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Genome of *Alcanivorax* sp. 24: A hydrocarbon degrading bacterium isolated from marine plastic debris

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ABSTRACT

Alcanivorax is an important member of the hydrocarbonoclastic group known for using alkanes and other related compound as their preferred carbon source. Here we report the genomic characteristics of *Alcanivorax* sp. 24 isolated from plastic marine debris. Its 4,765,873 bp genome, containing 4239 coding sequences, revealed the presence of all genomic features involved in alkane degradation (*i.e.* two cytochrome P450, three alkane monooxygenases AlkB and two enzymes involved in the degradation of long-chain alkanes AlmA) as well as other relevant enzymes that may play a role in the biodegradation of other polymers such as polyhydroxybutyrate. The genome features and phylogenetic context of these genes provide interesting insight into the lifestyle versatility of *Alcanivorax* sp. living in the plastisphere of marine plastic debris.

1. Introduction

Alcanivorax, a genus from the Alcanivoracaceae family within the class Gammaproteobacteria, is an important member of the hydrocarbonoclastic clade (Yakimov et al., 2019). Microorganisms associated to this clade have a strong preference for using hydrocarbon substrates such as alkanes and a variety of alkyl-benzene derivative compounds as a source of carbon and energy. Since the description of the genus *Alcanivorax,* all strains isolated to date have been reported to degrade aliphatic compounds *i.e.* both linear and branched alkanes (Rojo, 2009; Schneiker et al., 2006), however, some *Alcanivorax* spp. can also metabolise mono-aromatic compounds *i.e.* benzene and toluene (Hassan et al., 2012; Rahul et al., 2014). Although more labile compounds can be uptaken and metabolised by some members of this genus (Lai et al., 2011; Radwan et al., 2019), common carbohydrates such as glucose, fructose or mannose, and amino acids are generally not used by most *Alcanivorax* strains isolated to date (Liu and Shao, 2005; Yakimov et al., 1998). The ability for alkane degradation relies on key enzymes such as a cytochrome P450, different alkane monooxygenase AlkB homologues (Wang and Shao, 2013) and, as described more recently, a monooxygenase related to the flavin-binding family AlmA which is involved in the degradation of a broad range of long-chain alkanes (Throne-Holst et al., 2007).

Here we provide the genomic characterization of *Alcanivorax* sp. 24, isolated from marine plastic debris and able to catabolise alkanes of different chain lengths (*i.e.* C_{16}-C_{25}) as well as the polyester polyhydroxybutyrate.

2. Data description

2.1. Isolation, genome sequencing and phylogenetic affiliation

Alcanivorax sp. 24 was isolated from plastic marine debris collected from the high intertidal zone at La Rinconada beach, Antofagasta, Chile using Bushnell-Haas mineral medium (Bushnell and Haas, 1941) supplemented with 3% NaCl (*w/v*) and polyhydroxybutyrate (Sigma) as a source of carbon (Table 1). The isolate was then routinely grown with sodium succinate (0.5% *w/v*) as a carbon source.

Genomic DNA was extracted using the Qiagen DNeasy Plant Mini Kit implementing a bead-beating step for cell lysis. The genomic library preparation was performed using the Nextera XT kit prior to HiSeq 250–300 bp paired-end sequencing (Illumina®). Raw sequences were quality checked and trimmed (FASTQ Toolkit), assembled (SPAdes) and annotated (PROKKA) using Illumina's environment BaseSpace. The

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Table 1
Sampling, isolation and MIGS information.

