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ORIGINAL ARTICLE

Comparative genome analysis of *Oceanimonas* sp. GK1, a halotolerant bacterium with considerable xenobiotics degradation potentials

Reza Azarbaijani[1] · Laleh Parsa Yeganeh[1] · Jochen Blom[2] · Habibollah Younesi[3] · Seyyed Abolhassan Shahzadeh Fazeli[1,4] · Meisam Tabatabaei[1,5,6] · Ghasem Hosseini Salekdeh[1,5,7]

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Abstract The growing pollution by xenobiotic compounds generated through both natural and anthropogenic activities has endangered the environment. The advent of the next generation sequencing has provided fast and cost-effective tools to explore genomes to discover novel xenobiotic-degrading genes. A Gram-negative marine halotolerant *Oceanimonas* sp. GK1 was analyzed for main physiological and genetically important characteristics at the genome scale while being compared with six other phylogenetically-close sequenced genomes. This exploration revealed high potential of *Oceanimonas* sp. GK1 for biodegradation of xenobiotics compounds such as phenol. More specifically, the isolate utilizes phenol via the *ortho*-cleavage pathway as a carbon source in the citrate cycle. This was further confirmed by the significant shortage of carbohydrate active enzymes in *Oceanimonas* sp. GK1 genome, which has forced this bacterium during the course of evolution to change its metabolism and physiology to benefit unusual carbon and energy sources to survive under harsh conditions.

Keywords Xenobiotic pollution · Next generation sequencing · Genome annotation · Comparative study · Halotolerant

Reza Azarbaijani and Laleh Parsa Yeganeh contributed equally to this work.

✉ Ghasem Hosseini Salekdeh
hsalekdeh@yahoo.com

[1] Molecular bank, Iranian Biological Resource Center (IBRC), ACECR, No.80, West Howeyzeh St., Northern Sohrevardi Ave., Tehran, Iran

[2] Bioinformatics and System Biology, Justus-Liebig-University Giessen, Giessen, Germany

[3] Department of Environmental Science, Faculty of Natural Resources, Tarbiat Modares University, Noor, Mazandaran, Iran

[4] Department of molecular and cellular biology, faculty of basic sciences and advanced technologies in biology, University of Science and Culture, Tehran, Iran

[5] Agricultural Biotechnology Research Institute of Iran (ABRII), AREEO, Karaj, Iran

[6] Biofuel Research Team (BRTeam), Karaj, Iran

[7] Department of Molecular Systems Biology at Cell Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran

Introduction

The increasing level of environmental pollution is among the most catastrophic tragedies of the modern era which in turn has negatively impacted life quality in major parts of the world (Sekoai and Daramola 2015). Oil spills especially into marine ecosystems are regarded by many as the most dreadful disasters which have frequently occurred during the last decades. Apart from through the incidents, xenobiotic compounds, e.g., hydrocarbon aromatic compounds, phenol and their derivates are also the main constituents emitted into the environment by chemicals manufacturers as well as a number of other industries such as paper mills (Bonfá et al. 2013). Among the xenobiotic materials, phenol has been revealed to possess deadly affects on marine microorganisms and aquatic flora even at concentrations as low as parts-per-million (Tišler and Zagorc-Končan 1997; Saha et al. 1999; Kahru et al. 2002; Michałowicz and Duda 2007; Chen et al. 2010).

On the other hand, the enormous growth of contemporary science as well as industrial and technological revolutions have had a massive influence on attempts aimed at finding

novel ways to minimize the tragic effects of anthropogenic activities on the biota. Among the solutions offered, naturally-occurring tools and procedures seems to be more advantageous. For instance, Atlas and Philp (2005) and Sinha et al. (2011) in separate studies argued that xenobiotic-degrading microorganisms are the best way to control or reduce the mortal effects of such compounds. Such biodegrading microorganisms could be employed in bioremediation of phenolic effluents of various origins (Bonfá et al. 2013; Le Borgne et al. 2008; McGenity 2010).

In our previous study, *Oceanimonas* sp. GK1 (IBRC-M 10197), a Gram-negative, rod-shaped, motile, aerobic and marine halotolerant bacterium (up to 12 % NaCl) was isolated from the Gavkhouni wetland [with about 20 % (w/v) total salt; 32°25′N, 52°39′E] in Iran (Yeganeh et al. 2012). This wetland is a grabni hole covered by quaternary sediments and watered by the Zayandehroud river as the only water source of the Gavkhouni basin (Sabzevari et al. 2013). This water basin is severely polluted and contains a high level of phenolic compounds. Since 2001, when Brown et al. proposed to classify a distinct genus as *Oceanimonas*, the number of physiologically well-characterized and published species has been very limited (Brown et al. 2001; Ivanova et al. 2005). In fact, the closed molecular and physiological relationship between *Pseudomonas*, *Aeromonas* and *Oceanimonas* genera (Brown et al. 2001) make the isolation and registration of new species of this genus very difficult.

One of the most important features of *Oceanimonas* genus which Brown et al. (2001) stated for *Oceanomonas baumannii* sp. nov. and *Oceanomonas doudoroffii* (Baumann et al. 1983) comb. nov. is the phenol degradation capability of this bacteria via the *ortho*-cleavage pathway, and in the presence of elevated salinity [minimal medium containing 5 % NaCl (w/v) and 4 mM phenol]. On such a basis, the overall aim of this study was to conduct a genome-wide analysis of the halotolerant *Oceanimonas* sp. GK1 complete sequenced and annotated genome. This was the first complete sequenced genome analysis of this genus in order to characterize the genetic potentials of this bacterium to survive in an extreme environment with respect to phenol concentration. Moreover, other main physiological properties of this isolate were also surveyed.

