

Insights into the genome sequence of the glyphosate-degrading bacterium *Enterobacter* sp. Bisph1

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Abstract. Benserradj O, Benslama O, Ghorri S. 2022. Insights into the genome sequence of the glyphosate-degrading bacterium *Enterobacter* sp. Bisph1. *Biodiversitas* 23: 817-882. Glyphosate is by far the most extensively utilized herbicide in the world, and because of its negative effects on the environment and animal health, finding microorganisms with the ability to degrade this herbicide is one of the scientists' top priorities. *Enterobacter* sp. Bisph1 was isolated during prospecting glyphosate-degrading bacteria from a sandy soil of the region of Biskra in Algeria. To better understand the involvement of this strain in the degradation of glyphosate, a sequencing of the 4.5 MB genome of *Enterobacter* sp. Bisph1, as well as an annotation, and analysis of its genome sequence were performed in this study. Genomic DNA was sequenced on a MiSeq sequencer Illumina using paired-end sequencing with the Nextera XT protocol. The genome of strain *Enterobacter* sp. Bisph1 comprises one chromosome of 4,578,487 bp with a GC content of 53.6%. Its 4,330 genes (78.13%) were assigned a putative function. The genome contains two intact phages and no CRISPR was found. The genome inspection also revealed the presence of the carbon-to-phosphorus (C-P) bond lyase coding genes clusters that consists of eight genes phnFHIJKLMP. The draft genome of the *Enterobacter* sp. Bisph1 provides insight into the role of this strain as an important agent of bioremediation of glyphosate by examining the genes known to encode the biodegradation enzymes of this herbicide. The sequencing of the genome of Bisph1 allows a better understanding of the taxonomy of this strain within the Enterobacteriaceae family.

Keywords: *Enterobacter* sp. Bisph1, genome annotation, genome sequencing, glyphosate biodegradation

INTRODUCTION

Glyphosate [N-(phosphonomethyl) glycine] is the world's most widely used broad-spectrum herbicide (Duke and Powles 2008). It is a post-emergent and non-selective systemic herbicide (Gill et al. 2016). Glyphosate is a phosphonate compound with a stable carbon-phosphorus (C-P) bond (Sviridov et al. 2015). It is used for the irradiation of weeds in agriculture, forestry, and urban areas (Thiour-Mauprivez et al. 2019). Glyphosate's mode of action is to inhibit aromatic amino acid biosynthesis in the shikimate pathway by blocking the action of the enzyme 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS) (Herrmann and Weaver 1999), which leads to the death of target plants (Wang et al. 2014; Sviridov et al. 2015).

The use of glyphosate herbicides has greatly increased in the world over the last decades in particular, because of the Roundup-ready genetically modified crops, that are resistant to glyphosate (Coupe and Capel 2016), like wheat, maize, soy, cotton, and colza (Annett et al. 2014). Extensive use of glyphosate has led to negative effects for humans and environmental ecosystems (Wang et al. 2016). Due to its binding to soil particles, glyphosate was detected in surface and groundwater (Shushkova et al. 2010; Lupi et al. 2015). In addition, the Aminomethyl-phosphonic acid (AMPA), which is the main compound of glyphosate degradation (Grandcoin et al. 2017) has been reported to

alter the processes of DNA repair and mRNA synthesis in plants and animals (Guilherme et al. 2014).

In order to prevent the risks of glyphosate on human and animal health, as well as on the environment, finding an efficient and environmentally respectful means of degradation of the residual glyphosate is of crucial importance. Biodegradation of glyphosate by microorganisms is considered to be the most effective bioremediation strategy (Wang et al. 2016). Some previous studies have reported the potential of some bacterial species as an effective bioremediation agent for environments contaminated by glyphosate (Zhan et al. 2018), such as *Alcaligenes* sp. GL (Lerbs et al. 1990), *Rhizobia cecaemiloti* 1021 (Liu et al. 1991), *Pseudomonas* sp. 4ASW (Dick and Quinn 1995), *Bacillus cereus* CB4 (Fan et al. 2012), *Enterobacter* sp. Bisph2 (Benslama and Boulahrouf 2016), *Achromobacter* sp. MPK 7A (Ermakova et al. 2017), and *Comamonas odontotermitis* P2 (Firdous et al. 2017). Those species were reported as capable to use glyphosate as a source of phosphorus by cleaving its P-C bond.

