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Data in Brief

High-quality draft genome sequence of *Enterobacter* sp. Bisph2, a glyphosate-degrading bacterium isolated from a sandy soil of Biskra, Algeria

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ABSTRACT

Enterobacter sp. strain Bisph2 was isolated from a sandy soil from Biskra, Algeria and exhibits glyphosate-degrading activity. Multilocus sequence analysis of the 16S rRNA, *rpoB*, *hsp60*, *gyrB* and *dnaJ* genes demonstrated that Bisph2 might be a member of a new species of the genus *Enterobacter*. Genomic sequencing of Bisph2 was used to better clarify the relationships among *Enterobacter* species. Annotation and analysis of the genome sequence showed that the 5.535.656 bp genome of *Enterobacter* sp. Bisph2 consists in one chromosome and no detectable plasmid, has a 53.19% GC content and 78% of genes were assigned a putative function. The genome contains four prophages of which 3 regions are intact and no CRISPER was detected. The nucleotide sequence of this genome was deposited into DDBJ/EMBL/GenBank under the accession JXAF00000000.

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Specifications	
Organism/cell line/tissue	*Enterobacter* sp. Bisph2
Sex	Not applicable
Sequencer or array type	Illumina MiSeq
Data format	Assembled
Experimental factors	Genomic sequence of microbial strain isolated from soil
Experimental features	Description of the complete genomic sequencing and annotation with a set of features for strain *Enterobacter* sp. Bisph2.
Consent	Not applicable
Sample source location	Biskra, Algeria

1. Direct link to deposited data

http://www.ncbi.nlm.nih.gov/assembly/GCF_000814915.1/

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2. Introduction

Glyphosate (N-phosphonomethylglycine) is the most commonly used herbicide worldwide [1]. Because of concern regarding its toxicity for non-targeted species in soil, finding glyphosate-degrading bacteria in soil is of interest. A limited number of bacterial and fungal species grow when glyphosate is supplied as the sole phosphorus source [2–4]. Most of such isolates were identified as *Pseudomonas* species [5–8] and *Arthrobacter* species [9,10]. *Rhizobium* sp. [2] and *Agrobacterium* sp. strains [11] have been mentioned as using glyphosate as sole source of phosphorus in liquid phosphorus-free culture medium. In addition, *Bacillus megaterium* [8], *Alcaligenes* sp. [6], *Flavobacterium* sp. [9,12], and the thermophile *Geobacillus caldoxylosilyticus* [13], have been reported as utilizing the glyphosate as sole source of phosphorus. When investigating Saharian soil microbiota in Biskra, Algeria, we isolated a glyphosate-degrading organism for which first-line identification was unsuccessful. The isolate further appeared to be probably representative of a new species of the genus *Enterobacter*. The genus *Enterobacter* was created in 1960 [14]. To date, this genus is comprised of 19 species (excluding *E. aerogenes*) making it one of the largest genera within the family *Enterobacteriaceae*. *Enterobacter* is also one of the most rapidly expanding genera within the *Enterobacteriaceae*, with 50% of the novel species descriptions taking place in the last decade [15]. Members of the genus

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were isolated mostly from the environment, in particular from soil, plants and fruit, but also frequently isolated from humans, notably in health-care associated infection [16]. Here we present a summary classification and a set of features for strain *Enterobacter* sp. Bisph2, together with the description of the complete genomic sequencing and annotation.

3. Results

3.1. First-line characterization of strain Bisph2

Isolate Bisph2 consists of Gram-negative rods cells (Fig. 1), facultatively anaerobic and motile by peritrichous flagella, of 0.9 μm wide by 2.1 μm long and occur singly or in pairs (Fig. 2). Strain Bisph2 is catalase positive, oxidase negative and fermentative. Growth occurs after 18–24 h at 25–44 °C with an optimum growth at 37 °C on Columbia agar with 5% sheep-blood and chocolate agar.