Materials and methods

Bacterial strain, growth conditions, and genomic DNA extraction

The *Oceanimonas* sp. GK1 deposited in the Iranian Biological Resource Center (IBRC-M 10197; IBRC, Tehran, Iran) was characterized by using biochemical (i.e., catalase and oxidase reactions, nitrate reduction), physiological (i.e., motility, growth at different salt concentrations, pH and temperatures) and PCR tests. For genomic DNA extraction, cells were grown aerobically for 3–7 days in the DSMZ 10 % MH medium at 34 °C and 220 rpm . DNA extraction was conducted by the "IBRC Gram-negative bacterial genomic DNA extraction kit" (Cat no. MBK0041, Iran) following the manufacturer's instructions.

Genome sequencing and finishing

Whole genome sequencing of *Oceanimonas* sp. GK1 was performed on 454 GS-FLX titanium. In total, 93,921,861 bases of 247,884 random reads with an average read length of 378 nucleotides were obtained. The approximate coverage of *Oceanimonas* sp. GK1 genome was 30-folds. The sequence reads were assembled into 72 contigs using Newbler Assembler software v.2.3. Paired end sequencing resulted in 3 scaffolds with 47 gaps. All gaps were closed using sanger DNA sequencing (Sanger et al. 1977).

Genome annotation

Curation and annotation of the genome was carried out in two minimal and enriched levels following International Nucleotide Sequence Database Collaboration (INSDC) guidelines. At minimal level, the Glimmer v.3.02 (Delcher et al. 1999) and GenMark.hmm v. 2.8 (Lukashin and Borodovsky 1998) were used for prediction and finding of prokaryotic coding sequences (CDs), while RNAmmer v.1.2 (Lagesen et al. 2007) and tRNAscan-SE v.1.21 (Lowe and Eddy 1997) were used for identification of 5S, 16S, and 23S ribosomal RNA genes as well as tRNAs and their corresponding genes, respectively. The enriched annotation was performed by means of Rapid Annotation using Subsystem Technology (RAST) v.4.0 (Aziz et al. 2008), Bacterial Annotation System (BASys) v.1.0 (Van Domselaar et al. 2005) and NCBI Prokaryotic Genomes Automatic Annotation Pipeline (PGAAP).

Phylogenetic analysis

Phylogenetic analyses of the completely-sequenced genomes of *Oceanimonas* sp. GK1 (Acc. No. NC_016745) along with 6 members of the Aeromonadaceae family, including *Aeromonas salmonicida* ssp. *salmonicida* A449 (Acc. No. NC_009348), *Aeromonas hydrophila* ssp. *hydrophila* ATCC 7966 (Acc. No.: CP000462), *Aeromonas caviae* Ae398 (Acc. No. CACP01000001 to CACP01000149), *Aeromonas veronii* B565 (Acc. No. CP002607), *Aeromonas salmonicida* ssp. *salmonicida* 01-B526 (Acc. No. AGVO00000000), and *Tolumonas auensis* DSM 9187 (Acc. No. NC_012691) were performed based on the homology between their concatenated housekeeping genes (*adk*, *argS*, *gyrA*, *rpoB*, *rpoD*, *metS* and *dnaE*) and core genes of these genomes.

Growth culture preparation and phenol degradation experiments

The isolated bacterium was activated using the same DSMZ growth medium as described earlier. Distilled water was added to make a 1-L solution and the sterilized media were distributed into 250-ml Erlenmeyer flasks under aerobic conditions. The growth was monitored by measuring the turbidity of the cultures.

The isolated strain was first grown aerobically on the same medium containing 1000 mg/l phenol for acclimation and adaption to the environment for 5 days. Then, an acclimated seed culture was prepared and harvested at the mid-exponential phase. For phenol degradation experiments, a 3-ml cell suspension of the acclimated seed culture were transferred to fresh media (100 ml) with a gradual increase in phenol concentration (500–2000 mg/l), while the pH was adjusted to 7.0. The media were incubated at 25 °C on a rotary shaker (150 rpm). The cell growth was measured by the turbidity of the media using a spectrophotometer. A calibration curve was developed based on the cell dry weight with respect to optical density, and the cell dry weight of the samples was measured based on the calibration curve. For measuring the phenol concentration, the culture media was filtered through a filter paper (Whatman No. 42) to remove solid cells and the phenol concentration as indicated by chemical oxygen demand (COD) was monitored every 24 h.

Results and discussion

General genome features

The genome of *Oceanimonas* sp. GK1 (Acc. No.: NC_016745) as investigated in the present study comprises a single circular chromosome of 3,514,537 bp along with two plasmids of 8462 bp and 4245 bp in length. The average G+C content of the genome was measured at 61.08 %. Genome annotation revealed 3222 protein-coding genes with an average size of 966 bp. These coding regions cover 96.64 % of the genome and 2938 (88.12 %) were functionally assigned. Moreover, 122 rRNA genes were predicted out of which 22 were organized in 7 rRNA operons and the rest (90 of 122) were related to the tRNA genes.

Phylogenetic diversity

Phylogenetic analysis was performed based on the housekeeping genes (i.e., *adk*, *argS*, *gyrA*, *rpoB*, *rpoD*, *metS* and *dnaE*) and the core genes in the investigated genomes i.e., that of the present study and six from the literature: *Aeromonas salmonicida* ssp. *salmonicida* A449 (Acc. No. NC_009348), *Aeromonas hydrophila* ssp. *hydrophila* ATCC 7966 (Acc. No. CP000462), *Aeromonas caviae* Ae398 (Acc. No. CACP01000001 to CACP01000149), *Aeromonas veronii* B565 (Acc. No. CP002607), *Aeromonas salmonicida* ssp. *salmonicida* 01-B526 (Acc. No. AGVO00000000), and *Tolumonas auensis* DSM 9187 (Acc. No. NC_012691). The results obtained placed *Oceanimonas* sp. GK1 and *Tolumonas auensis* DSM 9187 in the same clade well separated from the *Aeromonases* (Fig. 1a, b).