Items	Description
General features	
Classification	Domain *Bacteria* Phylum Proteobacteria Class *Gammaproteobacteria* Order *Oceanospirillales* Genus *Alcanivorax*
Gram stain	Negative
Cell shape	Rod
Motility	Motile
Pigmentation	No-pigmented
Submitted to INSDC	GenBank SNUA00000000
Investigation type	Bacteria
Project name	*Alcanivorax* sp. 24
Sampling reference	
Geographical location (LAT/LONG)	23.463658S, 70.504668W
Geographical location (country)	Pacific Coastline, Chile
Sampling location (Area/region)	Shoreline, La Rinconada beach, Antofagasta
Collection date	29-October-2014
Isolation date	15-December-2014
Environment (biome)	Shoreline [ENVO:00000486]
Environment (feature)	Marine sub-littoral zone [ENVO:01000126]
Environment (material)	Plastic [ENVO:01000404]
Environmental package	Plastic pollution [ENVO:02500040]
Depth	0–1 cm (superficial)
Isolation conditions	
Culture medium	Bushnell-Haas (mineral medium)
Source of carbon	Poly(3-hydroxybutyrate)
Salt concentration	3% (w/v) NaCl
Temperature of incubation	30 °C
pH	7.0
Sequencing	
Sequencing method	Illumina HiSeq 250–300 bp
Assembly	*GS De Novo Assembly* (SPAdes)

complete genome sequence of *Alcanivorax* sp. 24 was deposited in GenBank database under the accession number SNUA00000000. Additionally, a list of 4239 codifying sequences including nucleotide and protein sequences was also generated (Table S1).

The BLASTN analysis of its 16S rRNA sequence (NCBI platform) identified the strain as part of the *Alcanivorax* genus, with 98.8% identity to both *Alcanivorax xenomutans* P40 and *Alcanivorax dieselolei* B5. The phylogenetic affiliation shows that *Alcanivorax* sp. 24 clusters together with other *Alcanivorax* species and is closely related to other hydrocarbon degrading organisms such as *Microbulbifer thermotolerans* (Lee et al., 2014) and *Oleiphilus messinensis* (Golyshin et al., 2002) (Fig. 1A).

2.2. Genome characteristics

The genome of *Alcanivorax* sp. 24 comprises 4,765,873 base pairs (bp) with 61.4% GC content. From the 4239 codifying sequences identified, 4188 code for proteins out of which 948 (22.6%) were annotated as proteins of unknown function (Table 2).

Further genome characterization done in BlastKOALA (Kanehisa et al., 2016) showed that *Alcanivorax* sp. 24 has no auxotrophies for amino acids, vitamins or cofactor, as it encodes the complete biosynthetic pathways for all these elements (Table S2). This was confirmed by its ability to grow on minimal media with no supplements. Despite encoding for all genes involved in glycolysis/gluconeogenesis, it lacks the phosphotransferase system required for the uptake and metabolism of extracellular sugars. Complete citric acid and glyoxylate cycles were found. The strain also contains transporters for ammonium and nitrate uptake as well as all genes required for nitrate dissimilatory reduction and denitrification, and assimilatory sulphate reduction (Table S2). Apart from *Alcanivorax borkumensis*, most *Alcanivorax* strains are motile and, as expected, *Alcanivorax* sp. 24 contains a complete flagellar machinery located within a single genetic locus (*i.e.* ALC24_1757 – ALC24_1812). A large number of fimbria and other proteins involved in adhesion are also present suggesting a lifestyle versatility. A number of mobile genetic elements were found in the genome *i.e.* 15 different transposases and six viral-like integrases.

A total of 32 genomic islands were detected in the genome of *Alcanivorax* sp. 24 using IslandViewer 4 (Bertelli et al., 2017) (Fig. S1 and Table S3). The size range was between 4 and 104 kbp and the maximum number of genes found in a single genomic island was 32. From the 265 genes encoded in these locations, half (*i.e.* 132 genes) encode for proteins of unknown function. Other elements encoded within the genomic islands were type II secretion systems, flagellar-like proteins and proteins with viral origin from prophage remnants. Additionally, the occurrence of transposases flanking some of the genomic islands suggests the potential genomic plasticity of *Alcanivorax* sp. 24. None of the genes involved in alkane degradation were found within these genomic islands (Table S3).

2.3. Genomic profile of alkane degradation

As expected, the genome of *Alcanivorax* sp. 24 encoded all genes involved in alkane degradation expected for this genus (Table S4). Among them, we identified two *cytochrome* P450 genes involved in the metabolization of short/medium-chain alkanes *i.e.* C_5–C_{11} (van Beilen et al., 2006) and three homologues of the alkane monooxygenase *alk*B (*i.e.* two copies of *alk*B1 and one of *alk*B2) which catalyse the first step of medium/long alkane hydroxylation (C_{10}–C_{20}). Additionally, we found two homologues of *alm*A which have been associated to longer alkane degradation ($> C_{20}$), and four outer-membrane transporters which showed similarities to the alkane transporter *fad*L (Gregson et al., 2018; Liu et al., 2011).