Using the API 20E and API ZYM system (bioMérieux, La Balme les grottes, France), positive reactions were obtained for β-galactosidase, indole production, Voges–Proskauer, glucose, mannose, sorbitol, rhamnose, saccharose, amygdalin, inositol, alkaline phosphatase, leucine arylamidase, trypsin and β-glucosidase. Whereas, negative reactions were obtained for arginine dehydrolase, lysine decarboxylase, ornithine decarboxylase, citrate, H_2S production, tryptophane deaminase and urease.

The isolate Bisph2 was identified by MALDI-TOF analysis as belonging to *Enterobacter* genus with a score of 1.6.

3.2. Phylogenetic analysis of strain Bisph2

Strain Bisph2 showed the highest 16S rRNA gene sequence similarity (>97%) with *Enterobacter asburiae* (GenBank Accession No. gb |JF772103.1|) and *Klebsiella pneumoniae* (GenBank Accession No. gb |EF197996.1|). The strain exhibited the highest *rpoB* gene sequence similarity of 94% with *E. cloacae* (GenBank No. gb |CP009756.1|). The result of *hsp60* gene analysis showed that Bisph2 shared 94.7% and 92.6% of similarity with *E. cloacae* (GenBank Accession No. emb|FN547033.1|) and *E. cowanii* (GenBank Accession No. emb|AJ567896.1|), respectively. Whereas, the highest *gyrB* sequence similarity (90.7%) was found with *E. cloacae* (GenBank No. gb |CP002272.1|). The result of *dnaJ* sequence analysis showed that strain Bisph2 exhibited 89.3% and 88.4% of similarity with *E. cancerogenus* (GenBank Accession No. dbj|AB272637.1|) and *E. cloacae* subsp. *cloacae* (GenBank Accession No. emb|FP929040.1|), respectively.

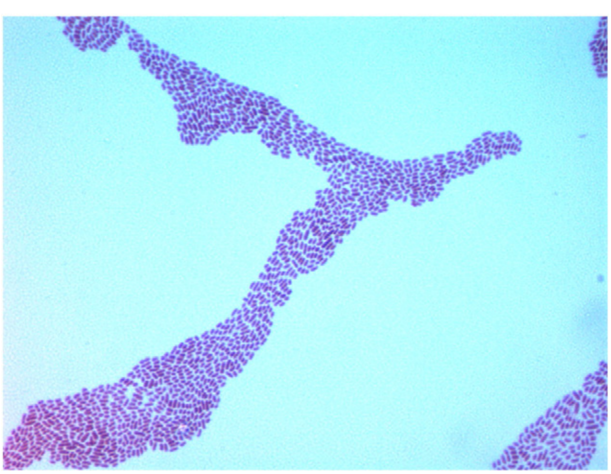

Fig. 1. Gram stain of *Enterobacter* sp. Bisph2.

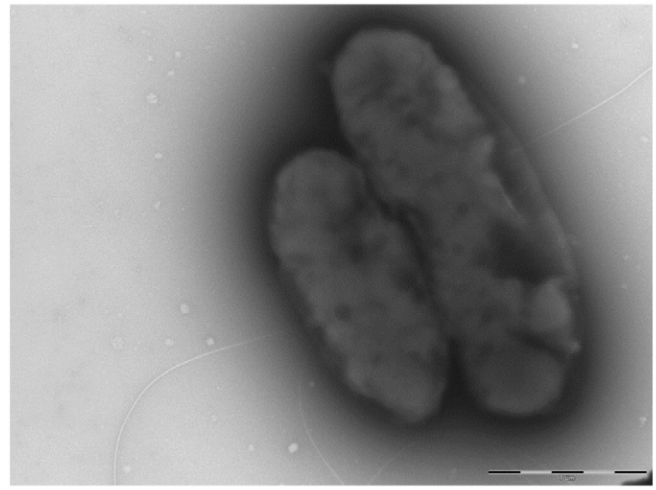

Fig. 2. Transmission electron micrograph of *Enterobacter* sp. Bisph2, taken using a Morgani 268D (Philips) at an operating voltage of 60 kV. The scale bar represents 1 μm.