During the screening for the degradation capacity of the herbicide glyphosate by bacterial strains isolated from Saharan soil from Biskra in Algeria, strain Bisph1 has shown a positive activity by growing on a minimum mineral medium containing glyphosate as the sole source of phosphorus. Results of morphological and physiological studies showed that strain Bisph1 presents the general

characteristics of the genus *Enterobacter*. MALDI-TOF analysis was showed that Bisph1 was not a member of known species of *Enterobacter*. Strain Bisph1 demonstrated high 16S rRNA gene sequence similarity with *Enterobacter* genus members. According to previous studies, assigning a species to the genus *Enterobacter* is difficult on the basis of 16S rRNA gene sequence analysis (Stephan et al. 2007; Brady et al. 2013).

In order to better understand the genomic and metabolic determinants involved in the glyphosate biodegradation capacity of the strain *Enterobacter* sp. Bisph1, we performed detailed sequencing and annotation of its genome. This study, therefore, aims to provide insight into the role of this strain as a potential glyphosate bioremediation agent by closely examining the genes known to encode the biodegradation enzymes of this herbicide. On the other hand, this study allows us a better understanding of the taxonomy of the strain Bisph1 within the Enterobacteriaceae family.

MATERIALS AND METHODS

Isolation of glyphosate-degrading strains

Soil samples were taken from a sandy ground in the Biskra province in northeastern Algeria, near the northern edge of the Sahara Desert (Figure 1). In order to isolate bacterial strains capable of degrading glyphosate, the modified method was used as previously described by Benslama and Boulahrouf (2016). In this method, a minimum medium containing glyphosate as the sole source of phosphorus was prepared and the concentrations of glyphosate were gradually raised until reaching 12 g L⁻¹ of glyphosate. A nutrient agar medium was inoculated and incubated at 37°C for 24 hours. The isolate Bisph1 was purified in order to sequence its genome.

Genomic DNA extraction and purification

Enterobacter sp. strain Bisph1 was inoculated on Columbia agar (bioMérieux) and incubated aerobically at 37°C. After growth, the bacteria were inoculated in a suspension of Tris-EDTA buffer, then treated with lysozyme and proteinase K. the genomic DNA was extracted three times with the phenol-chloroform and then precipitated with the ethanol at -20°C for purification. After centrifugation, the genomic DNA was resuspended in the Tris-EDTA buffer (Wright et al. 2017).

Genome sequencing and assembly

After extraction and purification of the genomic DNA of Bisph1 strain, the sequencing step was carried out using the MiSeq sequencer (Illumina Inc., San Diego, CA, USA) by the Nextera XT paired-end method. In order to prepare the paired-end library, a tagmentation step was performed, the DNA was fragmented with an average size of 1.4 kb, then tag adapters and dual-index barcodes were added to the fragments by a PCR step. The library was then normalized as reported by the protocol of Nextera XT. The paired-end sequencing with dual index reads was executed in a single run of 2x250-bp. Finally, the obtained reads were assembled using the Abyss software with a 10 × coverage cutoff.

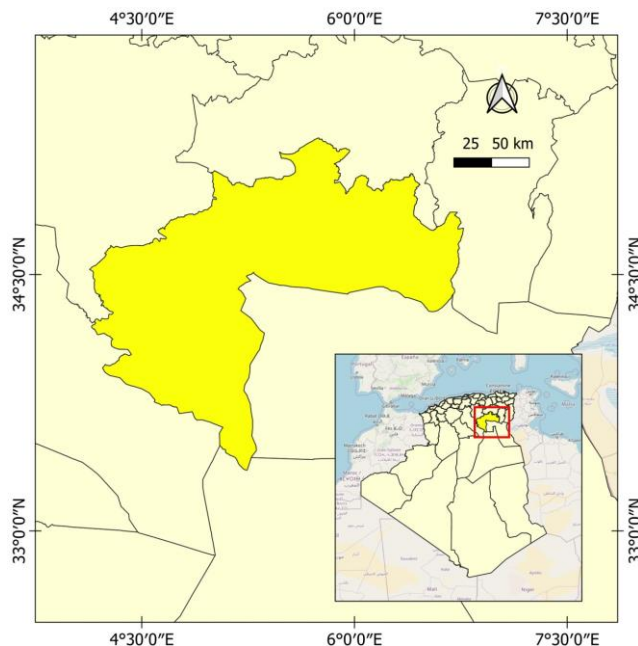


Figure 1. Geographical location of the region of Biskra, Algeria

Data analysis

The open reading frames (ORFs) were first predicted using the Prodigal program (Hyatt et al. 2010). The Aragorn (Laslett et al. 2004) and RNAmmer programs were then applied for the prediction of tRNA and rRNA, respectively. The functional prediction of the detected ORFs was carried out by the RPS-BLAST (Marchler-Bauer et al. 2002) against the cluster of orthologous groups (COG) database (Tatusov et al. 2000) and the Pfam database (Sonnhammer et al. 2000). The genes encoding the transmembrane helices were predicted by the TMHMM program (Krogh et al. 2001), and the genes with peptide signals were predicted by the signalP program (Bendtsen et al. 2004). To predict the presence of the bacteriophage genomes, the PHAST software has been used (Zhou et al. 2011).

