Complete genome sequence of *Chryseobacterium* sp. ZHDP1 with protease activity

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Abstract

This study reported a complete genome of *Chryseobacterium* sp. ZHDP1 isolated from the soils of a seafood market. The ZHDP1 genome with a size of 4,917,748 bp and a GC content of 35.95% possessed 4,478 coding genes, 5 rRNA genes, 26 sRNA genes, and 89 tRNA genes. The 16S rRNA gene sequence of ZHDP1 had a maximum similarity of 99.07% with that of *C. gambrini* 5-1St1a. The maximum values of average nucleotide identity and DNA-DNA hybridization of ZHDP1 genome were 91.39 and 47.8, respectively, which were lower than the thresholds for a new genome. Different protease genes were annotated in the genome of ZHDP1, and the protease activity was also detected in the fermentation broth of ZHDP1. Furthermore, the activity of protease in the fermentation broth was optimized through temperature, pH, and metal irons, and the results showed that 60°C and pH 7.0 were the optimum conditions and Fe^{3+} could positively increase the protease activity of ZHDP1. This study provides the first insight into the novel genomic information of *Chryseobacterium* sp. ZHDP1 and its protein-degrading ability, thereby broadening our knowledge of the industrial potentials in genus *Chryseobacterium* strains.

1. Introduction

Genus *Chryseobacterium* strains have been widely isolated from clinical specimens (Nicholson,Gulvik et al. 2020), soils (Dahal, Chaudhary et al. 2021), wastewater (Kämpfer, Dreyer et al. 2003), and plant roots (Park et al. 2006). The taxonomic information and functions of genus *Chryseobacterium* are also reported in former studies. Usually, genus *Chryseobacterium* strains emerge as causative agents in infected animal and human tissues (Lin, Lai et al. 2017). However, genus *Chryseobacterium* strains are recently verified with other properties. For example, *C. antibioticum* RP-3-3 isolated from Arctic soil shows antimicrobial activity against Gram-negative pathogens (Dahal, Chaudhary et al. 2021). A novel alkali-stable α-amylase has been isolated from *C. taeanense* TKU001 which possesses the ability of starch degradation (Wang, Liang et al. 2011). *Chryseobacterium* sp. RBT isolated from the soils is able to degrade keratinous waste including silk, human hair, wool, and chicken feathers (Gurav, Jadhav et al 2013). Genus *Chryseobacterium* strains are potential sources of microbial proteases which are of great values in pharmaceutical, food, waste disposal, and detergent industries (Boominadhan, Rajakumar et al. 2009). Many *Chryseobacterium* strains, such as *C. taeanense* TKU001 (Wang, Liang et al. 2008), *C. aquaticum* PUPC1 (Gandhi Pragash, Narayanan et al. 2009), *C. indologenes* TKU014 (Wang, Liang et al. 2008), and *C. oranimense* H8 (Hantsis-Zacharov, Shaked et al. 2008) are found with protease activities. In this work, *Chryseobacterium* sp. ZHDP1 with a novel genome was isolated, and the protease genes were annotated in this genome and the protease activity was also optimized. Our study supplements a potential new protein-degrading member and its genomic information in *Chryseobacterium* genus, which lays a foundation for the future study on the industrial application of this strain.

2. Materials And Methods

2.1 Isolation, purification, and genomic DNA extraction of ZHDP1

The sample for isolation was the soils collected from a seafood market located in Shenjianmen, Zhoushan City, China (29.95°N, 122.30°E). Approximate 10 g soils were collected into a sterile centrifuge tube by using a sterile medicine spoon. The sample was stored on ice and was immediately transferred back to the lab. The sample was kept at 4°C in the lab and the isolation must be finished in 7 days for ensuring the freshness of the sample. The sample was diluted 10^6 times with sterile physiological saline and 1 mL of diluent was spread on Casein Agar medium (10.0g/L Casein, 3.0g/L beef extract powder, 5.0g/L NaCl, 2.0g/L Na_2HPO_4, 15.0g/L agar, 0.05g/L bromothymol blue in 1 L distilled water). The plates were cultured at room temperature for 24 h. The single colonies with clear zones were selected as protein-degrading strains and were streak on Casein Agar medium three times for purification. The genomic DNA of the selected strains was extracted by using TIANamp Bacteria DNA Kit (Tiangen, China). The integrity and purity of the extracted DNA were verified by 1% agarose gel electrophoresis and Nanodrop 2000, respectively.