Genomic islands, insertion sequence elements and transposases

Four genomic islands (GEIs) were identified on the single chromosome of *Oceanimonas* sp. GK1. These GEIs cover 1.96 % of genome length with 62 coding sequences. Three of the GEIs identified contained genes related to phage recombination factors and one had insertion sequence (IS) elements. Characteristics of these GEIs are presented in Table 1 and Fig. 2. The GC content range of these islands was measured between 49.31 and 55.81 %.

The results of the blastp similarity analyses obtained for the GEIs' recombination factors showed that the GEI1 included four coding sequences (i.e., GU3_08700, GU3_08735, GU3_08745 and GU3_08760) which were highly similar to their counterparts in *Aeromonas* phage phiO18P. GEI2 contained 4 encoding genes for conjugal transfer i.e., *Tra*J (GU3_09380), *Tra*I (GU3_09385), *Trb*J (GU3_09390) and *Trb*L (GU3_09400) in addition to a single phage integrase-encoding gene (GU3_09355). All these genes were of close proximity to those of *Vibrio* genus. Among the 13 coding region sequences indentified in GEI3, 3 codings, integrase (GU3_10290), *Zonula occludens* toxin (*Zot*)(GU3_10305) and phage replication initiation factor (GU3_10320), were of significance in terms of recombination and pathogenic virulence characteristics. In fact, the protein sequences of these genes suggested high similarities with those of their homologues in *Tolumonas auensis* DSM 9187, *Shewanella baltica* OS625 and *Vibrio* phage VCY-phi, respectively.

Zot protein is a toxin which increases the intestinal permeability by altering the structure of intercellular tight junctions causing diarrhea. This was first reported in an infection caused by *Vibrio cholerae* (Baudry et al. 1992; Johnson et al. 1993; Rivera et al. 1995). As for the family Aeromonadaceae, within the complete sequenced genomes to date, the presence of *Zot* protein has only been reported in *Aeromonas salmonicida* ssp. *salmonicida* A449 (Reith et al. 2008; Beaz-Hidalgo and Figueras 2013). However, the protein was found non-functional due to a 1-bp frame shift and an incorporation of an IS element in the coding region (Reith et al. 2008). In the present study, aligning the known *Zot* proteins reported in *Vibrio* sp. strains with that of the *Oceanimonas* sp. GK1 revealed the presence of the catalytic region of *Zot* protein

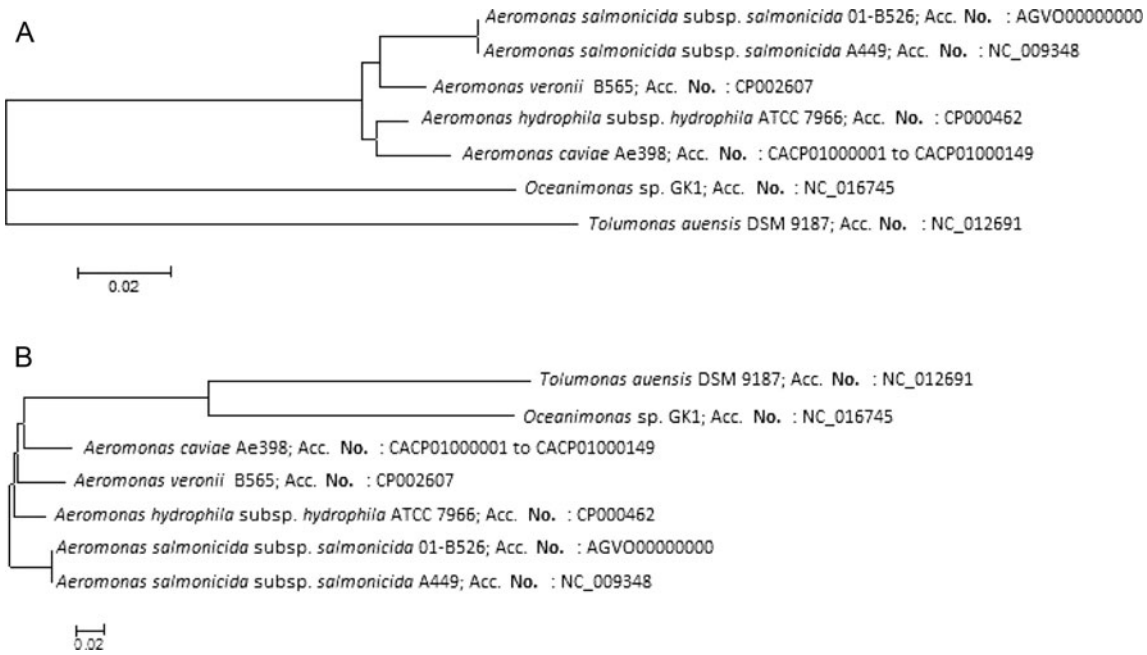

Fig. 1 **a**: Phylogenetic tree showing the association between *Oceanimonas* sp. GK1 and closely-related members of the Aeromonadaceae family. The maximum liklihood analysis was performed based on **a** the homology between their concatenated amino acid sequences of housekeeping genes (*adk*, *argS*, *gyrA*, *rpoB*, *rpoD*, *metS* and *dnaE*) and **b** core genes of these genomes using the EDGAR programs (Blom et al. 2009)

marking the *Oceanimonas* sp. GK1 as a potentially pathogenic microorganism.

