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Original article

Signs of biofilm formation in the genome of *Labrenzia* sp. PO1Madiha Zaynab<sup>a,b</sup>, Huirong Chen<sup>b</sup>, Yufei Chen<sup>b</sup>, Liao Ouyang<sup>a,b</sup>, Xuwei Yang<sup>b</sup>, Zhangli Hu<sup>b</sup>, Shuangfei Li<sup>b,\*</sup><sup>a</sup> College of Physics and Optoelectronic Engineering, Shenzhen University, Shenzhen 518060, China<sup>b</sup> Shenzhen Key Laboratory of Marine Bioresource & Eco-environmental Sciences, College of Life Sciences and Oceanography, Shenzhen University, Shenzhen, Guangdong 518071, China

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## ABSTRACT

*Labrenzia* sp. are important components of marine ecology which play a key role in biochemical cycling. In this study, we isolated the *Labrenzia* sp. PO1 strain capable of forming biofilm, from the *A. sanguinea* culture. Growth analysis revealed that strain reached a logarithmic growth period at 24 hours. The whole genome of 6.21813 Mb of *Labrenzia* sp. PO1 was sequenced and assembled into 15 scaffolds and 16 contigs, each with minimum and maximum lengths of 644 and 1,744,114 Mb. A total of 3,566 genes were classified into five pathways and 31 pathway groups. Of them, 521 genes encoded biofilm formation proteins, quorum sensing (QS) proteins, and ABC transporters. Gene Ontology annotation identified 49,272 genes that were involved in biological processes (33,425 genes), cellular components (7,031 genes), and molecular function (7,816 genes). We recognised genes involved in bacterial quorum sensing, attachment, motility, and chemotaxis to investigate bacteria's ability to interact with the diatom phycosphere. As revealed by KEGG pathway analysis, several genes encoding ABC transporters exhibited a significant role during the growth and development of *Labrenzia* sp. PO1, indicating that ABC transporters may be involved in signalling pathways that enhance growth and biofilm formation.

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## 1. Introduction

Bacteria and phytoplankton are two important marine ecosystem components (Sarmiento and Gasol, 2012). During the past few decades, several investigations found interactions between phytoplankton-bacteria and their potential activities during harmful algal bloom (HAB) occurrence and termination (Buchan et al., 2014; Teeling et al., 2012). Research findings have already shown that bacteria live in free and particulate attached forms and interact with phytoplankton in the aquatic world. Specific interactions often occur in the phycosphere, a micro-environment that defines close spaces around phytoplankton cells, in which bacteria of phycosphere grow according to the extracellular products secreted by phytoplankton (Bell and Mitchell, 1972). In return, bacteria generate important components (Bertrand et al., 2015; Kazamia et al.,

2012) to promote phytoplankton growth (Amin et al., 2012). Phytoplankton-bacteria symbiosis is considered a phenomenon of significant importance in the availability of nutrients and essential biogeochemical cycles (Buchan et al., 2014; Durham et al., 2019).

In terms of our knowledge, bacterial behaviour processes for the control of phycosphere bacterial attachment and motility, have not been thoroughly studied. The widespread HAB community has long been established as strong associations between dinoflagellate and bacterial phycosphere while bacteria's pivotal function in dinoflagellate growth has been already confirmed (Alavi et al., 2001). It has long suspected that the related bacteria are responsible for the host dinoflagellates' toxic production (Lafay et al., 1995). Some researchers have questions about the association between dinoflagellate toxicity and bacteria (Green et al., 2010; Jauzein et al., 2015). Most of the studies support the most prevalent bacterial community within the marine diatom and dinoflagellate phycosphere being *Rhodobacteraceae*, particularly the roseobacter clade (Geng and Belas, 2010). Successful phycosphere colonisation can be improved by unique genetic characteristics, such as motility, chemotaxis and surface attachment (Raina et al., 2019; Slightom and Buchan, 2009). Chemotaxis allows bacteria to sense differences in local food concentrations and thus regulates motility. Thus genes related to motility and flagellar seem essential for attachment and biofilm formation in many roseobacter (Bruhn et al., 2007; Miller and Belas,

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2006). Many bacteria have a biphasic 'swim-or-stick' lifestyle in the phycosphere to easily find the food sources, thus minimising energy costs until finding the food. Bacteria use chemotaxis to find phytoplankton cells in the motile phase (Seymour et al., 2017). In the phycosphere, 'switch' is turned on, which allows the bacteria to migrate into the sessile lifestyle, the phenotype entails the loss of flagella and the further development of biofilm (Geng and Belas, 2010).

Microalgae-bacteria interactions studied thus far; nutrient seems to play a significant role (Fuentes et al., 2016). Overall, the association of microbial cells with the substratum surface at the seawater/surface interface under specific nutritional conditions would possibly lead significantly to the initiation and progress of microbial colonisation in marine environments (Dang and Lovell, 2016). This colonisation depends on the the species' ability to acquire essential nutrients from the surrounding environment (Naik et al., 2018). Vitamin B12 and iron are critical for phytoplankton development (Croft et al., 2005), and Iron is another essential bacteria micronutrient.

Acquisition of iron and transportation have significant phycosphere consequences. Some bacteria associated with dinoflagellate allow dinoflagellates to assimilate iron (Amin et al., 2009; Roe et al., 2013). For transporting these nutrients, transporter proteins are required and are a significant factor in host-bacterial interactions (Bleves et al., 2020; Eisenreich et al., 2019).

Furthermore, various transporters have identified as responsible for the transportation of signalling molecules. Quorum sensing (QS) is one of the most well-studied bacterial cell-to-cell communication signalling mechanism. Many bacteria have demonstrated QS by secreting small signalling molecules called autoinducers, which can evaluate changes in the populations of bacteria and co-ordinate the gene expression in the population as a whole (Waters and Bassler, 2005). Autoinducers of bacteria are acyl-homoserine lactones (AHLs) used to regulate functions that are collectively beneficial to perform, including the motility, virulence, and biofilms (Antunes et al., 2010; Daniels et al., 2004; Hammer and Bassler, 2003).

*Akashiwo sanguinea* is a common alga that causes environmental issues worldwide. It has been the most prevalent producer of harmful algal blooms (HABs) in China's coastal regions (Menden-Deuer and Montalbano, 2015; White et al., 2014) (Lu and Hodgkiss, 2004). Observations of the epibiotic microbial communities of laboratory cultures of *A. sanguinea* revealed that the species abundance and composition of the microbial community are directly related to the physiological state of the dinoflagellates. These insights are the foundation of our ongoing investigations to understand further the functions of different phycosphere bacteria (Bolch et al., 2011). Genus *Labrenzia* is a member of the class *Alphaproteobacteria* (Amiri Moghaddam et al., 2018). *Labrenzia* consists of more than 100 genera and over 300 species (Pujalte et al., 2014a, 2014b; Xu et al., 2019). Representatives of *Rhodobacteraceae* exhibit very diverse physiology in marine environments and are the leading players in biogeochemical cycling (Pujalte et al., 2014a, 2014b; Xu et al., 2019). Genomes of *Labrenzia* sp. PHM005 and *Labrenzia* sp. CP4 strains and transcriptome of *Labrenzia aggregata* LZB033 have been well studied previously (Galan et al., 2019). However, information about the genome of *Labrenzia* species is limited. In the present study, we quantitatively examined the *De novo* genome of *Labrenzia* sp. PO1 isolated from the logarithmic growth phase of *A. sanguinea* algae. *De novo* genome assembly enables functional genomics research in species with limited genetic information, especially in non-model organisms. In the present study, the complete genome sequence of *Labrenzia* sp. PO1 was obtained using second- and third-generation sequencing technologies. Further, pathway enrichment, as well as enrichment of broader gene ontology categories, were performed. The current

results provide new insight into *Labrenzia* sp. PO1 at the genomics level, suggesting several gene targets that might be involved in the nutrient transport and biofilm formation.

## 2. Materials and methods

### 2.1. Bacterial strain isolation

Plate streaking and serial dilution methods were used to isolate the culturable bacteria from the cultures of *A. sanguinea* under Log growth phase. The isolated bacteria have been maintained at 30 °C on Zobell marine agar 2216E (agar: 15 g, ferric phosphate: 0.5 g, yeast extract: 0.5 g) in per litre seawater and R2A (agar: 15 g, MgSO<sub>4</sub>·7H<sub>2</sub>O K<sub>2</sub>HPO<sub>4</sub>: 0.3 g, sodium pyruvate: 0.3 g, soluble acid: 0.5 g, casamino acids: 0.5 g, protease peptone: 0.5 g and yeast extract :0.5 g) in per litre distilled water.