Phenotype microarray (PM) (BIOLOG Inc., Hayward, CA, USA) plates were used to analyze utilization of carbon, pH stress, and chemical sensitivity to a wide variety of antibiotics, antimetabolites, heavy metals, and other inhibitors. PM experiments were conducted according to the manufacturer's instructions. In brief, fresh overnight cells from an agar plate of the isolate Bisph1 were suspended in the inoculating fluid and the turbidity was adjusted to 85 %. All PM plates were placed in an aerobic OmniLog incubator reader (BIOLOG Inc., Hayward, CA, USA) set at 37°C and monitored automatically every 15-min for color changes in the wells. Readings were recorded for 48 hours, and data were analyzed with Omnilog-PM software (release OM_PM_109M), which generated a time course curve for Biolog Redox Dye mixA color formation. The isolate was analyzed in duplicate, and a consensus result was obtained. The exported measurement data were further analyzed for statistically estimating parameters from the respiration

curves and automatically translated into negative, positive, and weakly positive reactions.

RESULTS AND DISCUSSION

Genome sequencing analysis

The whole-genome Shotgun project of strain *Enterobacter* sp. strain Bisph1 has been deposited at GenBank with the accession number JXAG000000000. The genome comprises one chromosome of 4,578,487 bp with a GC content of 53.6% (Figure 2). It is composed of 54 large contigs (37 scaffolds). From the 4,339 predicted genes, 4,262 were protein-coding genes and 77 (1.77 %) were RNAs. The total number of genes with a putative function is 4,330, i.e., a percentage of 78.13%. The rest of the genes were annotated as hypothetical proteins or proteins with no known function. The genome sequence of *Enterobacter* sp. strain Bisph1 reveals that this strain encounters two intact phages, *Salmonella* phage RE-2010-NC_019488 and *Enterobacteria* phage P2 NC_001895.1, with the absence of CRISPR. The summary list of the genome properties of the Bisph1 strain is shown in Table 1. The assignment of genes to the different functional categories of COGs is summarized in Table 2.

The comparison of the genome of *Enterobacter* sp. strain Bisph1 with the closely related species such as *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. Bisph1 is smaller than those of *E. massiliensis*, *E. aerogenes*, *E. cancerogenus*, *E. cloacae*, *E. cloacae* subsp. *dissolvens*, *E. hormaechei*, and *E. lignolyticus* (4.57, 4.92, 5.28, 4.80, 4.79, 4.96, 4.80, and 4.81Mb, respectively), but larger than that of *E. asburiae* (3.81Mb). In addition, *Enterobacter* sp. Bisph1 has a G+C content lower than all species mentioned previously (53.6, 55.1, 54.8, 55.54, 54.54, 55.1, 55.2, 57.02, and 53.8%, respectively) and has the smallest number of predicted genes (4,339, 4,724, 5,021, 4,495, 4,740, 4,646, 4,779, 4,558 and 4,805, respectively) (Table 3).

Table 1. Genome statistics of *Enterobacter* sp. strain Bisph1

Attribute	Value	Total percentage
Genome size (bp)	4,578,487	100
DNA coding (bp)	4,002,759	87.42
DNA G+C (bp)	2,455,194	53.6
DNA scaffolds	37	100
Total genes	4,339	100
Protein coding genes	4,262	98.23
RNA genes	77	1.77
Genes assigned to COGs	4,330	78.13
Genes with Pfam domains	3,794	89.01
Genes with signal peptides	385	8.8
Genes with transmembrane helices	844	19.45
CRISPR repeats	0	0