Finally, the GEI4 was found different from the other 3 GEIs in terms of both nature and components. More specifically, this GEI had no regions coding phage recombination factors while it contained a number of IS630 sequences with high similarity to those of *Shewanella oneidensis* as determined by NCBI blastn similarity analysis.

Thirty-one IS elements with transposase activity were dispersed throughout the single chromosome. These ISs were classified into 4 main families, IS116/110/902 (7), IS4 (5), IS3/IS911 (16) and IS630 (3). The NCBI blastn similarity search against IS finder database (www-is.biotoul.fr) (Siguier et al. 2006) showed that most of these IS elements were more highly similar to their orthologes in *Shewanella* sp. than the other microorganisms.

Pan and core genome analysis

The genetical and biochemical characteristics and capabilities of the Aeromonadaceae family members investigated in this study were significantly variable. Pan and core genome analysis of these bacterial genomes assisted in making orthology comparisons in order to identify core, dispensable and unique genes. The gene content analyses conducted among the 7 genera of the family of Aeromonadaceae showed enormous variability i.e., 3222 CDS in *Oceanimoans* sp. GK1 to 4724 CDS in *Aeromonas salmonicida* ssp. *salmonicida* 01-B526 . A high similarity was observed in terms of CDS number between *Oceanimoans* sp. GK1 and *Tolumonas auensis* DSM 9187 while these two genomes were significant apart from the other five members of the Aeromonadaceae family.

In order to compute pan genome, core genome and singletons, Edgar was used (Blom et al. 2009). In total, there were 9481 CDSs as the pan genome of the mentioned 7 bacterial genomes with the average genome of the *Oceanimoans* sp. GK1 selected as the reference genome. This value was 2–3 times larger than the CDS numbers for each of the investigated bacteria. This great variability can be attributed to the phylogenetical distance and differences in physiological characteristics. The core genome analysis of these bacteria as

Table 1 General features of 4 GEIs of *Oceanimonas* sp. GK1

GEI number	Start	End	Size	GC content (%)	Most similar genome
1	1,894,044	1,905,233	11,189	51.75	*Shewanella* sp.
2	2,035,828	2,063,114	27,286	49.31	*Vibrio* sp.
3	2,234,026	2,245,074	11,048	53.44	*Aeromonas* and *Pseudomonas*
4	2,286,768	2,306,397	19,629	55.81	*Vibrio cholerae*

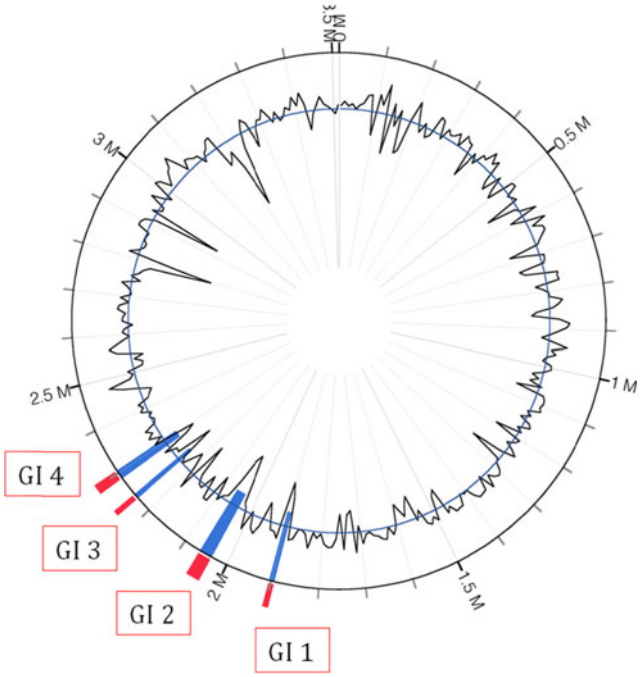

Fig. 2 Genomic islands distribution in *Oceanimonas* sp. GK1 genome. Four genomic islands (GEIs) were identified on the single chromosome of *Oceanimonas* sp. GK1, covering 1.96 % of genome length with 62 coding sequences

compared with iterative pairwise comparison resulted in 1137 CDSs with *Oceanimonas* sp. GK1 used as the reference. Also, there were 1130 unique singleton genes in *Oceanimonas* sp. GK1 genome, which were not present in the other investigated genomes. Oof those unique singleton genes, 365 CDSs were annotated as hypothetical proteins which could be the origin of the signature characteristics of *Oceanimonas* sp. GK1. Due to EDGAR's limitation of a maximum 5 genomes for the generation of Venn diagrams, only the genome sequences of *Aeromonas caviae* Ae398, *Aeromonas hydrophila* ssp. *hydrophila* ATCC 7966, *Aeromonas salmonicida* ssp. *Salmonicida* A449, *Oceanimonas* sp. GK1, and *Tolumonas auensis* DSM 9187 were used to construct the diagram (Fig. 3). For the selected genomes and based on the resulting diagram, 8189 and 1169 CDSs were detected as pan and core genome, respectively.

In addition, the *Tolumonas auensis* DSM 9187 and *Oceanimonas* sp. GK1 with 1343 and 1265 CDSs, respectively, were found to possess the most strain-specific genes among these 5 genomes. Also, the binary comparison to assess the shared genes for the paired genomes revealed that *Oceanimonas* sp. GK1 had 1770, 1751, 1741 and 1277 homologues with *Aeromonas caviae* Ae398, *Aeromonas salmonicida* ssp. *salmonicida* A449, *Aeromonas hydrophila* ssp. *hydrophila* ATCC 7966 and *Tolumonas auensis* DSM 9187, respectively (Fig. 3).