Slow-growth of less culturable strains, was promoted by streaking the cultures of *A. sanguinea* are streaked on agar plates of R2A and 2216E medium and incubated for over one month until single colonies were obtained. Bacterial DNA (Omega, USA) Kit was used to extract the genomic DNA following the manufacturer's instructions.

The ribosomal sequences 16S subunits were amplified with primers 27F/1492R (27F: 5' -AGATTGATCTGCTCAG-3' -1492R: 5' -TACGGCTACCTGTTACGACT -3' um) with initial denaturation at 94 °C for 5 minutes, accompanied by 30 cycles of 94 °C for one minute each, annealing at 55 °C for one minute, extension at 72 °C for 1 minute, and final elongation at 72 °C for 10 min. The taxon of bacteria was defined in GenBank using BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) for *Labrenzia* PO1 sequence.

### 2.2. Bacterial strain growth

*Labrenzia* sp. PO1 isolates were preserved on Zobell marine agar 2216E (per L of seawater, 15 g of agar, 0.1 g of ferric phosphate, 1 g of yeast extract, 5 g of peptone) at 30 °C before collecting single colonies. PO1 subcultured on 2216E agar. A single colony was reinoculated on 100 mL 2216E and cultured at 30 °C and 200 rpm for at least 18 h. One hundred µL of this crop was inoculated on 100 mL of fresh 2216E agar and cultivated at 30 °C. Samples of bacteria were obtained from 10-fold serial dilution in autoclave-purified water at 0 and 2 h, respectively, from 12 to 42 h after inoculation. By patting the ideal dilutions on 2216E agar, colony-forming units were quantified. The growing sample was examined three times, and the experiment was repeated three times.

### 2.3. Scanning electron microscopy method

The *Labrenzia* sp. PO1 streaked, and a single colony was used to inoculate 100 mL of 2216E medium at 30 °C and 200 rpm for 24 h. One mL of bacterial solution was taken and centrifuged at 8000 rpm for 3 min; after discarding the supernatant, the bacterial pellet was washed three times with phosphate buffer. Then 1 mL 2.5% pentanediol fixing solution was added and fixed at four-degree Celcius for 4 h, again centrifuged at 8000 rpm for 3 min, and discarded the supernatant. Washing three times with phosphate buffer. Dehydrated with 15%, 50%, 80%, and 100% ethanol gradients respectively. Each gradient was allowed to stand for 7 min and then centrifuged as mentioned above, and the upper phase was discarded. After dehydration, resuspended in 1 mL sterilised pure water, taking 1 µL bacterial suspension onto a dehydrated silicon wafer, and freeze-dried for 12 h. After drying and dehydration, the sample was sprayed with a conductive coating, and its morphology was observed by scanning electron microscope.

#### 2.4. Bacterial biofilm formation

A single colony was selected from streaked bacteria and inoculated in sterilised 2216E medium and incubated at 200 rpm, 30 °C for 24 h. 200 µL of the bacterial solution was added to the wells of 96-well plates, and three biological replicates were performed for each strain. After incubation at 30 °C for 12 h, wells were washed three times with 0.9% physiological saline to remove free bacteria. After drying at 60 °C, bacteria were stained with 0.1% crystal violet for 5 min and washed with 0.9% physiological saline three times. After drying, 250 µL of 33% acetic acid was added to each well. After the crystal violet was fully dissolved, absorbance at OD590 was determined with a microplate reader (OD590 > 0.2 is positive). Isolated and purified bacteria were incubated in 100 mL of 2216E liquid medium at 200 rpm and 30 °C with shaking to the bacterial suspension logarithmic phase (concentration of the bacteria reached 108 cell mL<sup>-1</sup> after 24 h of incubation). The bacterial solution to be tested was mixed with *A. sanguinea* liquid (algae concentration up to 104 cell mL<sup>-1</sup>, logarithmic growth period) in a ratio of 1:1, i.e., add 1 mL of bacterial liquid and 1 mL of algae liquid to each well of a 12-well plate. A blank control group was set up, and 1 mL of algal solution and 1 mL of 2216E medium were added to the control. Kept in a light incubator and shake manually at regular intervals 2 to 3 times a day (within 2 to 3 days). They were observed under the microscope to determine whether the isolated bacteria have algae dissolving ability.

#### 2.5. Preparation of DNA and library construction

The PacBio (BGI, Shenzhen, China) and MGI2000 (MGI, Shenzhen, China) sequencing platforms were used to sequence *Labrenzia* sp. PO1 genome. A 50-mL culture of *Labrenzia* sp. PO1 was centrifuged for 10 min at 8000 rpm for genomic DNA extraction. The cell pellets were ground in liquid nitrogen and treated with 5 mL DNA extraction buffer. After that, the cells were resuspended in 5 mL phenol/chloroform/isoamyl alcohol (25:24:1) and centrifuged twice at 9000 rpm for 16 min. The cells were subsequently resuspended in an equivalent volume of chloroform and centrifuged for 16 min at 12,000 rpm. Finally, the pellet was resuspended in 2.5 × volume of 100% ethanol to precipitate genomic DNA. The third-generation PacBio sequencing technology was used to sequence *Labrenzia* sp. PO1. The purity, integrity, and concentration of genomic DNA were determined using gel electrophoresis (1.2% agarose gel, 120 V, 50 min) and Qubit fluorometer version 2.0 (Invitrogen, Carlsbad, CA, USA) before library construction. A total of 10 µg genomic DNA was fragmented using g-TUBE (Covaris, Woburn, MA, USA) for library construction, and 10-kb DNA fragments were obtained using the Agencourt AMPure XP-Medium kit (Beckman Coulter, Brea, CA, USA). Exonuclease ExoVII (New England Biolabs, Ipswich, MA, USA) digestion was performed to generate the DNA fragments and subject to end repair were linked to the hairpin structure on both sides to form the SMRTbell dumbbell structure. The library was constructed successfully for PacBio sequencing after purification and sorting of DNA fragments. After the annealing and binding polymerase steps, qualified library fragments were sequenced. The genome sequencing data of *Labrenzia* sp. PO1 were uploaded to the National Center for Biotechnology Information Search database (NCBI) (BioProject ID: PRJNA579065; BioSample accession: SAMN13135800; SRA accession: PRJNA579065). The genome sequence data of *Labrenzia* sp. PO1 was uploaded to the National Center for Biotechnology Information Search database (NCBI) (SUB7234302PRJNA614627 SAMN14435810JAAXCR0000000000 *Labrenzia* sp. PO1). The library construction methods used for MGI2000 (MGI, Shenzhen, China) and PacBio (BGI, Shenzhen, China) platforms were identical.

#### 2.6. Fragment assembly and gene annotation

The HGAP (Chin et al., 2013) software used to correct the sequencing data, high-quality error-corrected data used for assembly, and Finally, it optimised the assembly results to obtain high-quality genome assembly sequences. Canu (Koren et al., 2017) is an assembly software specially adapted for PacBio sequences. It corrects algorithm reads and trims suspicious regions, and finally assembles high-quality genome sequences. At the present study present, we use SMRT Analysis software's P\_ModificationDetection and P\_MotifFinder modules for genome base modification and Motif analysis. Glimmer (Gene Locator and Interpolated Markov ModelER) (Delcher et al., 1999) is a tool for finding microbial DNA genes. The software uses interpolated Markov models (IMMs) to identify coding regions and distinguish them from non-coding DNA, with low false-positive predictions. Glimmer is the preferred software for microbial gene structure prediction in TIGR (The Institute for Genomic Research) database. So far, Glimmer has successfully annotated the gene structure of more than 100 bacteria. We used Glimmer version 3.02 software to predict the gene structure of the bacterial genome. tRNAscan-SE (Lowe and Eddy, 1997) can scan the whole genome of tRNA. The software integrates various analysis tools, which can identifying 99% of tRNA genes with extremely low false positives. While providing tRNA prediction results, the software can also predict the secondary structure of tRNA. We use tRNAscan-SE version 1.3.1 for tRNA prediction of the bacterial genome. RNAmmer (Lagesen et al., 2007) integrates a hidden Markov model, predicting 5 s/8s, 16 s/18 s, 23 s/28 s ribosomal RNA of the whole gene. We use RNAmmer version 1.2 to predict the rRNA of the bacterial genome. The Rfam database can be used to identify non-coding RNA (ncRNA) of prokaryotes. The database contains a large number of RNA family data information, and each RNA family contains a large number of multiple sequence alignments and covariance models (CMs). The Rfam 12.0 version (Griffiths-Jones et al., 2005), which contains 2450 families. This version is the first version based on Infernal 1.1. Use cmscan in Infernal to find ncRNA. We use Rfam 12.0 and Infernal to find the ncRNA structure of the genome. To explore the repetitive sequences in the bacterial genome, we use RepeatMasker to find various types of repetitive sequences in the whole genome. We use trf407b Linux (Benson, 1999) and MISA to search for the tandem repeat TR and microsatellite sequence SSR of the strain. CRISPR (clustered regularly interspaced short palindromic repeat sequences) is a cluster of regularly spaced short palindromic repeat sequences. Mining CRISPRs in Environmental Datasets, (MinCED) is a tool for analysing bacterial genome and metagenomic CRISPR elements. We used MinCED0.2.0 ([github.com/ctSkennerton/minced](https://github.com/ctSkennerton/minced)) to predict the CRISPR structure of the bacterial genome. For the generation corresponding functional annotation data, these sequences were compared with protein databases, such as SwissProt (Version: release-2017-07), Kyoto Encyclopedia Genes and Genomes (KEGG) (Version: 81), and Gene Ontology (GO) (Version: releases\_2017-09-08) using BLAST. Because each alignment result was more than one, therefore we selected the optimal alignment result to ensure that the biological significance as gene annotation. A separate BLAST search was conducted to compare sequence with the Eukaryotic Cluster of Orthologous Groups (KOG) and KEGG databases against both the KEGG (<https://www.genome.jp/kegg/>) and the KOG (<http://www.ncbi.nlm.nih.gov/COG/>) databases. The SwissProt ([https://web.expasy.org/docs/swiss-prot\\_guideline.html](https://web.expasy.org/docs/swiss-prot_guideline.html)) and GO databases (<https://www.ebi.ac.uk/ols/ontologies/go>) were also used for GO and SwissProt annotations. The kinase database (<http://ekpd.biocuckoo.org/faq.php>) and HMMER (v.3.0) with default parameters were used for the annotation and predic-