2.4. Phylogenetic context of the alkane degradation genes in Alcanivorax sp. 24

Alkane degraders can harbour different *alk*B homologues with a large range of divergence between copies (*i.e.* 27.7 to 99.7% similarity at the amino acid level; Nie et al., 2014). *Alcanivorax* sp. 24, like *A. xenomutans* and *A. dieselolei* (Fu et al., 2018; Lai et al., 2012), encodes for three different *alk*B genes in its genome. These three different *alk*B genes are considerably divergent (between 35% and 44% similarities at the amino acid level) and group in three distinct phylogenetic branches in the phylogenetic tree (Fig. 1B), suggesting a potential functional distinctness between copies as previously described (Liu et al., 2011; Marín et al., 2003).

The two FAD-dependent monooxygenases present in *Alcanivorax* sp. 24 were closely related to the flavoprotein alkane hydroxylase AlmA, which was shown to be involved in long-chain alkane degradation in *Acinetobacter* sp. (Minerdi et al., 2012; Throne-Holst et al., 2007) and *Alcanivorax dieselolei* (Liu et al., 2011). Both enzymes related to AlmA diverge from each other (*i.e.* 52% similarity) and group in two distinct phylogenetic clusters together with the two *alm*A-like genes present in strains *A. xenomutans* and *A. dieselolei* (Fig. 1C). Low similarities were also observed between the two cytochromes P450 genes identified in *Alcanivorax* sp. 24 (13%) suggesting different functions and/or substrate specificity.

2.5. Additional catalytic functions involved in biodegradation

The genome mining of *Alcanivorax* sp. 24 revealed a large number of other enzymes that could play an important role in other biodegradation processes, *i.e.* 13 esterases, 17 peroxidases, and a predicted laccase-like protein (Table S5). To the best of our knowledge, no laccase or esterase activity has been previously characterized in the genus