General adaptive features

Membrane transporters

Membrane transport systems are crucial for the translocation of proteins and acquisition of organic nutrients and play vital roles in primary cellular processes such as preservation of ion

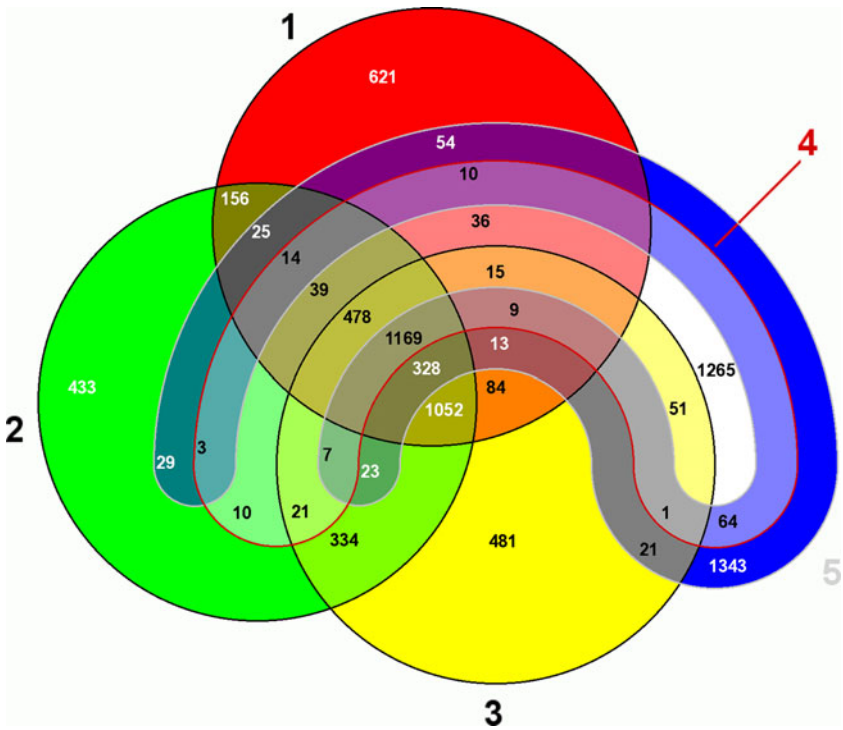

Fig. 3 Venn digram for pan and core genome analysis of 5 genomes of the Aeromonadaceae family using EDGAR. *1 Aeromonas caviae* Ae398, *2 Aeromonas hydrophila* ssp. *hydrophila* ATCC 7966, *3 Aeromonas salmonicida* ssp. *salmonicida* A449, *4 Oceanimonas* sp. GK1, and *5 Tolumonas auensis* DSM 9187. A total number of 9481 CDSs as the pan genome of the mentioned 7 bacterial genomes with the average genome of the *Oceanimoans* sp. GK1 selected as the reference genome were identified. The core genome analysis resulted in 1137 CDSs with *Oceanimonas* sp. GK1 used as the reference. Also, there were 1130 unique singleton genes in the *Oceanimonas* sp. GK1 genome which were not present in the other investigated genomes

homeostasis, cell signaling and environmental sensing (Ren and Paulsen 2005). Whole-genome transporter analyses were carried out on 5 of the 7 studied genomes whose protein sequences in FASTA format were available. For each of the investigated organisms, Transporter Automatic Annotation Pipeline (TransAAP) (Ren et al. 2007) analysis characterized the complete set of membrane transport systems, predicted their functions, and categorized them into protein families based on the transporter classification system. The results obtained are tabulated in Table 2. In general, larger genomes possessed a relatively greater number of transport systems while the number of transporters per Mb genome was approximately identical. In all genomes, ATP-dependent transporters and secondary transporters occurred in the most frequencies (collectively 83.2–92.2 %). In contrast to the other genomes, the secondary transporters of *Oceanimonas* sp. GK1 contributed a greater portion (47.6 %) to its whole transporters.

ABC Transporters

ABC type transporters are the extensive part of translocators in all organisms (Tomii and Kanehisa 1998). They are typically composed of two functional parts to bind and hydrolyze ATP (Nucleotide Binding Domain, NBD) and translocate via the multiple transmembrane segments (Membrane Spaning Domain, MSD) (Davidson and Chen 2004). The MSD domains of ABC transporters are involved in signal transduction processes using the extracytoplasmic loop. Thus, ABC-type transporters could play a role in the adaptive bacterial response to environmental changes (Seo et al. 2002; Coumes-Florens et al. 2011). Comparative analysis of ABC translocation capabilities as realized by using the complete sequenced genomes of the Aeromonadaceae family revealed various adaptation systems depending on environmental differences and physiological characteristics.

In the present study, the ABC transporters of all 7 genomes were assigned based on substrate specificity. The assignment is summarized in Table 3. In short, based on in silico analysis, the *Oceanimonas* sp. GK1 revealed the potential for ABC transpotation of iron (II)/manganese and disability for ABC transportation of maltose/maltodextrin and methyl-galactoside in contrast to other genomes.