tion of Protein Phosphatases Database (EKPD) and kinase domains in the Eukaryotic Protein Kinases.

### 2.7. Phylogenetic Analysis

For sequence alignment studies, 18S rRNA sequences were retrieved from the NCBI database. The NCBI blast feature was used to determine the similarity of *Labrenzia* sp. PO1 sequence with that of the reference strains. The accession numbers of the reference strains are listed in (Table 5). A Phylogenetic tree was constructed for the reference strains and *Labrenzia* sp. PO1 using the MEGA software (Version: 7.0.26; algorithm: neighbour-joining method, the maximum composite likelihood method for the evolutionary distances; related website: <https://www.megasoftware.net/>).

### 2.8. Statistical Analysis

Analysis of variance (ANOVA) was performed, and statistically significant differences among group mean at  $P < 0.05$  were determined by the least significant difference (LSD).

## 3. Results

### 3.1. *Labrenzia* sp. PO1 Isolation

Bacterial strain *Labrenzia* PO1 was isolated and identified in the present study. Their 16S rRNA sequence accession numbers and other related 16S rRNA sequences in the NCBI GenBank database shown in (Table 1). This strain was isolated in the log Phase, this isolated strain being *Rhodobacteraceae* members, particularly the *Roseobacter* clade (Table 1).

### 3.2. Growth and characteristics of *Labrenzia* sp. PO1

The *Labrenzia* sp. PO1 strain belonged to the *Labrenzia* and was isolated from the *A. sanguinea* culture. Growth analysis (Fig. 1 A) revealed that strain reached a period of logarithmic growth. For *Labrenzia* sp. PO1, the maximum number of cells,  $2.0 \times 10^4$ , was reached at 24 h. The *Labrenzia* sp. PO1 morphological characteristics were evaluated by SEM (Fig. 1). The colony of *Labrenzia* sp. PO1 is round, with smooth edges. The PO1 strain exhibited behavior and shape on the plate shown in (Fig. 2). However, their color PO1 was milky white. If the plate was left for more than a week, a blackening phenomenon occurred from the edges to the single colony's centre. *Labrenzia* sp. PO1 cells can form a biofilm (Table 1).

### 3.3. Genome sequencing and de novo assembly

A total of 6.21813 Mb raw data and 6005 annotated genes were generated by PacBio (BGI, Shenzhen, China) and MGI2000 (MGI, Shenzhen, China) platforms. After eliminating short adapter sequences and poor-quality data, high-quality subreads were generated by the PacBio platform. The statistical data for *Labrenzia* sp. PO1 subreads are shown in (Table 2), and the subreads' length distribution in (Fig. 3). As shown in (Table 2), 15 scaffolds and 16 contigs were generated following the sequencing assembly. A total of 73,939 subreads were generated from 545,739,862 bases (Table 3). The scaffolds and contigs N50 values were 1,576,508 bp and 1,576,508, respectively. The GC content of the scaffolds and contigs was 59.12%. The GC content and GC depth Poisson distributions

analysis showed that sequence bias or DNA contamination absence indicated high-quality genome assembly (Fig. 4). The  $(G - C)/(G + C)$  method of calculation was used to analyse GC skew, distribution and ncRNA annotation. Fig. 5 shows a genomic diagram with variables such as genome length, gene density, non-coding RNA (ncRNA) density, and GC rate. The *Labrenzia* sp. PO1 contains 5,527,806 CDSs. The annotation and assembly results were used to predict 119 ncRNA copies, which included including nine rRNA and 53 tRNA copies (Table 4).

### 3.4. Annotation of genome sequence

All *Labrenzia* sp. PO1 gene sequences were blasted in five databases, including COG, Swiss-Prot, NR, KEGG, and GO. Table 5 shows the number and percentage of annotated genes in each database. The Gene Ontology Association (GOA) had developed the GO database in 1988 and included the following three categories: biological process, molecular function, and cellular components. The cellular component category can provide information about macromolecular complexes, subcellular structures, and their locations. Gene and product of gene described by molecular function. The biological process described ordered combination of molecular functions to achieve broader biological functions. A total of 49,272 genes of *Labrenzia* sp. PO1 were annotated based on the GO database (Fig. 6). Of all genes within the biological process category, 1459 and 2017 were involved in metabolic and cellular processes, respectively. Moreover, one gene was involved in the extracellular activity. The top three categories with most genes were membrane (1197 genes), membrane part (1115 genes), and cellular (833 genes), respectively. In the molecular function category, 1458 and 2290 genes were involved in binding and catalytic activities, respectively. Furthermore, only nine genes were associated with the regulation of molecular function. To understand the biological system's important functions, molecular information about genes was obtained using high-throughput techniques and genome sequencing. This molecular information was also used to generate the KEGG database. Biological pathways are divided into eight categories, which are further subdivided. Each subdivision is annotated with a related gene that is displayed graphically. The accession numbers of the reference strains are listed in (Table 6).

In the present study, 3566 genes of *Labrenzia* sp. PO1 were categorised into 5 KEGG classes: cellular processes (12.43%), metabolic processes (65.49%), environmental information processing (18.01%), organismal systems (1.98%), and genetic information processing (6.04%) (Fig. 7). Metabolic processes were further divided into the following 12 subclasses: amino acid metabolism, global maps, energy metabolism, carbohydrate and lipid metabolism, vitamin and cofactor metabolism, nucleotide metabolism, biodegradation of xenobiotic compounds, metabolism of other amino acids, biosynthesis of secondary metabolites, terpenoid biosynthesis, and polyketide and glycan biosynthesis. Biofilm formation and QS-related genes were identified by further analysis of cellular processes. A total of 47, 134, and 340 genes were responsible for biofilm formation, QS, and ABC transport, respectively.

### 3.5. Predict genome CRISPR structure

CRISPR structure is a short palindrome repeat sequence in regular clusters; the length of the repeat sequence is generally 21–47 bp. There are three CRISPR elements on the chromosome of

**Table 1**  
Characteristics of *Labrenzia* sp. PO1.

Number	GenBank accession No.	Reference sequence	% similarity	Taxonomic family	Species	Isolation stage	Biofilm forming ability
PO1	MH443361	KP301106.1	9%	<i>Rhodobacteraceae</i>	<i>Labrenzia</i> sp.	Log	+

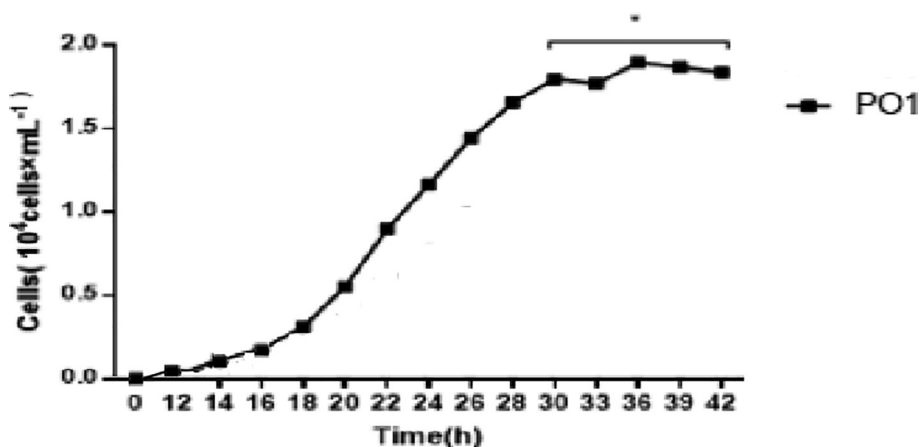


Fig. 1. Growth curve of *Labrenzia* sp. PO1.