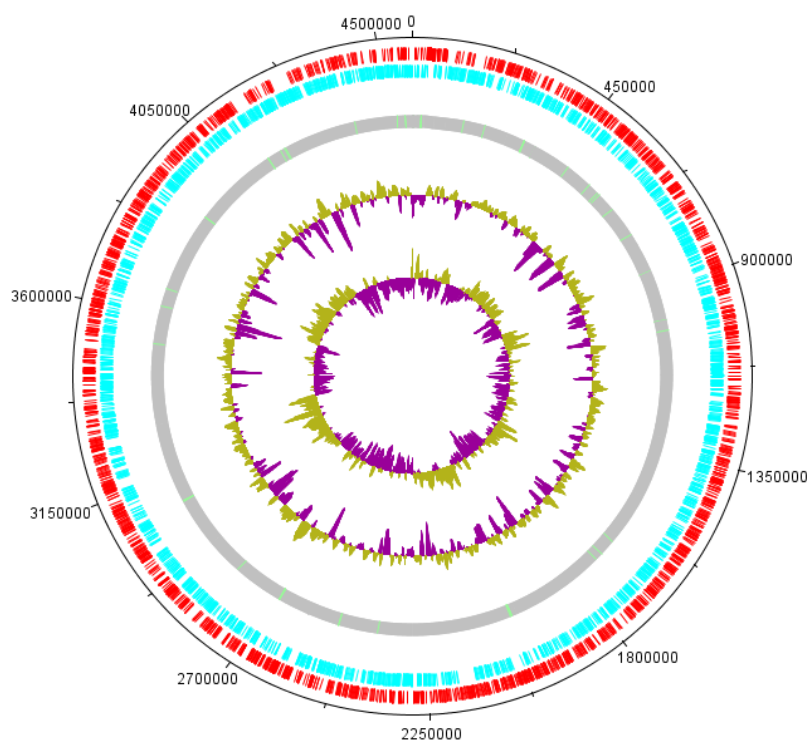


Figure 2. Graphical circular map of *Enterobacter* sp. strain Bisph1 genome. From outside to the center are as follows: genes on the forward strand, genes on the reverse strand, RNA genes (tRNAs green, rRNAs grey), GC content, and GC skew

Table 2. Number of genes associated with general COG functional categories

Val	Percent-	Description
ue	age	
153	3.59	Translation, ribosomal structure, and biogenesis
1	0.02	RNA processing and modification
258	6.05	Transcription
139	3.36	Replication, recombination, and repair
0	0	Chromatin structure and dynamics
30	0.70	Cell cycle control, cell division, chromosome partitioning
40	0.94	Defense mechanisms
196	3.97	Signal transduction mechanisms
203	4.76	Cell wall/membrane biogenesis
78	1.74	Cell motility
64	1.50	Intracellular trafficking and secretion
122	2.86	Posttranslational modification, protein turnover, chaperones
191	4.48	Energy production and conversion
314	7.37	Carbohydrate transport and metabolism
304	7.13	Amino acid transport and metabolism
73	1.71	Nucleotide transport and metabolism
152	3.57	Coenzyme transport and metabolism
106	2.49	Lipid transport and metabolism
210	4.93	Inorganic ion transport and metabolism
67	1.57	Secondary metabolites biosynthesis, transport and catabolism
383	8.99	General function prediction only
277	6.50	Function unknown
932	21.87	Not in COGs

Note: the total is based on the total number of protein-coding genes in the genome.

Phenotypic microarray analysis

Phenotype microarray analysis showed that the strain Bisph1 seems able to use a wide range of organic and inorganic carbon sources such as D-trehalose, tween 80, adenosine, fumaric acid, bromosuccinic acid, dihydroxyacetone, L-alaninamide, citric acid, lactic acid, glycerol, D-gluconic acid, malonic acid, L-serine, L-alanine, D-psicose, and uridine. Furthermore, strain Bisph1 was resistant to the higher doses of several antibiotics such as amikacin, amoxicillin, bleomycin, capreomycin, erythromycin, ceftriaxone, gentamicin, carbenicillin, rolitetracycline, ampicillin, rifampicin, spiramycin, spectinomycin, polymyxin B, and azlocillin. The isolate is also resistant to certain fungicides such as 1-hydroxypyridine-2-thione (pyrithione), domiphen bromide, dichlofluanid, and 4-chloro-3,5-dimethyl-phenol, as well as to certain organic acids such as ethylene glycol bis-acid (b-aminoethylether) tetraacetic -NNN⁺N⁺, boric

acid, and nalidixic acid, and to other toxic compounds such as benzethonium chloride, dodecyltrimethylammonium bromide, metaborate sodium, sodium cyanate, chloride of cadmium, sanguinarine chloride, nitrite sodium, cesium chloride, sodium selenite, aluminum sulfate, chloride of chromium (III), and ferric chloride. However, the strain Bisph1 was susceptible to the lowest dose of the antibiotic novobiocin, cefepime, ticarcillin+clavulanic acid, and imipenem, of the chelating agent 2,2-dipyridyl, the toxic anion of sodium orthovanadate, and sodium metavanadate. The strain Bisph1 was relatively resistant to acidic pH, tolerating a pH of about 4.0, and can grow under alkaline pH up to 10.