3.3. Genome sequencing analysis

The whole genome Shotgun project of Bisph2 strain has been deposited at DDBJ/EMBL/GenBank under the accession number JXAF00000000. Assembling yielded 27 scaffolds and 46 large contigs (>500 bp), generating 159.85× genome equivalents of a 5.3 Mb-genome. The genome consists of one circular 5.535.656 bp chromosome without detected plasmid with a 53.19% GC content (Fig. 3). A total of 5.248 genes (78%) were assigned a putative function. The remaining genes were annotated as either hypothetical proteins or proteins of unknown function. No CRISPER was detected. The genome contains four prophages of which three regions are intact, including PHAGE_Salmon_SP_004_NC_021774, PHAGE_Cronob_ENT47670_NC_019927, PHAGE_Entero_HK225_NC_019717 and one incomplete PHAGE_Aggreg_S1249_NC_013597. The distribution of genes into COGs functional categories is presented in Table 3. The properties and the statistics of the genome are summarized in Tables 2 and 3.

4. Discussion

Results of morphological and physiological studies showed that strain Bisph2 presents the general characteristics of the genus *Enterobacter*. For the MALDI-TOF analysis, a score enables the presumptive identification and discrimination of the tested species from those in a database: a score ≥2 with a validly published species enable the identification at the species level, a score ≥1.7 but <2 enabled the identification at the genus level, and a score <1.7 did not enabled any identification. For strain Bisph2, the score was 1.6, suggesting that this isolate was not a member of known species of *Enterobacter*.

Bisph2 differ from their nearest neighbors by several properties including the negative test to ornithine decarboxylase and arginine dihydrolase, positive for indole production and the ability to growth on dulcitol, D-arabitol, 5-ketogluconate and malonate.

The isolate was initially identified by the first-line identification tools (morphological, physiological studies, MALDI-TOF and API system) as belonging to *Enterobacter* genus. Further identification to the species level was not possible.

The analysis of 16S rRNA gene sequence indicated that strain Bisph2 belongs to the family of *Enterobacteriaceae.* Comparison of the sequences of conserved genes, most commonly those encoding 16S rRNA, is used for bacterial genotypic identification. Currently, *Enterobacter* has been shown to be polyphyletic based upon 16S rRNA gene sequence analysis [17,18], making it difficult to assign novel species to *Enterobacter* [15]. Among