Fig. 2. SEM image of *Labrenzia* sp. PO1.

Table 2  
Statistical data of sample subreads.

Sample Name	Seq Type (#)	Total Number (#)	Total Length (Mb)	N50 Length (Mb)	N90 Length (Mb)	Max Length (Mb)	Min Length (Mb)	GC Content (%)
PO1	Scaffold	15	6,218,140	1,576,508	352,075	1,744,114	644	59.12
PO1	Contig	16	6,218,130	1,576,508	352,075	1,744,114	644	59.12

Total number (#): Total number of contigs or scaffolds. Total length (Mb): Total length of assembly. N50 Length (Mb): Used to determine the assembly continuity; the higher the better. N50 is a weighted median statistic indicating that 50% of the total genome length is contained in transcripts that are equal to or larger than this value. N90 length (Mb): similar to N50 length. Max length (Mb): the maximum length of a scaffold or a contig. Min length (Mb): the minimum length of a scaffold or a contig. Gap number (Mb): number of gaps in the sequence. GC content (%): the percentage of G and C bases in the assembly.

PO1 genome, indicating that PO1 also has a bacterial-specific CRISPR immune system, which can effectively defend against foreign DNA infestation (Table 7).

### 3.6. Identification of key genes

Genes involved in biofilm formation were identified from the genomic sequence and protein annotation data. *waaA* encodes 3-deoxy-D-manno-octulosonic acid transferase, which is responsible for inner core assembly and phosphorylation. *Labrenzia* sp. PO1 *ompF* and *lpxC* (encodes a protein involved in lipid biosynthesis) gene sequences were obtained in this study. Curli is another extracellular protein fibre involved in cell-surface, cell-cell interaction and colonisation of host cells. *csgD* is a master regulator of biofilm formation and can activate curli fimbriae synthesis. Hence, the *csgD* gene was sequenced and annotated in this study. Lipoproteins

encoding genes are that participate in adhesion and biofilm formation were annotated and sequenced in *Labrenzia* sp. PO1. *Labrenzia* PO1 genomes contained five chemotaxis genes in a single operon-like structure (*cheA*, *cheR*, *cheW*, *cheB* and *cheY*) (Table 8). Flagellar genes were present in the genome of *Labrenzia* PO1, *flg*, *MotA* and *MotB*. In addition, *Labrenzia* PO1 contained genes related to pilus formation. Gene such as *tadD* that are involved in PGA biosynthesis in *Labrenzia* sp. PO1 were annotated and sequenced (Table 8). Several gram-negative bacteria use ATP-binding cassette transporters (ABCs) to facilitate intermembrane transport of substrates. Substrates such as ions, amino acids, antibiotics, and materials required for biofilm formation are often recognised and transported by ABC transporter proteins.

The activity of *dppB* is regulated by five substrate-binding proteins (SBP) that function in the periplasm. These SBPs affect biofilm formation because four other SBPs bind to ABC transporter dipep-

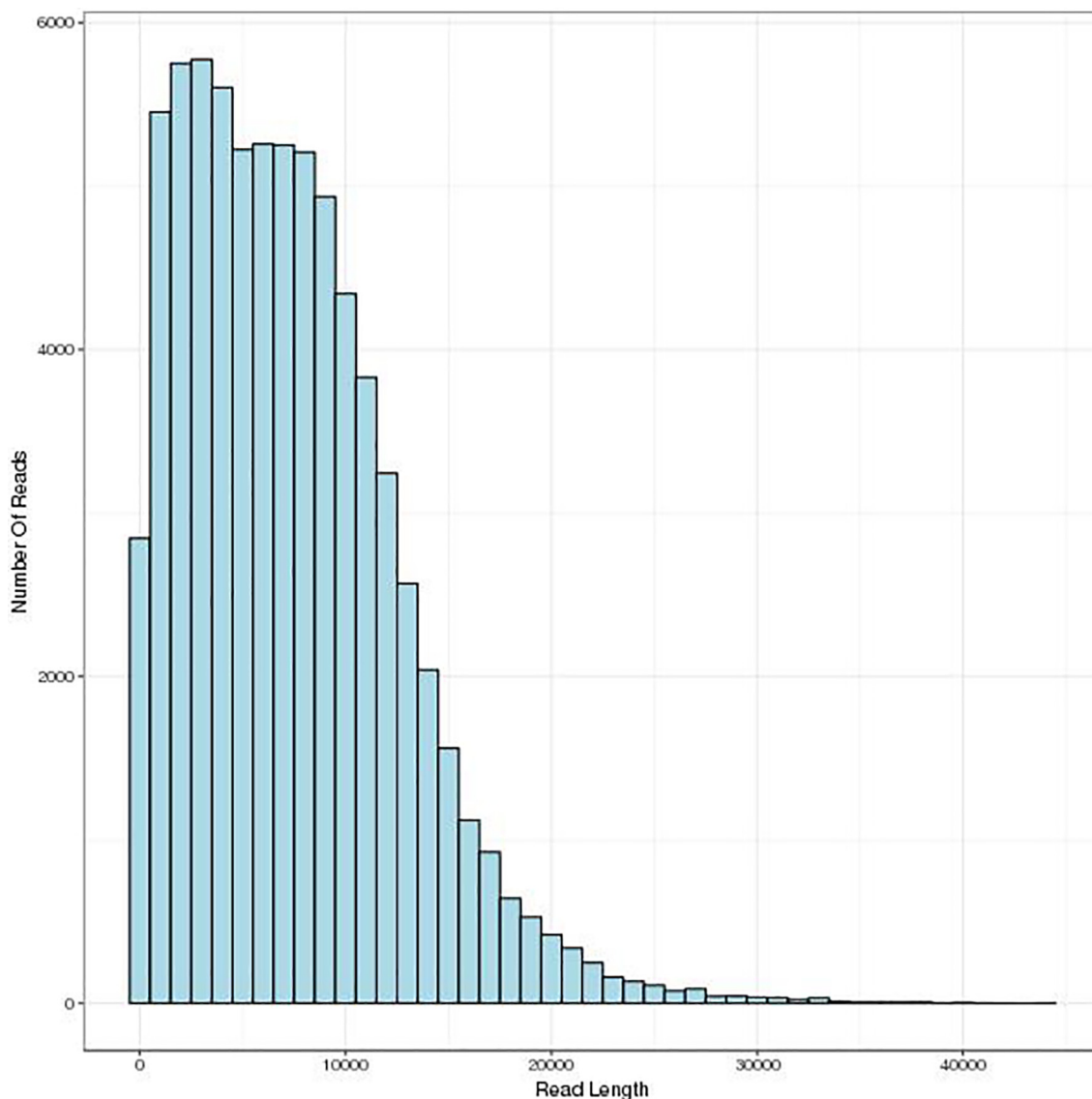


Fig. 3. *Labrenzia* sp. PO1 subread length distribution.

Table 3  
Summary of clean data assembly for *Labrenzia* sp. PO1.

