 (Fig. 2B).

Ten genomes from other strains belonged to genus Microbulbifer 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. 2C). Therefore, strain YPW1 possessed more versatile abilities for polysaccharide utilization than other representative strains belonged to genus Microbulbifer.

**Denitrification ability of strain YPW1**

According to the genomes from genus Microbulbifer, 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 (N₂O). No N₂O reductase was found in the genome of YPW1. Therefore, N₂O served as the end-product of the denitrification process of strain YPW1 (Fig. 2A and Table 2).

**Discussion**

In this work, strain YPW1 assigned into genus Microbulbifer 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 genus Microbulbifer, 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 et al. 2004; Kim et al. 2011; Vijayaraghavan and Rajendran 2012; Swift et al. 2014) demonstrated that genus Microbulbifer 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 that 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 harbor mangroves.

Besides, YPW1 was the only strain that can denitriﬁ nitrate to N₂O among the 11 genomes analyzed in our work (Fig. 2A and Table 2). As the results shown, strain YPW1 was a special member in genus Microbulbifer that can produce N₂O, a greenhouse gas more powerful that CO₂ (Kampschreur et al. 2009). Therefore, strain YPW1 was not only a center for various polysaccharide degradation but also a potential denitriﬁer for nitrogen cycle in mangroves. 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.

**Conclusion**

In this study, a potential new species belonged to genus Microbulbifer 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 genus Microbulbifer, YPW1 have the genetic materials that reduce nitrate into N₂O, a powerful greenhouse gas, as the end-product of its denitrification process. Therefore, strain YPW1 played a potential special ecological role in energy source providing and greenhouse gas emission, and the abundance and biological activity of this strain could have implications for the livings in this mangrove and climate warming.
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 sp. YPW1 | 5 | 2 | 1 | 0 |
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Availability of data and material The genome sequence has been deposited in Genbank database under the accession no. CP055157.

Code availability Not applicable.

Declarations

Conflict of interest Authors have no conflict of interest to declare.

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