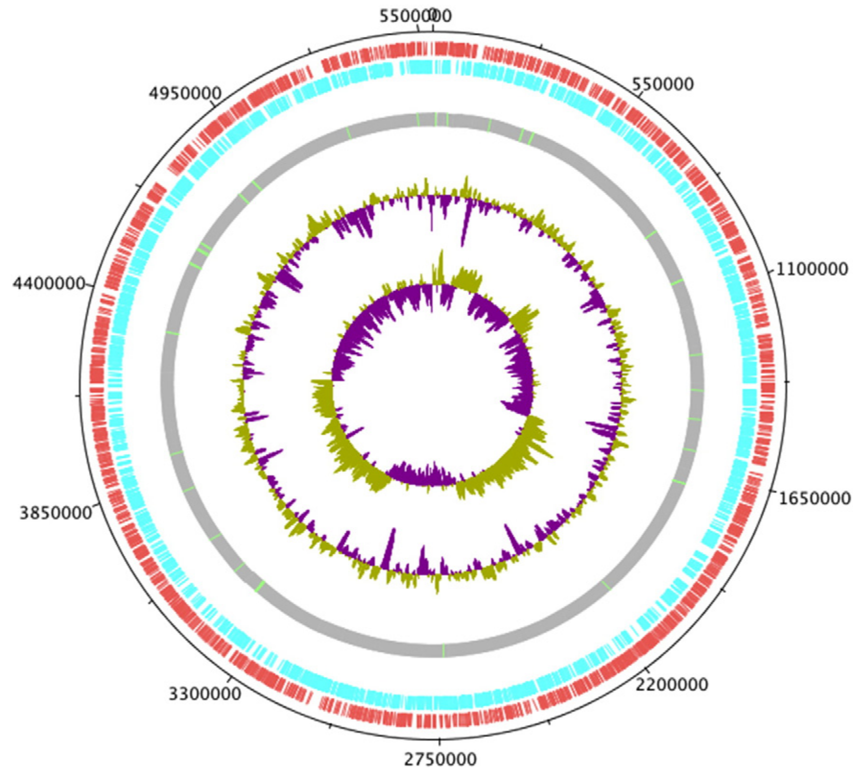

Fig. 3. Graphical circular map of *Enterobacter* sp. Bisph2 genome. From outside to the center: Genes on the forward strand, genes on the reverse strand, RNA genes (tRNAs green, rRNAs red), GC content, and GC skew.

Enterobacteriaceae, variation within 16S rRNA gene does not allow confident species identification. In common with ribosomal RNA-encoding gene, the *rpoB* encoding the bacterial RNA polymerase β-subunit is universal [19]. It was showed by a study of Mollet et al. that the levels of divergence between the *rpoB* sequences of different strains of *Enterobacteriaceae* were markedly higher than those between their 16S rRNA genes. The comparison of partial sequences of the *rpoB* gene was more sensitive than the 16S rRNA gene and represents between 1% to 15.4% more variability [20]. As the utility of *rpoB* gene for species identification and discrimination between members of the family *Enterobacteriaceae* has been demonstrated previously by several authors [18,20], this approach was used to determine the taxonomic position of the strain Bisph2. The result of *rpoB* gene analysis demonstrated that the isolate shared similarities with *Enterobacter* species below the determined cut-off (97.7%) [20]. However, even the increased resolution of the *rpoB* gene fails to resolve *Enterobacter* and its closest phylogenetic relatives in monophyletic clade. Multilocus sequence analysis (MLSA), based on partial sequencing of the protein-encoding gene has been used to address several taxonomic issues [15].Thus, three additional protein-encoding genes *gyrB* [21], *hsp60* [22,23] and *dnaJ* [24] were sequenced to further describe the phylogenetic relationships of strain Bisph2 with other members of the genus *Enterobacter*. Based on Dauga's studies, similarities between *gyrB* sequences from all *Enterobacter* species ranged from 84.8 to 97.3% [21]. Strain Bisph2 exhibited a *gyrB* sequence similarity between 84 and 97.3% with *E. cloacae*. According to the study of Nhung et al., the degree of divergence of the *dnaJ* gene in the family of *Enterobacteriaceae* was approximately six times greater than that of the 16S rRNA gene. In addition, the greater divergence of the *dnaJ* sequences was particularly evident for species not well differentiated by other gene analysis [24]. *E. hormaechei* for example, showed 0.9% sequence difference for 16S rDNA gene to those of *E. cloacae* [25], but 11.1% *dnaJ* sequence difference was found between these two species. Strain Bisph2 showed 10.7% of *dnaJ* sequence difference with *E. cloacae*. Thus, it is likely that strain Bisph2 represents a new *Enterobacter* species, supporting the results of *rpoB*, *hsp60* and *gyrB* sequences analysis.

Enterobacter sp. Bisph2 isolated from soil collected in Biskra, Algeria grow in a mineral salt medium containing the glyphosate as sole source of phosphorus and can resist to the high concentration of the herbicide. Thus, this isolate might therefore be useful for bioremediation of glyphosate-contaminated environments. Because of the ability to bioremediation of the strain Bisph2 regarding glyphosate, we performed detailed genome sequencing and annotation.