Insights into the genome sequence

A large number of genes assigned to COG functional categories for the transport and metabolism of carbohydrates (7.37%) and amino acids (7.13%) were identified. Genomic analysis of strain Bisph1 showed an abundant presence of gene cluster encoding for enzymes involved in the breakdown, utilization, and biosynthesis of carbohydrates. The result of phenotypic microarray analysis reveals that strain Bisph1 can use a multitude of carbohydrate and amino-acid compounds such as D-mannose, D-sorbitol, L-proline, glycerol, L-rhamnose, thymidine, uridine, and gentiobiose. In addition, analysis of the genome sequence of *Enterobacter* sp. strain Bisph1 revealed the presence of a high number of genes associated with biosynthesis of exopolysaccharides such as genes putatively associated with cellulose synthesis (*BcsA*, *BcsB*, *BcsC*, and *BcsE*) (Omadjela et al. 2013).

Glyphosate is a phosphonate compound containing a carbon-to-phosphorus (C-P) bond (Singh and Walker 2006). The C-P linkage is found to be heavily resistant to non-biological degradation in the environment (Hayes et al. 2000). One of the metabolic biodegradation pathways of glyphosate involves the enzyme C-P lyase. This enzyme breaks the C-P bond, producing inorganic phosphate and sarcosine, which is metabolized to glycine by sarcosine oxidase (Dick and Quinn 1995; Sviridov et al. 2011). The inspection of strain Bisph1 genome confirmed the presence of C-P lyase coding genes clusters. The *phn* operon that governs the C-P lyase activity (Poehlein et al. 2013) in strain Bisph1 consists of 8 genes *phnFHIJKLMP*. This finding supports the ability of strain Bisph1 to grow on a minimal medium containing glyphosate as the sole phosphorus source.

Table 3. Result of Comparative genomics between *Enterobacter* sp. strain Bisph1 and related species

Related species	Genome size (Mb)	G+C%	Number of predicted genes	Reference
<i>Enterobacter</i> sp. strain Bisph1	4.57	53.6	4,339	<i>This research</i>
<i>E. massiliensis</i> strain JC163 T	4.92	55.1	4,724	Lagier et al. (2013)
<i>E. aerogenes</i> strain KCTC 2190	5.28	54.8	5,021	Shin et al. (2012)
<i>E. asburiae</i> strain LF7a	3.81	55.54	4,495	Lau et al. (2014)
<i>E. cancerogenus</i> strain YZ1	4.80	54.54	4,740	We et al. (2013)
<i>E. cloacae</i> strain EcWSU1	4.79	55.1	4,646	Humann et al. (2011)
<i>E. cloacae</i> subsp. <i>dissolvens</i> strain SDM	4.96	55.2	4,779	Xu et al. (2012)
<i>E. hormaechei</i> strain ATCC49162	4.80	57.02	4,558	O'Hara et al. (1990)
<i>E. lignolyticus</i> SCF1	4.81	53.8	4,805	DeAngelis et al. (2011)

The investigation of the functional genes of strain Bisph1 revealed the presence of the membrane protein TonB-ExbB-ExbD complex, involved in the synthesis of siderophores and their transport into the cell in its chelated form (Postle 2007). Thus, the strain can ensure its iron requirement. The genome sequence of *Enterobacter* sp. strain Bisph1 reveals that this strain encounters two intact phages, *Salmonella* phage RE-2010-NC_019488, and *Enterobacteria* phage P2 NC_001895.1. This invasive presence is probably due to the absence of a defense system against phages (CRISPR-Cas) (Ratha et al. 2015).

In conclusion, the genome of *Enterobacter* sp. strain Bisph1 is successfully characterized. This research confirms that the genome of the strain *Enterobacter* sp. Bisph1 contains genes involved in the biodegradation of glyphosate, which reflects the interest of this strain as a bioremediation agent in environments contaminated by this herbicide. Analysis of the genome of Bisph1 also shows that this strain has a great metabolic potential managed by an entire range of genes and operons.

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