Sample	Total bases (bp)	Number of subreads	Average length (bp)	N50 (bp)
<i>Labrenzia</i> PO1	545,739,862	73,939	7380	10,292

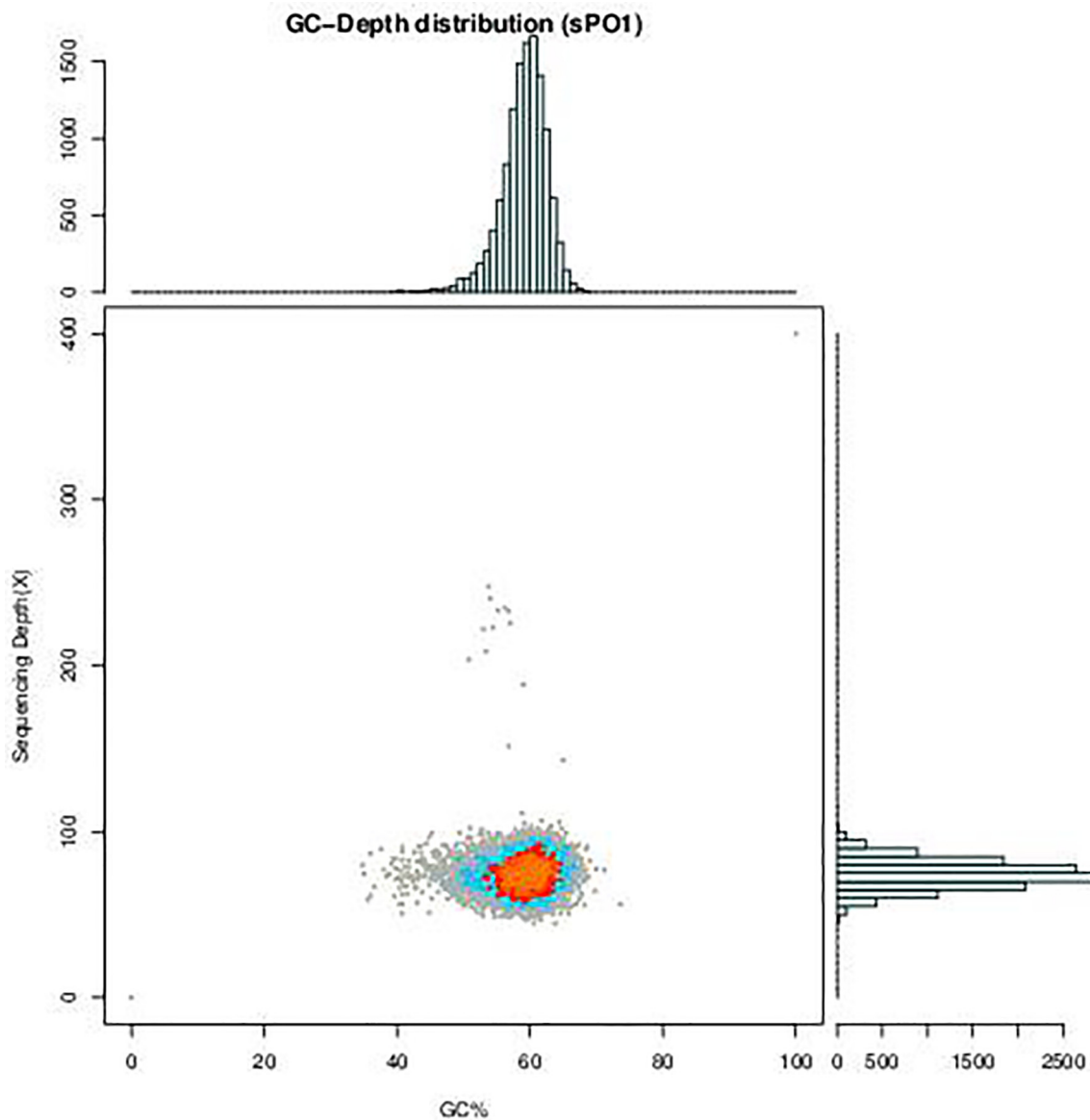
tides. Genes encoding SBPs and zinc ABC transporter SBP *znuC* of *Labrenzia* sp. PO1 was sequenced and annotated. Furthermore, genes encoding putative ABC transporter permease, which can increase biofilm formation, were also sequenced and annotated. There are three stages of biofilm formation: initial attachment, microcolony formation, and community development. The bacterial cells in biofilms, therefore, are metabolically distinct from their free-living forms. It assumed that extracellular signalling molecules (autoinducers of QS) could regulate specific transcription factors to modulate bacterial expression in biofilms and induce the transition from a planktonic to a sessile lifestyle. The transcriptional regulator *luxR* a key player of QS regulates several genes, including those that encode proteins involved in biofilm formation.

Furthermore, *mll1393* was sequenced and annotated in the present study. The QS-related *mll1393* gene encodes proteins for the

biosynthesis of phenazine compounds, promoting biofilm formation. The bacterial response to challenging environmental conditions during biofilm formation. This response requires sensor and regulator proteins during each phase of biofilm formation and genes *cpxA* encoding sensory histidine kinase in *Labrenzia* sp. PO1 was also sequenced and annotated.

### 3.7. Phylogenetic analysis of *Labrenzia* sp. PO1

A phylogenetic tree was constructed using the MEGA program with 18S rRNA sequences of *Labrenzia* sp. PO1 sample and reference strains. A neighbour-joining analysis showed that *Labrenzia* sp. PO1 have a common ancestor with all reference strains (Fig. 8). The evolutionary position of *Labrenzia* sp. PO1 is lower than that of *Tropicibacter multivorans*, *Roseovarius pacificus*, *Labren-*



**Fig. 4.** Correlation between the GC content and read depth distribution for *Labrenzia* sp. PO1. Abscissa, GC content; ordinate, average depth. The scatter plot shows a Poisson distribution of sequencing data with low GC bias.

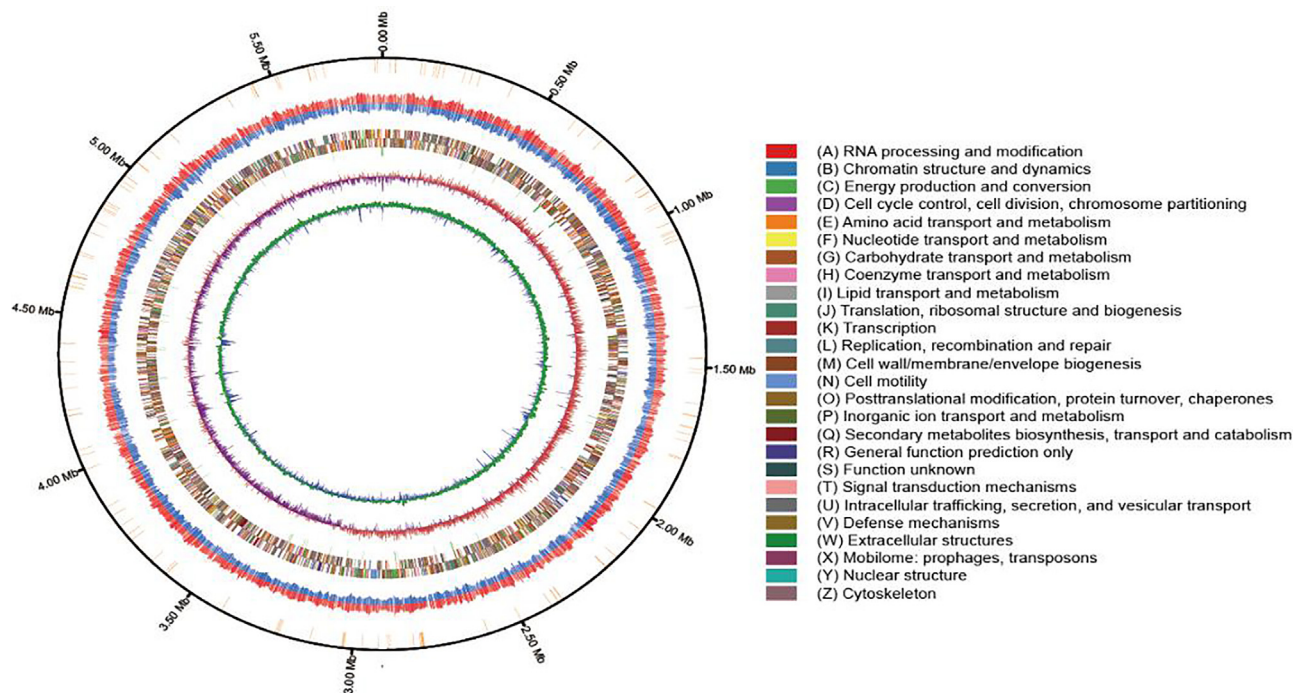
*zia alba*, *Labrenzia alexandrii*, *Stappia indica*, *Labrenzia suaedae*, and *Stappia stellulata*. In terms of kinship, *Labrenzia* sp. PO1 showed a higher kinship than *Labrenzia aggregata*.

#### 4. Discussion

*Rhodobacteraceae* is one of the most diverse aquatic bacterial groups (Garrity, 2005; Giovannoni and Rappé, 2000) and is often found in the maritime environment. Members of this family are phenotypically and ecologically diverse (Pujalte et al., 2014a) and play a key role in biochemical cycling. We isolated *Labrenzia* sp. PO1 a member of *Rhodobacteraceae* from *A. sanguinea*. Microbial interaction studies showed that *Labrenzia* sp. PO1 coexists with *A. sanguinea*, a prominent species found in the South China Sea that forms large blooms (Lu and Hodgkiss, 2004). A phylogenetic tree revealed that *Labrenzia* sp. PO1 is phylogenetically linked to *Tropiobacter multivorans*, *Roseovarius pacificus*, *Labrenzia alba*, *Labrenzia alexandrii*, *Stappia indica*, *Labrenzia suaedae*, and *Stappia stellulata*

strains. We sequenced and analysed *Labrenzia* sp. PO1 and submitted to the GenBank database under the accession number JAAXCR000000000.