The comparison of the genome of *Enterobacter* sp. strain Bisph2 with those of *E. massiliensis* strain JC163T, *E. aerogenes* strain KCTC 2190, *E. asburiae* strain LF7a, *E. cancerogenus* strain YZ1, *E. cloacae* strain EcWSU1, *E. cloacae* subsp. *dissolvens* strain SDM, *E. hormaechei* strain ATCC49162 and *E. lignolyticus* SCF1 showed that the draft genome of *Enterobacter* sp. Bisph2 is larger than those of *E. massiliensis*, *E. aerogenes*, *E. asburiae*, *E. cancerogenus*, *E. cloacae*, *E. cloacae* subsp. *dissolvens*, *E. hormaechei* and *E. lignolyticus* (5.53, 4.92, 5.28, 3.81, 4.80, 4.79, 4.96, 4.80 and 4.81 Mb, respectively). *Enterobacter* sp. Bisph2 has a G + C content lower than all the species mentioned previously (53.19, 55.1, 54.8, 53.8, 55.54, 54.54, 55.1, 55.2 and 57.02%, respectively) and has the greatest number of predicted genes (5.248, 4.724, 5.021, 4.805, 4.495, 4.740, 4.646, 4.779 and 4.558, respectively).

5. Materials and method

5.1. Enrichment and isolation of glyphosate-degrading strains

Soil specimens were collected from a sandy field located in the region of Biskra between 34°51′01″ north latitude and 5°43′40″ east longitude in northeastern Algeria on the northern edge of the Sahara Desert. Samples of about 1 kg were taken from the first 15 cm of depth and then pooled and sieved. Samples were air dried and stored in sterile plastic bags at 4 °C until use. About 5.0 g of soil were added to 95 mL of sterile minimal medium in 250 mL flasks with the addition

Table 1
Project information.

Property	Term
Finishing quality	High-quality draft
Libraries used	One paired-end 454 3-kb library
Sequencing platforms	454 GS FLX Titanium
Fold coverage	159.85×
Assemblers	Abyss version 1.3.4–3
Gene calling method	Prodigal
Genbank ID	JXAF00000000
GenBank date of release	January 09, 2015
GOLD ID	Gp0109567
BIOPROJECT	PRJNA270819
Source material identifier	Bisph2
Project relevance	Study of pesticide soil degrading bacteria

of glyphosate as the sole phosphorus source at a final concentration of 500 mg/L and incubated in the dark at 30 °C under shaking condition for seven days. A 5 mL volume of this suspension was then transferred to fresh sterile minimal medium containing 1 g/L of glyphosate and incubated for seven days. Three additional successive transfers were made into medium successively containing 3, 6 and 12 g/L of glyphosate. The appropriate dilutions of enriched sample were plated on nutrient agar supplemented with 1 g/L of glyphosate. The plates were incubated at 37 °C for 24 h. Strain Bisph2 was isolated and obtained in pure culture.

5.2. First-line characterization of strain Bisph2

For morphological and physiological studies, Bisph2 was grown in aerobically and anaerobically atmospheres at different temperatures (25 °C–44 °C) on Columbia agar 5% sheep-blood media (Biomérieux, la Balme-les-Grottes, France). Motility and morphology after Gram staining and after negative staining for transmission electron microscopy were observed. Observation by electron microscopy was done as previously described [26]. Briefly, strain Bisph2 was suspended and then washed in phosphate buffer and stained with 1% (*w*/*v*) phosphotungstic acid. Afterwards examination was carried on using Morgagni 268 D (Philips) electron microscope at an operating voltage of 60 kV. Physiological studies were performed using the API 20E and API ZYM system (bioMérieux, La Balme les grottes, France).