2.2 Genome sequencing and annotation

The sequencing of genomic DNA was conducted by Nanopore (Oxford Nanopore technologies, UK) and Illumina (Illumina, Inc., USA) platforms. The pass reads from Nanopore and clean reads from Illumina were selected for further assembly. The assembly of the genome was performed with unicycler software (0.4.8). Then, Prodigal, Aragorn, barrnap, and Infernal were used for predicting the coding genes, tRNA, rRNA, and mRNA genes, respectively. BLAST software was used for function annotations of genes against Cluster of Orthologous Groups of proteins (COG, http://blast.ncbi.nlm.nih.gov/Blast.cgi), Kyoto Encyclopedia of Genes and Genomes (KEGG, https://www.kegg.jp/), UniProt (https://sparql.uniprot.org/), and Reference Sequences (Refseq, ftp://ftp.ncbi.nlm.nih.gov/refseq/release/) databases and HMMER software was used for annotating the gene functions against Pfam (http://pfam.xfam.org/) databases. PHASTER (http://phaster.ca/) and IslandViewer 4 (http://www.pathogenomics.sfu.ca/islandviewer/) were used for the prediction of prophages and gene islands in the genome. The values of average nucleotide identity (ANI) and DNA-DNA hybridization (DDH) were calculated based on the genomes of *Chryseobacterium* strains and ZHDP1 by using the websites of https://www.ezbiocloud.net/tools/ani and https://tygs.dsmz.de/user_requests/new, respectively. The genome sequence was deposited at NCBI Genbank under accession no. CP075682.

2.3 Determination and optimization of the protease activity in the fermentation broth of ZHDP1

Strain ZHDP1 was cultured in the liquid medium of casein at 28°C for 36 h. The supernatant of the fermentation broth of ZHDP1 was collected by centrifugation at 3500 rpm and 4°C for 10 min and the supernatant was treated as crude protease solution for further research within 12 h. One milliliter of protease solution was mixed with 1.0 mL of 1% casein solution in 0.02 M phosphate and incubated in a

water bath at 30°C for 10 min. Two milliliters of 0.4 M trichloroacetic acid were added into the mixture to terminate the reaction, then the mixture was centrifuged at 6,000 ×g for 5 min. Next, 1 mL of the solution, 5 mL of Na_2CO_3 (0.4 M), and 1 mL of Folin reagent were mixed into a 10 mL clean tube and was incubated at 35°C for 15 min. The absorbance value of the solution was detected at 680 nm using a UV-visible spectrophotometer. The control sample was prepared by adding trichloroacetic acid solution prior to adding protease solution. In this work, the unit of enzyme activity (U) was defined as the amount of enzyme required to decompose 1 ug tyrosine per minute under certain conditions. The protein concentration was determined by folin-ciocalteu method with casein as standard.

3. Results And Discussion

3.1 Description of strain ZHDP1 and its genomic information

A colony with a clear zone was obtained during the isolation process, indicating the protease activity of this strain named ZHDP1. The sequencing depths were ~230.57X and ~204.67X for the reads from Illumina and Nanopore platforms, respectively. A complete genome of ZHDP1 without any gap was assembled. The size of this genome was 4,917,748 bp and the GC content was 35.95%. A total of 4,478 coding genes was annotated in the genome, and 5 rRNA genes, 26 sRNA genes, and 89 tRNA genes were predicted. Nine gene islands and 1 prophage were found in the genome. Most genes were assigned into “Global and overview maps” (241 genes), “Carbohydrate metabolism” (252 genes), and “Amino acid metabolism” (235 genes) pathways according to the results of KEGG annotation (Figure 1A). A total of 160 and 138 genes were annotated into “Translation, ribosomal structure and biogenesis” and “Amino acid transport and metabolism” COG groups, respectively (Figure 1B). Both of the annotation results showed the genes related to amino acid metabolism were abundant in the genome, indicating that strain ZHDP1 had a developed metabolic system that was used to treat the hydrolysates of proteases, which endows this strain with the potential in the food industry (Neis, Dejon et al. 2015, Lin, Liu et al. 2017). The general characteristics of the genome were listed in Table 1 and Figure 1C.