Secretion system

Secretion of extracellular proteins is one of the most important mechanisms in bacteria in order to adjust their interactions with the surrounding environments for adaptation, survival and pathogenecity (Glenn 1976; Tseng et al. 2009). In fact, bacterial species develop different strategies to secret their extracellular enzymes and toxins. Six highly specialized pathways are so far known including: type I: signal sequence-independent pathway; type II: the main terminal branch of general secretion pathway; type III: the contact-dependent pathway; type IV: filamentous hemagglutinin secretion pathway; type V: autotransporter pathway (Binet et al. 1997; Collazo and Galán 1997; Burns 1999; Cornelis and Van Gijsegem 2000; Sandkvist 2001; Wooldridge et al. 2005; Deane et al. 2010; Silverman et al. 2011; Büttner 2012; Korotkov et al. 2012; and finally; type VI whose structure is like the bacteriophage cell-puncturing machine and is widely distributed in pathogenicGgram-negative bacteria such as *Vibrio cholerae* and *Pseudomonas aeruginosa* (Pukatzki et al. 2007, 2009; Bingle et al. 2008; Boyer et al. 2009; MacIntyre et al. 2010).

The type II secretion system (T2SS) is just present in the Gram-negative proteobacteria phylum in order to secret proteases, cellulases, phospholipases, pectinases, lipases as well as toxins (Filloux 2004; Cianciotto 2005). The expression and secretion of most of these extracellular enzymes, with the cell wall degrading and virulence effects, are firmly controlled by the environmental needs (Sandkvist 2001).

Table 2 Classification of membrane transporters using TransAAP analysis

Transporter type	*Oceanimonas* sp. GK1	*Aeromonas hydrophila* ssp. *hydrophila* ATCC 7966	*Aeromonas salmonicida* ssp. *salmonicida* A449	*Aeromonas veronii* B565	*Tolumonas auensis* DSM 9187
ATP-dependent (%)	44.6	44.6	49.7	44.6	53.3
Ion channels (%)	3.9	4.7	5.3	4.5	3.3
Outer membrane porins (%)	0.7	0.5	0.6	0.8	0.2
Phosphotransferase system (PTS) (%)	1.6	5.6	5.5	6	11.4
Secondary transporter (%)	47.6	41.9	36	41.3	29.9
Unclassified (%)	1.6	2.2	2.4	2.3	1.6
Total transporters	439	554	531	516	428
No. of transporters per Mb genome	0.12	0.12	0.11	0.11	0.12

Table 3 The assignment of ABC transporters of all 7 genomes

ABC transporters		*Oceanimonas* sp. GK1	*A. salmonicida* ssp.*salmonicida* 01-B526	*T. auensis* DSM 9187	*A. salmonicida* ssp. *salmonicida* A449	*A. hydrophila* ssp. *hydrophila* ATCC 7966	*A. caviae* Ae398	*A. veronii* B565
Mineral and organic ions	Sulfate	+	+	+	+	+	+	−
	Tungstate	−	−	−	+	+	−	+
	Nitrate/nitrite/cyanate	−	−	+	−	+	+	−
	Taurine	−	−	−	−	+	−	−
	Molybdate	+	+	+	+	+	+	+
	Iron (III)	+	+	+	+	+	+	+
	Thiamin	–	+	+	+	+	+	+
	Spermidine/putrescine	+	+	–	−	−	+	+
	Putrescine	+	+	+	+	+	+	+
	Glycine betaine/proline	−	+	−	+	+	+	−
	Osmoprotectant	+	−	−	−	−	−	−
Oligosacharides and polyols	Maltose/maltodextrin	−	+	+	+	+	+	+
	Cyclodextrin	−	−	−	+	−	−	−
Monosacharides	Ribose	+	+	+	-	+	+	+
	L-Arabinose	−	+	+	+	+	+	−
	Methyl-galactoside	−	+	+	+	+	+	+
	D-Allose	−	−	+	−	−	−	−
Phosphate and amino acids	Phosphate	+	+	+	−	+	+	+
	Phosphonate	+	−	−	+	+	−	−
	Lysine/arginine/ornithine	−	+	+	−	−	+	−
	Arginine	+	+	−	+	+	+	+
	General L-amino acid	+	−	+	−	−	−	−
	Arginine/ornithine	+	+	+	+	+	+	+
	Branched-chain amino acid	+	+	+	+	+	+	+
	D-Methionine	+	+	+	+	+	+	+
Peptide	Oligopeptide	+	+	−	+	+	+	+
Metalic cations, iron siderophore and vitamin B12	Iron complex	+	+	+	+	+	+	+
	Vitamin B12	+	+	+	+	+	+	−
	Zinc	+	+	−	+	+	+	+
	Iron (II)/manganese	+	−	−	−	−	−	−
	Cobalt	−	−	+	−	−	−	−
BC-2 and other transporters	Antibiotics	+	+	+	+	+	+	+
	Capsular polysaccharide	+	−	−	−	−	−	+
	Lipopolysaccharide	−	+	−	+	+	+	−
	Lipoprotein	+	+	+	+	+	+	−
	Heme	+	+	−	+	+	+	+
	Cell division	+	+	+	+	+	+	+
Macrolide exporters	Macrolide exporters	+	+	+	+	+	+	+
Other	YojI (putative ABC transporter)	−	−	+	+	+	−	+
Putative ABC transporter	YddA (putative ABC transporter)	−	−	+	−	−	−	−

The *Oceanimonas* sp. GK1 also secrets its extracellular enzymes through T2SS composed of 10 T2SS specific proteins [Secretin (GspD), Inner Membrane Proteins (GspF, GspG, GspH, GspI, GspJ, GspK, GspL and GspM) and ATPase (GspE)], 10 s-SRP [Inner Membrane Proteins (SecD/F, SecE, SecG, SecY, YajC and YidC), ATPase (SecA), SRP receptor (FtsY) and Targeting protein (SecB and ffh)] and 3 Twin arginine targeting (Tat) proteins [Inner Membrane Proteins (TatA, TatB and TatC)].