The molecular and genetic pathways for biofilm formation are not well understood in *Labrenzia* sp. PO1. Hence, whole-genome sequencing of *Labrenzia* sp. PO1 was performed to investigate the molecular mechanism of biofilm formation. GO and KEGG pathway enrichment was performed to identify essential gene-encoding proteins that are involved in biofilm formation. Motility, chemotaxis, and attachment are likely to lead to efficient phytoplankton surface colonisation. We recognise genes involved in bacterial quorum sensing, attachment, motility, and chemotaxis to investigate bacteria's ability to interact with the diatom phycosphere. Flagellar motors were regulated by signal transduction cascade that mediated by CheAYW. CheB and CheR regulate methyl-accepting chemotaxis proteins methylation state together (Wuichet et al., 2007). These data indicate that *Labrenzia* PO1 can chemotaxis, pili, and flagella structures construction because we find genes of chemotaxis, pili, and flagella in this strain. Extracellular polymeric



**Fig. 5.** Genomic diagram of *Labrenzia* sp. PO1. Outer to inner: Genome (sorted by length), gene density (in 50,000 bp non-overlapping windows), ncRNA density (in 100,000 bp non-overlapping windows), GC rate (in 20,000 bp non-overlapping windows), GC skew (in 20,000 bp non-overlapping windows).

**Table 4**  
RNA distribution of *Labrenzia* sp. PO1.

Type	Total bases (bp)	Copy number	Average length (bp)	% of genome
CDS	5,527,806	6005	920.53	88.64
rRNA	12,050	9	1338.89	0.19
tRNA	4143	53	78.17	0.07
ncRNA	52,328	119	439.73	0.84

CDS: coding sequence; rRNA: ribosomal RNA; tRNA: transfer RNA; ncRNA: non-coding RNA

**Table 5**  
Gene annotation results of *Labrenzia* sp. PO1 according to the database.

Database	NR	SwissProt	KEGG	GO	COG
Number	5622	3819	2831	3887	4878
%	93.62	63.60	47.14	64.73	81.23

NR: Non-redundant protein database; KEGG: Kyoto Encyclopedia of Genes and Genomes; GO: Gene Ontology Database; COG: Clusters of Orthologous Groups.

matrix biosynthesis activates the function of curli subunit gene D (*csgD*) a central transcriptional regulator in the formation of biofilm; curli allows bacterial cells to migrate from the planktonic stage into multicellular condition. *CsgD* is important for forming biofilms; individual bacteria do not express *CsgD* at levels similar to the multicellular (Liu et al., 2014). Bistable characteristics of *CsgD* expression was high in a biofilm subpopulation, while *CsgD* expression was low in another bacterial subpopulation. The upregulated *CsgD* gene bacteria create the extracellular matrix and shape the biofilm structure that supports the low *CsgD* expressing bacteria because bistable *CsgD* high expression saves energy in biofilm formation, it can provide the bacterial community as a whole with additional benefits while maintaining population growth capacity (Grantcharova et al., 2010). Ironically, *csgD* encoding a DNA-binding transcriptional regulator were annotated and sequenced in the *Labrenzia* PO1 strain (Table 8). These factors are responsible for the transport and assembly of the curli fibres and maybe play an important role in biofilm formation (Xie et al., 2016). Surface

polysaccharides often play a significant role in biofilm formation (Beloin et al., 2008; O’Toole et al., 2000). The evolutionary conserved *waa* gene is responsible for inner core assembly and phosphorylation in *Labrenzia* sp. PO1 (Whitfield et al., 1999); this gene was sequenced in this study (Table 8). For attachment, genes involved in exopolysaccharide production and biofilm formation were distributed in the bacteria’s genome (Table 8). The initial steps in biofilm formation are reversible fixation and motility (Wood, 2009). Furthermore, the genes involved in adhesion, such as *tadC*, can promote biofilm formation. Several studies have shown that tight adherence (*tad*) genes are required for adhesion to the surfaces of the bacterium as well as for rough colony autoaggregation, morphology, and Flp pilli development (Bhattacharjee et al., 2001; Clock et al., 2008; Kachlany et al., 2000; Perez et al., 2006; Planet et al., 2003). Our data show that genome of *Labrenzia* sp. PO1 contains *TadD* gene which supports adhesion of this strain. QS autoinducers AHLs have widely been shown to modulate important biological functions motility and biofilm formation



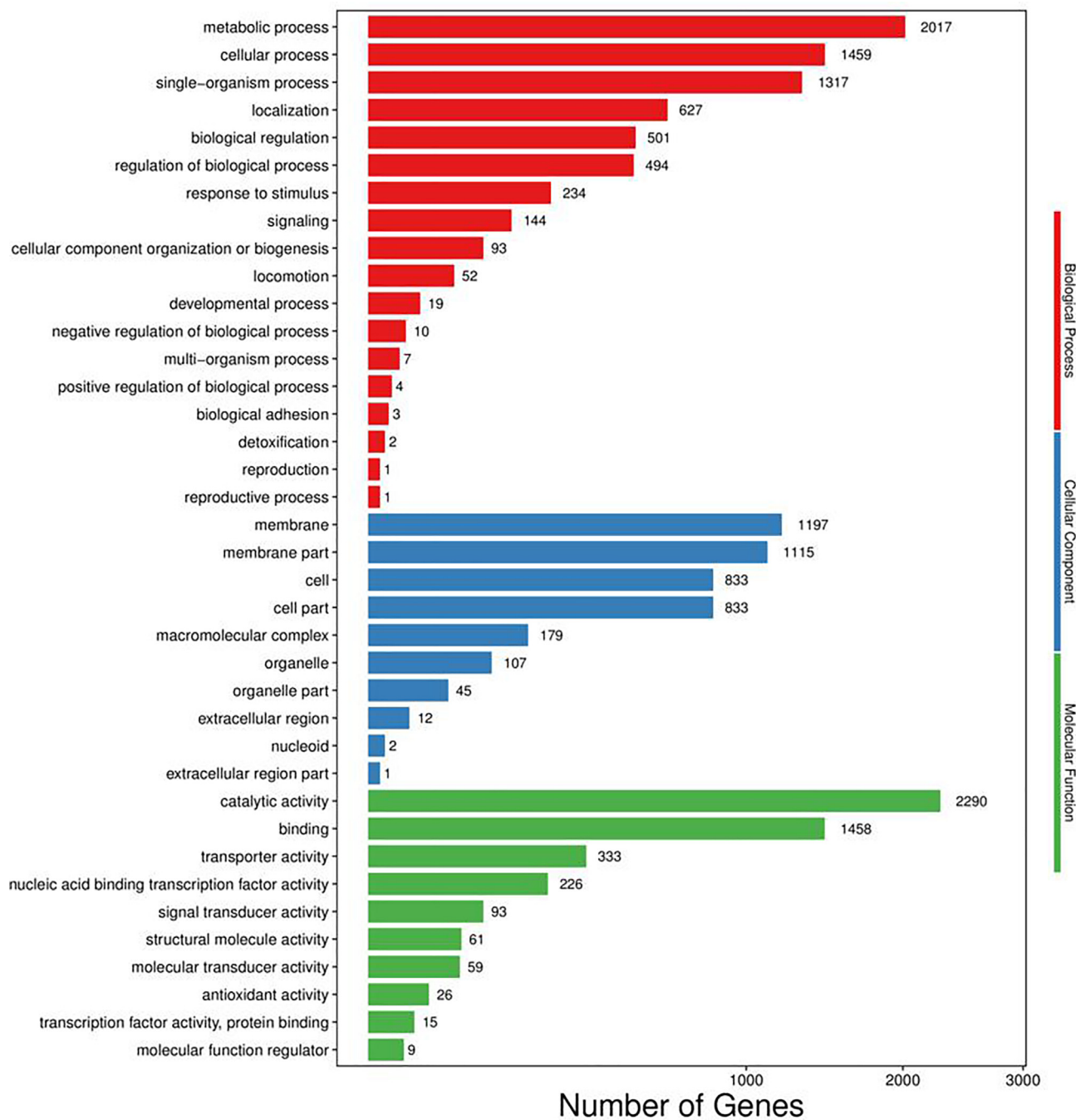


Fig. 6. Distribution of functional annotations in the GO database. The ordinate is the annotation item and the abscissa is the number of corresponding genes.