3.2 Identification of strain ZHDP1 based on the genomic information

Based on the 16S rRNA gene sequences, strain ZHDP1 had a maximum identity of 99.07% and was phylogenetically closed to *C. gambrini* DSM 18014 (Figure 2). Therefore, strain ZHDP1 was assigned into genus *Chryseobacterium*. The colony of strain ZHDP1 was circular, orange, and raised, which is similar with the former species (Kämpfer, Dreyer et al. 2003, Park, Jung et al. 2006, del Carmen Montero-Calasanz, Göker et al. 2013). Nevertheless, the maximum values of ANI and DDH of ZHDP1 genome that calculated with other genomes from genus *Chryseobacterium* were 91.39 and 47.8, respectively, which were lower than the thresholds (ANI < 95%-96%; DDH < 70%) (Goris, Konstantinidis et al. 2007, Yoon, Ha et al. 2017) for a new genome (Table 2).

3.3 Protease genes and activity optimization of strain ZHDP1

A total of 27, 22, 23 protease genes, including the genes of metalloprotease, serine protease, lon protease, thiol protease, tail-specific protease, rhomboid protease, and alkaline protease, were annotated in the genome of this potential new species by using Uniprot, Pfam, and Refseq databases, respectively, indicating that strain ZHDP1 is a novel protein-degrading member in genus *Chryseobacterium* in addition to previously reported strains. Except for the analysis at genome level, the activity of protein degradation by ZHDP1 was verified and optimized in this study. The factors of temperature, pH, and metal ions could affect the protease activity in the fermentation broth of ZHDP1. The results showed that the optimum temperature and pH were 60°C and 7.0, respectively. Fe^{3+} could mostly increase the protease activity in the broth. Finally, the maximum activity reached 25.7 U/mL under the optimum conditions (Figure 3). The protease genes annotated in the genome not only are beneficial for the related industries but also are possible participants in glycoprotein link (Parente, Casabuono et al. 2014), immune response (Lad, Yang et al. 2007), biofilm formation (Marr, Overhage et al. 2007), and pathogenesis (Miyoshi, Shinoda 2000). Therefore, the protease genes make strain ZHDP1 become a potential novel member of genus *Chryseobacterium* with values for industrial production, medical treatment, ecological research, and molecular biology.

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. The general features and MIGS information of the genome of ZHDP1.

Items	Description
MIGS data	
Geographic location	Shen jiamen seafood market (29.95°N, 122.30°E)
Collection date	6/2020
Isolation source	Soils
Habitat	Soils
Sequencing platform	Illumina Miseq, Nanopore
Assembler	unicycler (0.4.8)
Coverage	230.57X (Illumina)□204.67X (Nanopore)
General feature	
Classification	
Domain	Bacteria
Phylum	Bacteroidetes
Class	Flavobacteriia
Order	Flavobacteriales
Family	Weeksellaceae
Genus	*Chryseobacterium*
Genomic feature	
Contigs	1
Genome Size	4,917,748
GC Content	35.95
CDS	4478
RNAs	83

Table 2. The ANI and DDH values that were calculated based on the genomes of ZHDP1 and other *Chryseobacterium* strains.

Stains	ANI	DDH
C. gambrini DSM 18014	91.39	47.8
C. caeni DSM 17710	74.58	28.9
C. arachidis DSM 27619	81.97	25.8
C. gleum NCTC 11432	80.21	25.6
C. gleum ATCC 35910	80.79	25.5
C. hispalense DSM 25574	80.56	25.5
C. cheonjiense RJ-7-14	80.25	24.7
C. takakiae DSM 26898	80.42	24.4
C. geocarposphaerae DSM 27617	80.33	24.3
C. taichungense DSM 17453	80.74	24.2
C. taihuense CGMCC 1.10941	79.38	23.6
C. limigenitum SUR2	80.09	23.1
C. polytrichastri DSM 26899	77.66	22.7
C. daecheongense DSM 15235	78.72	22.2
C. defluvii DSM 14219	78.51	22.1
C. zeae DSM 27623	73.67	21.4