Resistance to toxic compounds

A wide-range in-silico analysis conducted on the 7 studied genome sequences provided their profiles with respect to

resistance to toxic compounds such as antibiotics and heavy metals. The detoxifying mechanism of the heavy metal resistant bacteria involves the utilization of an inducible ion efflux system for reduction of intracellular concentration of a specified ion via active export. This mechanism is contrary to that of the thermophilic or psycrophilic organisms only produce xenobiotic-degrading enzymes under extreme circumstances (Nies 2000). Figure 4 represents each organism prospective to degrade, survive, and adapt to toxic compounds.

Xenobiotic degradation

The growing pollution by xenobiotic compounds generated through both natural and anthropogenic activities has endangered the environment, especially the aquatic ecosystems and consequently has posed serious threats to the public health. Xenobiotics including phenols, phenyl carbonates, benzoate, biphenyl and the other aromatic compounds make up a major part of pesticides and herbicides, petroleum (oil), solvents, alkanes, polycyclic hydrocarbons (PAHs), antibiotics, and synthetic azo dyes which are increasingly being introduced to the environment (Ojo 2007; Sinha et al. 2011). These substances are toxic and carcinogenic even at trace concentrations. Among the organisms with detoxifying capabilities, microorganisms, particularly bacteria, due to their wide range of diversity and flexibility for adaptation to xenobiotics, are the best candidates to recycle xenobiotic compounds back into natural biogeochemical cycles (Bonfá et al. 2013). Our analysis of *Oceanimonas* sp. GK1 genome revealed that the strain had high potentials to degrade xenobiotic compounds contained in wastewaters such as high-phenol content and oil wastewaters.

Phenol degradation: in silico analysis

Phenols and its derivatives as troublesome environmental contaminants are present in the commonly hypersaline wastewater of many industries such as petroleum industry and the chemical manufacturing plants (Lefebvre and Moletta 2006; Agarry et al. 2008; Nagamani et al. 2009; Bonfá et al. 2013). Such phenolic hypersaline effluents are commonly treated by physico-chemical means or biological methods (Dubey et al. 2013; Haddadi and Shavandi 2013). While physico-chemical techniques are usually energy-consuming and expensive, the biological removal of phenol and other dissolved organics from such effluents has been shown to be reasonably more advantageous (Lefebvre and Moletta 2006). However, biological treatment of hypersaline wastewaters requires prior desalinization for most microorganisms with the xenobiotic capabilities which cannot cope with hypersaline conditions. This pretreatment step involves expensive procedures or dilution with fresh water (Bonfá et al. 2013). The latter although less costly increases the volume of wastewater and also puts extra pressure on fresh water resources. As a result, halophilic microorganisms with xenobiotic degradation capabilities are the best choice to overcome these challenges. Unluckily, the known or characterized halophilic or halotolerant phenol-degrading bacteria are very limited until now (Bonfá et al. 2013).

Recently, Fathepure (2014) in an excellent review listed the main organisms of biodegradation capability for petroleum hydrocarbons in hypersaline environments. Fathepure also categorized 10 phenol-degrading species under moderate to highly saline conditions (0–30 %), i.e., the halophilic isolate

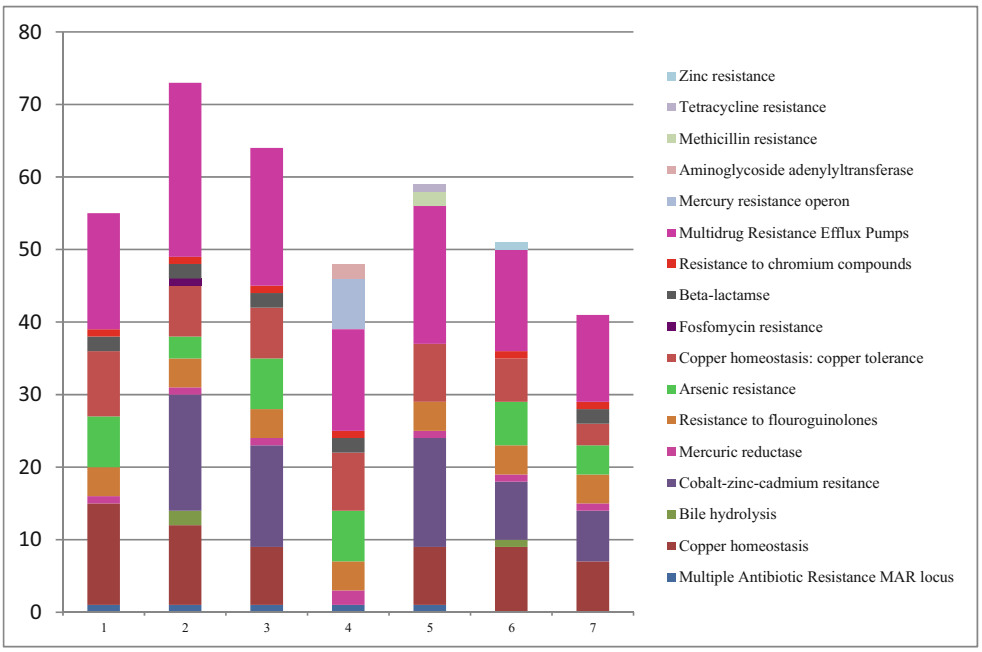

Fig. 4 A summary of the capabilities of the studied genomes for resistance to toxic compounds. *1 Aeromonas hydrophila* ssp. *hydrophila* ATCC 7966, *2 Aeromonas caviae* Ae398, *3 Aeromonas salmonicida* ssp. *salmonicida* 01-B526, *4 Aeromonas salmonicida* ssp. *salmonicida* A449, *5 Aeromonas veronii* B565, *6 Oceanimonas* sp. GK1, *7 Tolumonas auensis* DSM 9187