5.3. Matrix-assisted laser-desorption/ionization time-of flight analysis

The matrix-assisted laser-desorption/ionization time-of-flight mass spectrometer analysis (MALDI-TOF-MS) (Bruker Daltonics, Bremen, Germany) was carried-out as previously described [27]. Briefly, a pipette tip was used to pick one isolated bacterial colony from a culture agar plate, and to spread it as a thin film on a MTP 384 MALDI-TOF target plate (Bruker Daltonics, Leipzig, Germany). Twelve distinct deposits were done from twelve different colonies of strain Bisph2. Each smear was overlaid with 2 μl of matrix solution in 50% acetonitrile, 2.5% trifluoracetic-acid, and allowed to dry for 5 min. Measurements were performed with a Microflex spectrometer (Bruker). Spectra were recorded in the positive linear mode for the mass range of 2.000 to 20.000 Da. A spectrum was obtained after 675 shots at a variable laser power. The twelve spectra were imported into the MALDI BioTyper software (version 2.0, Bruker) and analyzed by standard pattern matching against the main spectra of 6.213 bacteria including 36 spectra from validly published *Enterobacter* species that were used as reference data in the BioTyper database. The method of identification includes the m/z 3.000 to 15.000 Da. For every spectrum, 100 peaks at most were taken into account and compared with spectra in the database.

5.4. 16S rRNA, rpoB, hsp60, gyrB and dnaJ genes amplification and sequencing

The 16S rRNA gene of Bisph1 was amplified using the primer pair fD1 (5′-AGAGTTTGATCCTGGVTCAG-3′) and P2 (5′-ACGGCTACCTTGTT ACGACTT-3′) [28]. PCR amplification was carried-out in a 50 μL volume containing 5 μL template, 50 mM KCl, 1.5 mM $MgCl_2$, 200 μM each dNTP, 0.2 μM each oligonucleotide primers and 0.5 units of *Taq* DNA polymerase (EuroblueTaq, Eurobio, Les Ulis, France). The thermal cycle consisted of an initial 5 min denaturation at 95 °C followed by 35 cycles of 30 s denaturation at 95 °C, primer hybridization at 52 °C for 30 s, elongation at 72 °C for 5 min and a final 5 min elongation step at 72 °C. PCR reaction was examined by electrophoresing 5 μL of PCR product on a 1% agarose gel stained with ethidium bromide. The gel was visualized using Gel Doc 1000 (Bio-Rad, California, USA). Successful PCR was transferred into PCR purification plate (Macherey Nagel Hoerdt, France). Purified PCR product was sequenced using BigDye® Terminator v1.1 cycle sequencing ready reaction kit (Applied Biosystems, Courtabeuf, France) and the primers 536F (5′-CAGCAGCCGCGGTAATAC-3′), 536R (5′-GTATTACCGC GGCTGCTG-3′), 800F (5′-ATTAGATACCCTGGTAG-3′), 880R (5′-CTAC CAGGGTATCTAAT3′), 1050F (5′-TGTCGTCAGCTCGTG-3′) and 1050R (5′-CACGAGCTGACGACA-3′). The *rpoB*, *gyrB*, *hsp*60 and *dnaJ* genes were amplified and sequenced as previously described by [20–22,24], respectively.

The nucleotide sequences were edited using ChromasPro software. The 16S rRNA, *rpoB*, *hsp60*, *gyrB* and *dnaJ* gene sequences of strain Bisph2 were deposited in GenBank with the accession number KC315994, KC316002, KC316000, KC315998 and KC315996, respectively.

5.5. Genomic DNA preparation

Strain Bisph2 was grown aerobically on 5% sheep-blood enriched Columbia agar (bioMérieux) at 37 °C. Four Petri dishes were spread, bacteria were harvested and resuspended in 4 × 100 μl of TE buffer. Then, 200 μl of this suspension was diluted in 1 ml TE buffer prior to being treated with 2.5 μg/μl lysozyme for 30 min at 37 °C, and then with 20 μg/μl of Proteinase K overnight at 37 °C. The DNA was then purified by 3 successive phenol–chloroform extractions followed by an ethanol precipitation at −20 °C overnight. Following centrifugation, the DNA was resuspended in 160 μl TE buffer. The yield and concentration were measured by the Quant-it Picogreen kit (Invitrogen) on the Genios Tecan fluorometer.