Figures

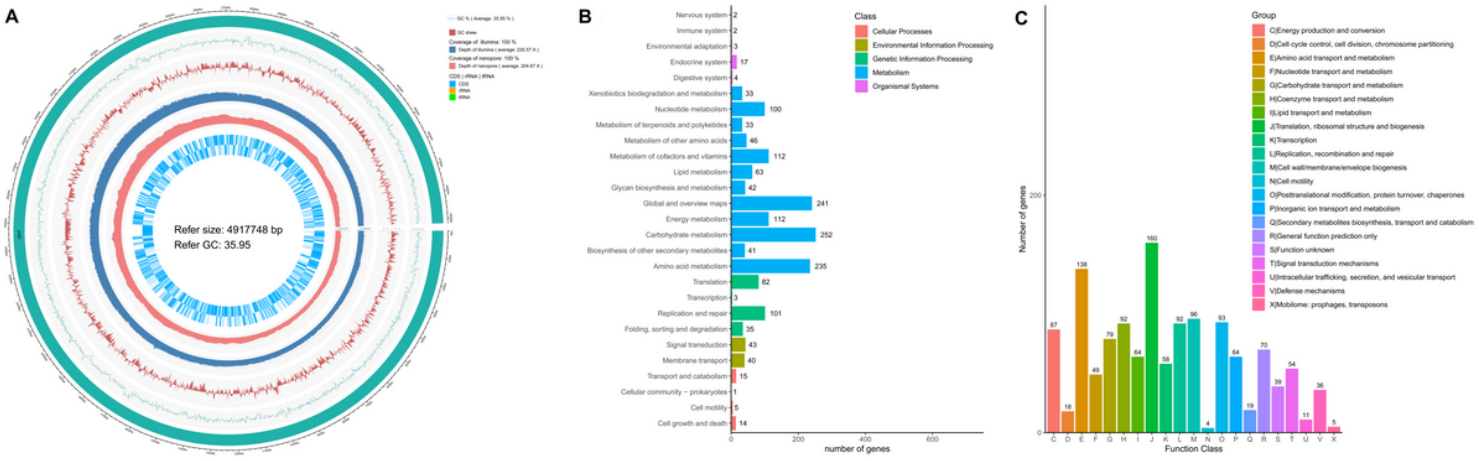

Figure 1

(A) Genome circular map of ZHDP1. The meanings of the circles from outside to inside are genome sequence scales, GC contents, GC skew, depth and coverage of sequencing of Illumina and Nanopore platforms, and genes (coding and non-coding) on the positive and negative strands, respectively; (B) Annotation results of the genes in ZHDP1 genome against KEGG database; (C) Annotation results of the genes in ZHDP1 genome against COG database.