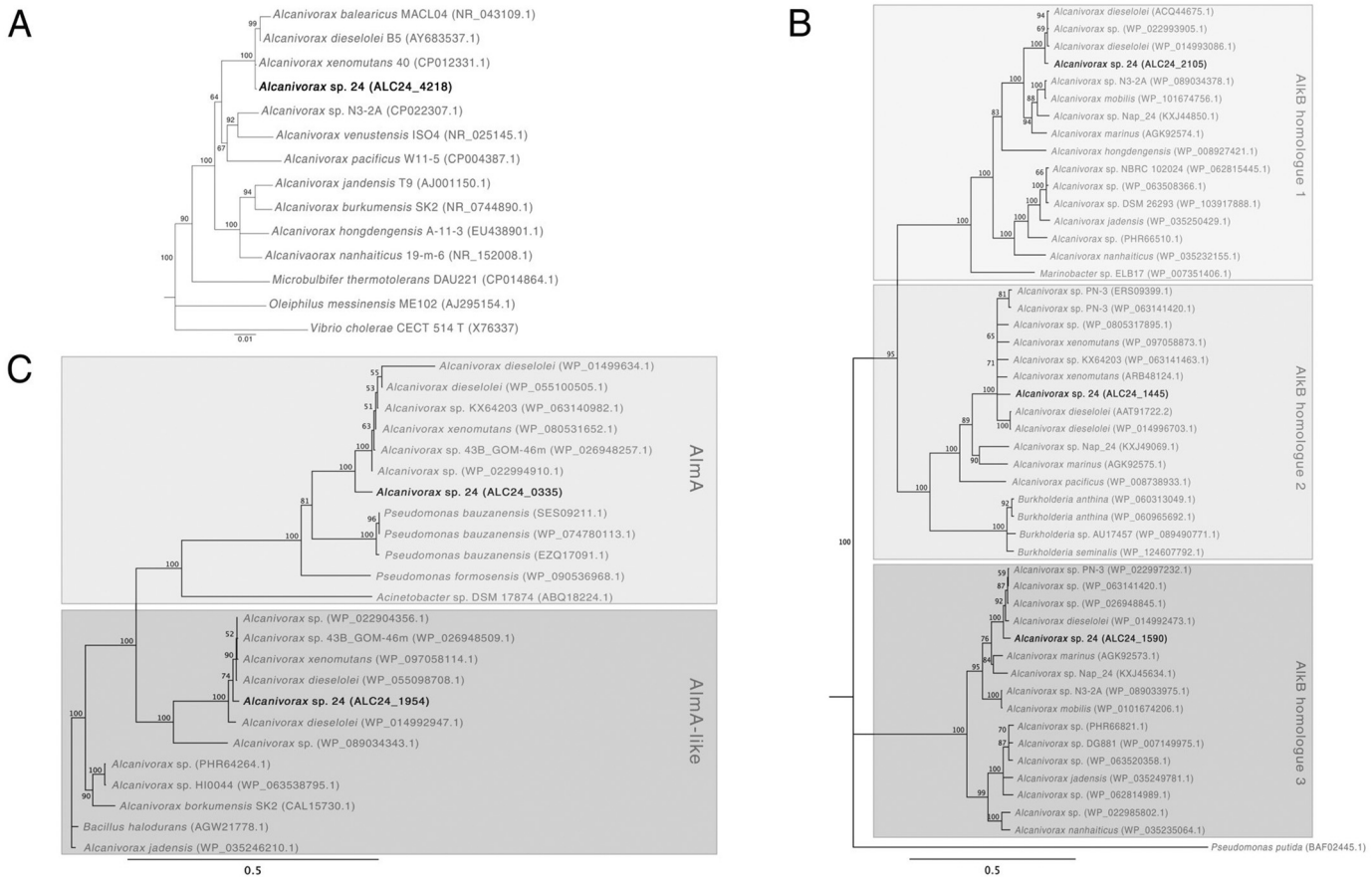

Fig. 1. Phylogenetic trees of Alcanivorax sp. 24 and its closest related species. A) 16S rRNA sequence analysis using Neighbour-Joining algorithm (*i.e.* Tamura-Nei Genetic Distance Model). The outgroup was V. cholerae CECT 514. B) AlkB tree showing the three homologues present in Alcanivorax sp. 24 in bold. Branches containing each one of the two copies of AlkB1 (ALC24_1445; ALC24_2105) and AlkB2 (ALC24_1590) are indicated. A xylene monooxygenase from P. putida was used as the outgroup of the rooted tree. C) AlmA tree showing the two AlmA-like homologues present in Alcanivorax sp. 24. Both FAD-containing monooxygenase EthA (ALC24_0335) and MymA (ALC24_1954) are highlighted in bold. Trees were built using the Neighbour-Joining algorithm with closely related amino acid sequences retrieved from a BLAST-p search of Alcanivorax sp. 24 alkane degrading proteins. The bootstrap value represents 1000 replicates and random seeding. Trees were built using Geneious v11.1.5.

Table 2
Genome features of *Alcanivorax* sp. 24.

Genome features	
Size bp	4,765,873
% GC content	61.4
Total coding sequences	4239
Protein coding sequences	4188
% Protein coding sequences	98.8
Hypothetical proteins	943
Function assigned	3235
% Function assigned	77.7
Codon elements	
Start codon	
% ATG	87.5
% GTG	8.8
% TTG	2.5
% Non-canonical codons	1.2
Stop codon	
% TGA	59.9
% TAA	23.7
% TAG	15.2
% Non-canonical codon	1.2
RNA elements	
rRNA	4 (5S, 23S, 2 × 16S)
tRNA	46

Alcanivorax. The esterase ALC24_4107 (Table S5) may be responsible for polyhydroxybutyrate hydrolysis, the natural polyester on which this strain was enriched, although further research is required to determine the role these enzymes really play *e.g.* in polymer degradation.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.margen.2019.05.001.

Authors contributions

V.Z., C.D., M.I.G and J.A.C-O created the framework of this study. V.Z. performed the experimental work and analysis with the help of J.A.C-O. The manuscript was written by V.Z and J.A.C-O., with input from C.D. and M.I.G.

Conflict of interest statement

The authors declare no conflict of interest.

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