5.6. Genome sequencing and assembly

Genomic DNA of strain Bisph2 was sequenced on a MiSeq sequencer (Illumina Inc., San Diego, CA, USA) using paired-end sequencing with the Nextera XT. To prepare the paired-end library, genomic DNA was diluted 1:3 to obtain a 1 ng/μl concentration. The “tagmentation” step

Table 2
Genome statistics.

Attribute	Value	% of total
Genome size (bp)	5,535,656	100
DNA coding (bp)	4,891,263	88.35
DNA G + C (bp)	2,944,511	53.19
DNA scaffolds	27	100
Total genes	5248	100
Protein coding genes	5174	98.58
RNA genes	74	1.41
Genes assigned to COGs	5.248	78
Genes with Pfam domains	4740	90.32
Genes with signal peptides	471	8.97
Genes with transmembrane helices	1006	19.16
CRISPR repeats	0	

Table 3
Number of genes associated with general COG functional categories.

Code	Value	%age	Description
J	162	3.09	Translation, ribosomal structure and biogenesis
A	1	0.02	RNA processing and modification
K	357	6.80	Transcription
L	175	3.33	Replication, recombination and repair
B	0	0	Chromatin structure and dynamics
D	33	0.63	Cell cycle control, cell division, chromosome partitioning
V	49	0.93	Defense mechanisms
T	220	4.19	Signal transduction mechanisms
M	234	4.46	Cell wall/membrane biogenesis
N	103	1.96	Cell motility
U	87	1.66	Intracellular trafficking and secretion
O	133	2.53	Posttranslational modification, protein turnover, chaperones
C	235	4.48	Energy production and conversion
G	463	8.82	Carbohydrate transport and metabolism
E	391	7.45	Amino acid transport and metabolism
F	80	1.52	Nucleotide transport and metabolism
H	166	3.16	Coenzyme transport and metabolism
I	119	2.27	Lipid transport and metabolism
P	243	4.63	Inorganic ion transport and metabolism
Q	94	1.79	Secondary metabolites biosynthesis, transport and catabolism
R	466	8.88	General function prediction only
S	299	5.70	Function unknown
–	1138	21.68	Not in COGs

The total is based on the total number of protein coding genes in the genome.

fragmented and tagged the DNA with a mean size of 1.4 kb. Then, a limited PCR amplification (12 cycles) completed the tag adapters and introduced dual-index barcodes. After purification on AMPure XP beads (Beckman Coulter Inc., Fullerton, CA, USA), the library was then normalized on specific beads according to the Nextera XT protocol (Illumina). The pooled single strand library was loaded onto the reagent cartridge and then onto the instrument along with the flow cell. Automated cluster generation and paired-end sequencing with dual-index reads were performed in a single 39 h run in 2×250 bp. Total information was obtained from a 574 K/mm cluster density with a cluster passing quality control filters of 95.4% of the clusters passing quality control filters. The passed-filter reads were assembled on the abyss software with $10\times$ coverage cutoff. A summary of the project information is shown in Table 1.

5.7. Genome annotation

Prodigal program was used to predict the open reading frames (ORFs) [29]. tRNAs were predicted using the Aragorn program [30] and rRNAs were predicted using RNAmmer. The predicted genes were Blasted against non-redundant database. The functional annotation of predicted ORFs was performed using RPS-BLAST [31] against the cluster of orthologous groups (COG) database [32] and Pfam database [33]. TMHMM program was used for gene prediction with transmembrane helices [34] and signalP program was used for prediction of genes with peptide signals [35]. PHAST software was used for bacteriophage detection [36].

Competing interest

The authors declare that they have no competing interests.

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