Table 6

The National Center for Biotechnology Information (NCBI) accession numbers of the reference strains for phylogenetic analysis.

Species ID	Genome (Mb)	Scaffolds	Contigs	GC%	Accession ID	No. of genes	No. of specific genes
<i>Labrenzia</i> sp. PO1	6.21813	15	16	59.12	MH443361	6005	538
<i>Labrenzia aggregata</i>	6.60619	3	3	58.9	CP019630.1	5657	391
<i>Labrenzia alba</i>	6.88095	32	41	56.4	CXWE00000000.1	6234	1619
<i>Labrenzia alexandrii</i>	5.4074	3	6	56.4	CM011002.1	4887	1033
<i>Labrenzia suaedae</i>	5.14283	14	14	60.2	FRBW00000000.1	4626	982
<i>Roseovarius pacificus</i>	4.54703	52	52	62	FRBR00000000.1	4482	1539
<i>Stappia indica</i>	4.62317	17	17	67	MBQE00000000.1	4572	816
<i>Stappia stellulata</i>	4.62317	12	15	64.7	AUIM00000000.1	4254	689
<i>Epibacterium multivorans</i>	4.17562	45	45	59.7	CYSD00000000.1	3926	1255

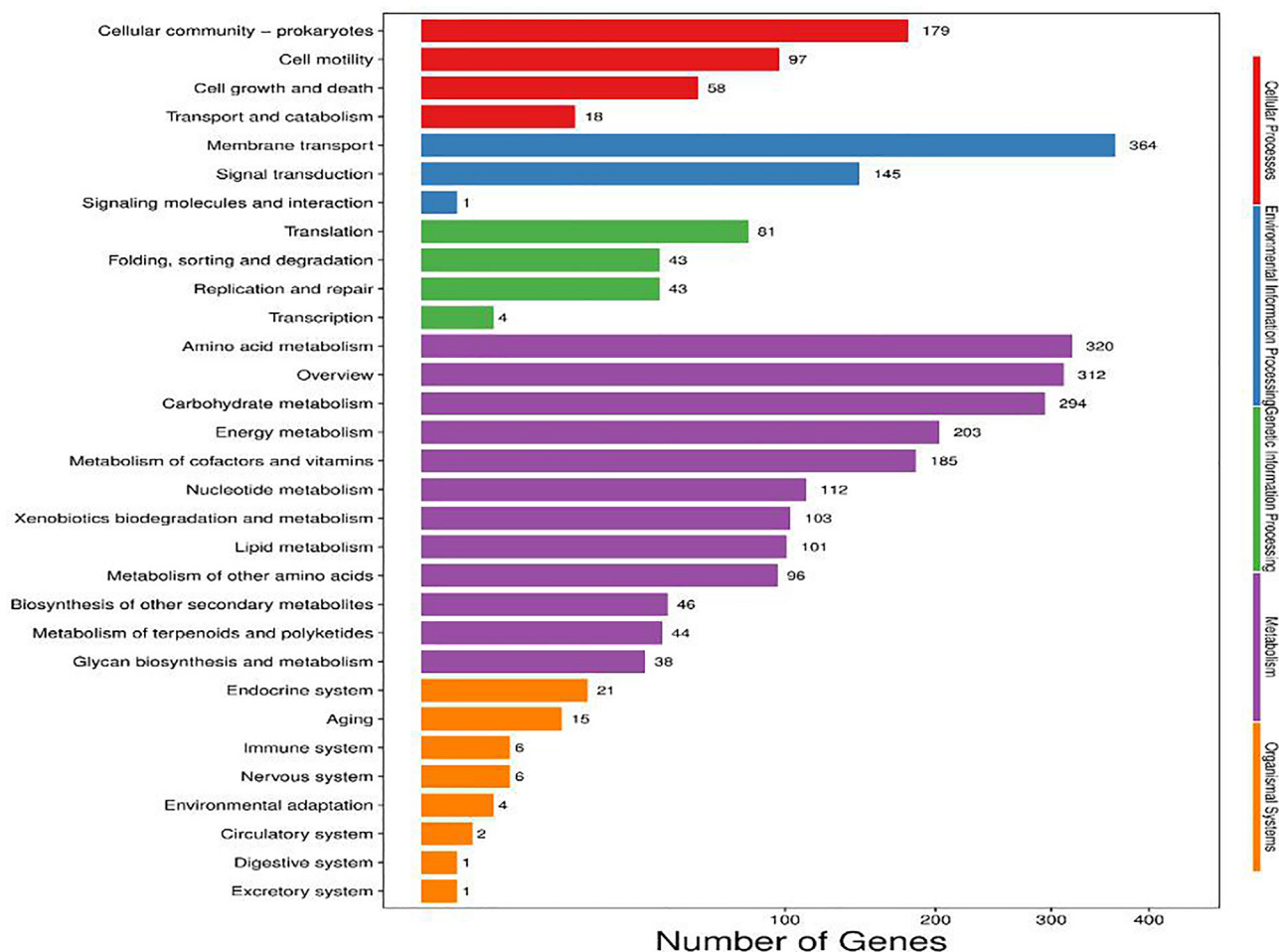


Fig. 7. Distribution of functional annotations in the KEGG database. The ordinate is the annotation item and the abscissa is the number of corresponding genes.

Table 7  
Predicting CRISPR element information on the genome.

Position on chromosome (bp)	Number of repeats	repeat average length	Spacer average length	Repeat sequence
1672458–1672732	5	23	40	CCGCCTTCACGGCCACCGCCGCC
2097288–2097446	3	27	39	GGTCGTGTCGATGGCCGGTAAACGCTG
3452386–3452635	4	34	38	TTGCCATTGCCACCGGAATTGCCGTTGCCGTTGC

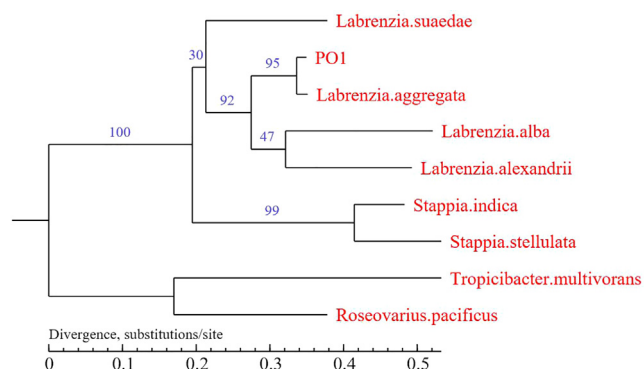
(Bassler, 2002; Waters and Bassler, 2005). In addition to AHL biosynthesis, a transcriptional regulator, encoded by a *luxR* family gene, is required to perceive AHLs and coordinate gene expression among bacterial populations. *Labrenzia* sp. PO1 genome contained *luxR* family genes. QS signals are required for biofilm formation. QS is a cell–cell signaling process that bacterial strains use to coordinate behavior (Dobretsov et al., 2009). Several QS gene regulation mechanisms occur in gram-negative bacteria via direct interaction of a self-inducer with a cytosolic transcription factor. LuxR protein is a transcriptional regulator that binds as a ligand to an autoinducer to induce the dimerisation of QS-regulated genes and promote its binding to DNA (Choi and Greenberg, 1991). Histidine kinase receptors activate a cascade signalling to upregulate *LuxR* transcription factor and induce QS gene expression (Miyamoto et al., 1996, 2003). Several gram-negative bacteria contain LuxR proteins that participate in QS signal generation (Fuqua et al., 1994). Bacteria with *luxR* genes have been hypothesised to identify and respond to exogenous signals from other bacterial populations (Hudaiberdiev et al., 2015). We identified Histidine kinase receptors and LuxR-type regulators in the genome

of *Labrenzia* sp. PO1 (Ball et al., 2017; Chen and Xie, 2011). We have found that phenazine production dramatically affects the bacterial colony's morphology (Dietrich et al., 2008). Several studies showed that phenazine involves in flow cell biofilm formations; a lack of phenazine production prevents cell attachment to surfaces and removes the formation of biofilms (Maddula et al., 2008, 2006). A previous study has shown that QS can modulate phenazine production to induce adhesion and formation of biofilms (Cao et al., 2017). Another recent study showed that *pyo* expression could greatly influence bacterial biofilm formation (Cao et al., 2017). We identified gene of Phenazine biosynthesis protein in the genome of *Labrenzia* sp. PO1 (Ball et al., 2017; Chen and Xie, 2011) which may be important for the attachment of the cell to surface. Sigma factors are dissociable subunits of prokaryotic RNA polymerase that regulate recognition and transcription of genes. Direct gene transcription from the promoter of genes needed to support basic cellular functions is primary sigma factors (Wilson and Lamont, 2006). In addition to primary sigma factors, bacteria also contain secondary sigma factors to respond to environmental stresses. The sigma factor that regulates biofilm formation in