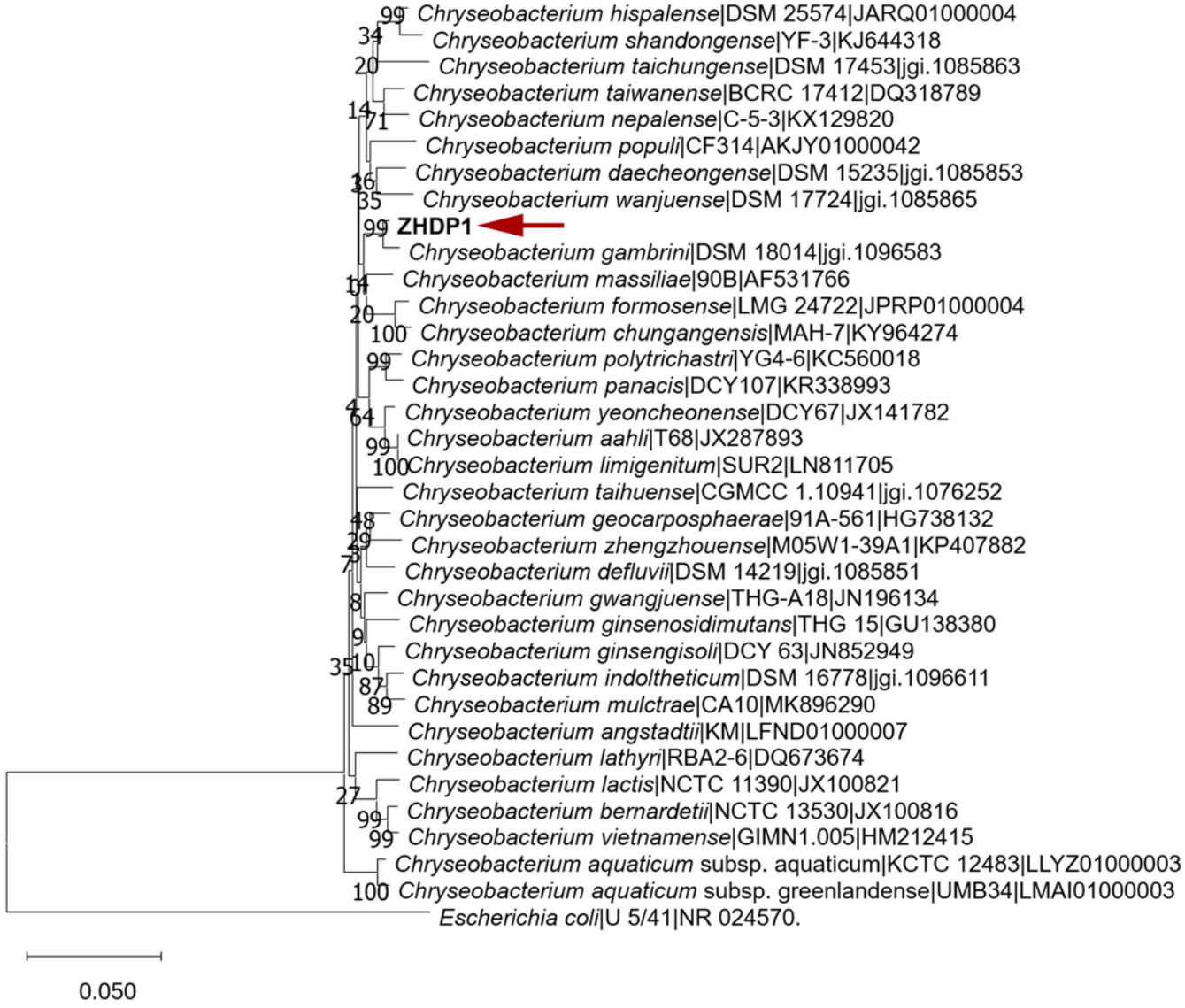

Figure 2

Phylogenetic tree constructed based on the 16S rRNA gene sequences of ZHDP1 and other genus Chryseobacterium strains by using neighborhood-joining method.

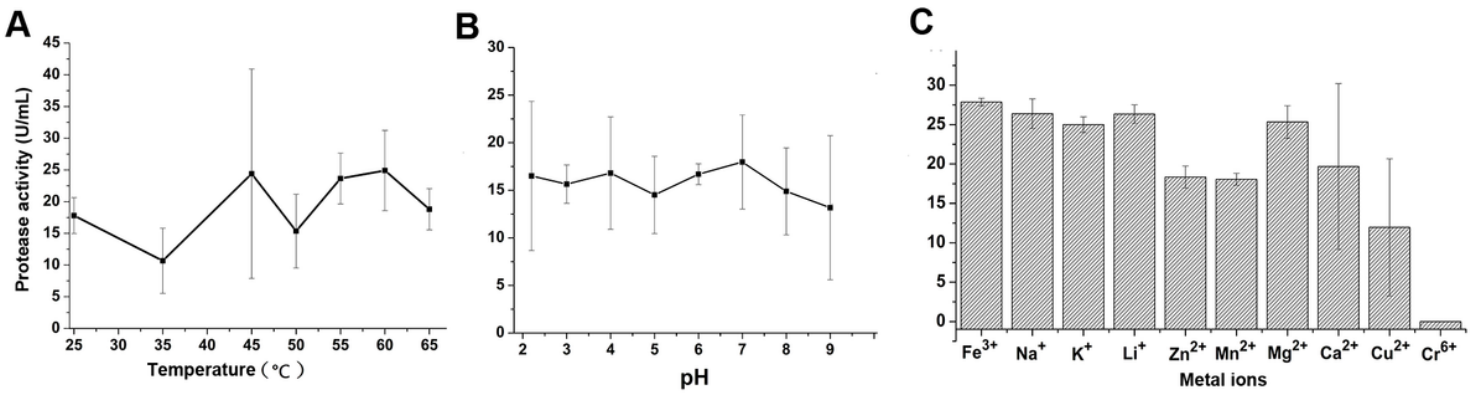

Figure 3

Optimization of the protease activity in the fermentation broth of ZHDP1 through temperature (A), pH values (B), and metal ions (C).