**Table 8**  
Genes involved biofilm formation and metabolism of *Labrenzia* sp. PO1.

Gene_ID	CDS_Length	CDS_GC(%)	Pep_Length	Gene	Protein
orf03123-1943	615	59.02439024	204	<i>lprI</i>	Lipoprotein
orf00150-5717	915	57.26775956	304	<i>yfhB</i>	Phenazine biosynthesis protein
orf03073-1913	912	63.26754386	303	<i>mll1393</i>	Phenazine biosynthesis protein
orf05066-3183	804	54.47761194	267	<i>aapP</i>	ABC transporter permease
orf06171-3887	1011	59.74282888	336	<i>dppB</i>	Dipeptide transport system permease protein DppB
orf04493-2810	1605	56.38629283	534	<i>ddpA</i>	ABC transporter substrate-binding protein
orf02722-1684	1989	58.57214681	662	<i>rpoD</i>	RNA polymerase sigma factor RpoD
orf05475-3441	1605	58.87850467	534	<i>mdlC</i>	Acetolactate synthase
orf07489-4715	1407	60.69651741	468	<i>thrC</i>	Threonine synthase
orf05456-3428	1845	59.94579946	614	<i>ilvD</i>	Dihydroxy-acid dehydratase
orf00218-127	780	62.17948718	259	<i>hisF</i>	Imidazole glycerol phosphate synthase
orf01654-1020	1179	62.68023749	392	<i>serC</i>	Phosphoserine aminotransferase
orf05907-3716	1311	59.72540046		<i>glyA</i>	Glycine/serine hydroxymethyltransferase
orf04860-3049	729	53.22359396	242	<i>ydhC</i>	HTH-type transcriptional regulator
orf03087-1922	1020	58.92156863	339	<i>ilvC</i>	Ketol-acid reductoisomerase
orf03820-2385	918	59.80392157	305	<i>ilvE</i>	Branched-chain-amino-acid aminotransferase
orf00217-126	744	62.90322581	247	<i>hisA</i>	Phosphoribosylformimino-5-aminoimidazole
orf04013-2510	522	60.53639847	173	<i>ydjA</i>	Putative NAD(P)H nitroreductase
orf00485-283	606	57.42574257	201	<i>leuD</i>	3-isopropylmalate dehydratase
orf05748-3611	1338	60.61285501	445	<i>DHS1</i>	Phospho-2-dehydro-3-deoxyheptonate aldolase
orf07227-4550	1920	61.71875	639	<i>dxs</i>	1-deoxy-D-xylulose-5-phosphate synthase
orf07242-4559	1299	61.0469592	432	<i>ribBA</i>	Riboflavin biosynthesis protein
orf07316-4609	1083	57.34072022	360	<i>oprF</i>	Outer membrane protein
orf05847-3680	1851	47.70394381	616	<i>NGR_a03550</i>	glycosyltransferase
orf07526-4736	1317	62.2627183	438	<i>waaA</i>	3-deoxy-D-manno-octulosonic acid transferase
orf00520-5950	1338	62.0328849	445	<i>cpxA</i>	Sensor protein
orf02281-1400	1143	53.10586177	380	<i>CsgD</i>	DNA-binding transcriptional regulator, CsgD
orf03001-1865	945	59.57671958	314	<i>lpxC</i>	UDP-3-O-acyl-N-acetylglucosamine deacetylase
orf02494-1535	606	62.04620462	201	<i>oprF</i>	Outer membrane porin F Precursor
orf07976-5019	777	62.03346203	258	<i>TadD</i>	Flp pilus assembly protein TadD
orf04743-2967	807	58.11648079	268	<i>yabD</i>	LuxR family transcriptional regulator
orf00446-262	534	61.2359550	177	<i>LuxR</i>	LuxR family transcriptional regulator
orf04516-2826	1143	56.86789151	380	<i>Atu1380</i>	Membrane-associated protease RseP, regulator of RpoE
orf05683-3570	843	61.32858837	280	<i>znuC</i>	Zinc import ATP-binding protein
orf04044-2527	588	57.65306122	195	<i>CheY</i>	chemotaxis protein
orf01688-1044	891	58.81032548	296	<i>CheR</i>	chemotaxis protein
orf01683-1041	492	60.36585366	163	<i>CheW</i>	chemotaxis protein
orf01681-1039	2595	60.61657033	864	<i>CheA</i>	Chemotaxis protein
orf01687-1043	1161	61.32644272	386	<i>cheB</i>	Chemotaxis response regulator protein-glutamate methyltransferase
orf05140-3228	1620	61.17283951	539	<i>MotB</i>	Motility protein B Chemotaxis protein
orf04616-2888	897	58.63991081	298	<i>MotA</i>	Motility protein A Chemotaxis protein
orf00027-5630	1911	56.41025641	636	<i>epsL1</i>	protein involved in exopolysaccharide biosynthesis

*Pseudomonas aeruginosa* has been identified previously. Sigma factor-related genes were identified in the present study.



**Fig. 8.** A phylogenetic tree diagram. The evolutionary history was inferred using the neighbor-joining method. The optimal tree with the sum of branch length of 0.01309240 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) is shown next to the branches. 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 analysis involved six nucleotide sequences. Codon positions included were 1st + 2nd + 3rd + noncoding. All ambiguous positions were removed for each sequence pair. The final dataset comprised 1,739 positions. Evolutionary analyses were conducted in MEGA7.

The bacterial capacity of biofilm formation is greatly enhanced upon the activation of ABC transporters (Zhu et al., 2008). Bacteria utilise transport proteins to transfer substrates via the internal and external membrane (Chung et al., 2009). Increased nutrient uptake via ABC transporters can help the bacteria adapt quickly to the evolving host microenvironment. Transporters also participate in signal transduction (Mai-Prochnow et al., 2015) and are required for bacterial survival (Garmory and Titball, 2004). Moreover, ABC transporters use ATP for intermembrane transport of substrates (Chung et al., 2009; Moussatova et al., 2008). Several ABC importers play a crucial role in the distribution of nutrients. ABC importers in *Yersinia pestis*, *Proteus mirabilis*, and *Brucella abortus* (Gutiérrez-Preciado et al., 2015; Vitreschak et al., 2002) import a wide variety of substrates such as carbohydrates, sugars, metals, peptide, amino acids, and other metabolites. ABC exporters are responsible for transporting substrates such as antibiotics, lipids, and proteins. It has been reported that limited nutrient availability might not promote the interaction between *A. sanguinea* and *Labrenzia* sp. PO1. Genes that may be involved in transport, namely transporters, were identified for the PO1 strain. In this study, we identified ABC transporter-related genes that are involved in regulating biofilm formation in *Labrenzia* sp. PO1 (Table 8). Earlier research in *Pseudomonas aeruginosa*, *Listeria monocytogenes*, *Burkholderia pseudomallei*, and *Rhizobium leguminosarum* bv. viciae 3841 revealed the importance of transporter proteins in bacteria (Lee et al., 2018; Pinweha et al., 2018; Vanderlinde et al., 2010;

Wang et al., 2009; Zhu et al., 2008). Gene sequencing of a zinc-specific transporter showed that it could enhance biofilm formation in *Labrenzia* PO1. Biofilm formation is a bacterial response against stressful environmental conditions and requires sensor and regulator proteins at each step of the biofilm formation process (Król et al., 2019). We have previously shown that *cpxA* encodes sensor proteins that might play a role in biofilm formation under environmental stress (Beloin et al., 2008).

## 5. Conclusions

In summary, this study provides useful insights into the genomic influence of *Labrenzia* sp. PO1 on biofilm formation. The *Labrenzia* sp. PO1 strain was isolated from the *A. sanguinea* culture. Growth analysis revealed that strain reached a period of logarithmic growth period at 24 hours. We identified genes related to transporter proteins, and quorum sensing that participates in biofilm formation in *Labrenzia* sp. PO1. To our knowledge, this is the first study that provides the complete genome sequence of *Labrenzia* sp. PO1 using a third-generation sequencing platform. We also analysed and predicted KEGG and GO terms for potential genes and proteins that promote biofilm formation by assembling contigs and scaffolds. These data may provide new insights into the molecular mechanisms of *Labrenzia* sp. PO1 and help develop innovative strategies for limiting the growth of the harmful red tide.

## Author Contributions

M.Z and Y.C conceived the study. M.Z and H.C contributed to the selection of the data. Y.C and H.C performed the pre-sequencing steps. M.Z and S.L write the manuscript. X.Y, Z.H and L.O revise the manuscript.

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## Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

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