(1–15 %) (Woolard and Irvine 1995), *Halomonas* sp. (1–14 %) (Hinteregger and Streichsbier 1997), *Candida tropicalis* (15 %) (Bastos et al. 2000), *Halomonas campisalis* (0–15 %) (Alva and Peyton 2003), *Halomonas organivorans* (1.5–30 %) (García et al. 2004, 2005b), *Thelassobacillus devorans* (7.5–10 %) (García et al. 2005a), *Arthrobacter* sp. (6–9 %) (Plotnikova

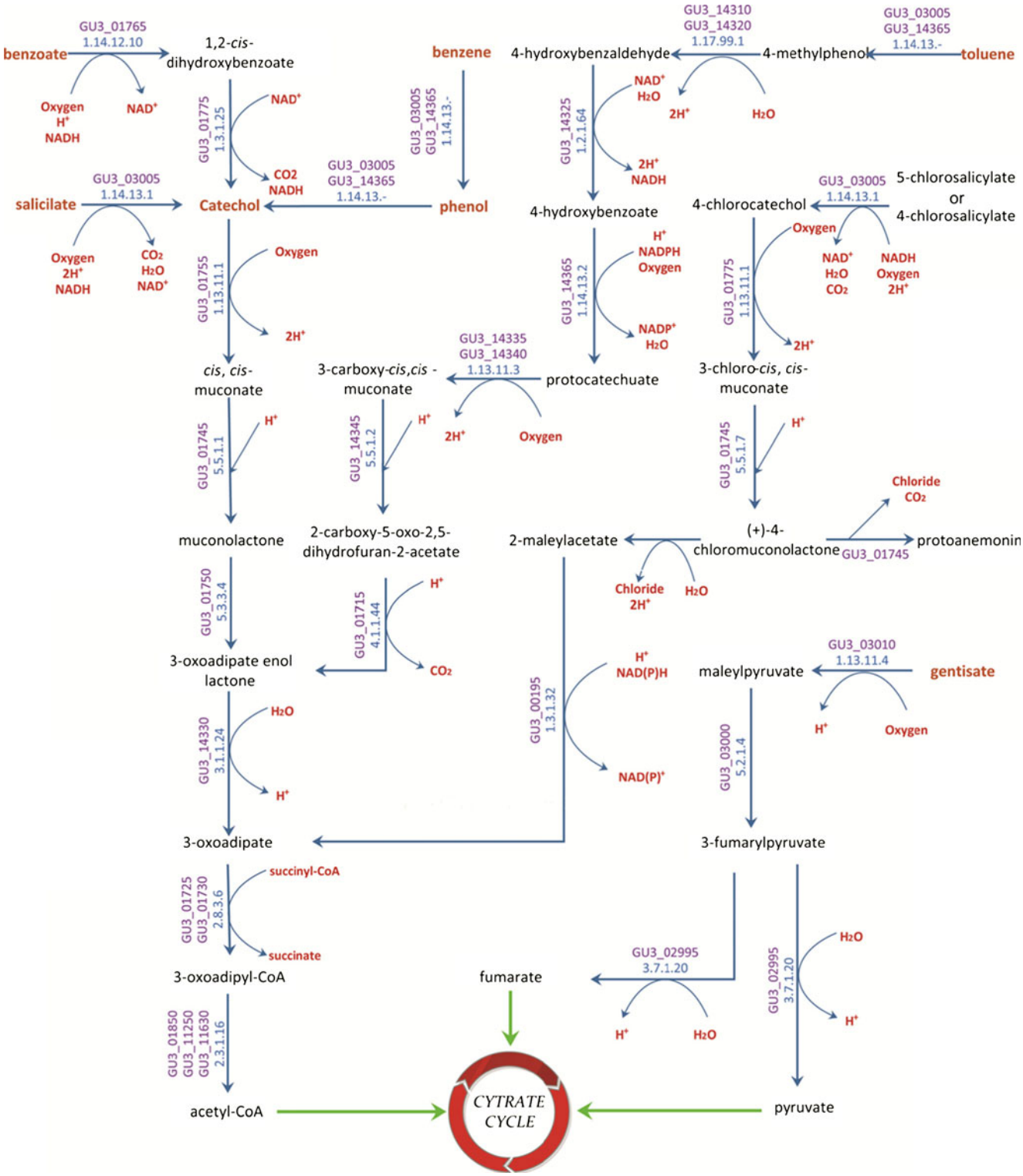

Fig. 5 Comprehensive view reavealing the potentials of *Oceanimonas* sp. GK1 for aromatic compounds biodegradation. *Oceanimonas* sp. GK1 was found capable of converting benzene to phenol, followed by its oxidization to catechol. Catechol then undergoes ring cleavage by catechol 1, 2-dioxygenase (EC 1.13.11.1) (GU3_01755) via the intradiol pathway

et al. 2011), *Halomonas organivorans*, *Arhodomonas aquaeolei* (10 %) (Bonfá et al. 2013), and *Modicisalibacter tunisiensis* Strain C5 (12 %) (Chamkha et al. 2008).

The comprehensive comparison of the 7 studied genomes in terms of their phenolic and other aromatic compounds degradation potentials well segregated *Oceanimonas* sp. GK1 from the others (Fig. 5). Comparative pathway analysis of *Oceanimonas* sp. GK1, *Aeromonas hydrophila* ssp. *hydrophila* ATCC 7966, *Aeromonas salmonicida* ssp. *salmonicida* A449, *Aeromonas veronii* B565 and *Tolumonas auensis* DSM 9187 using the Metacyc pathway tool v.18.0 (Caspi et al. 2008; Karp and Caspi 2011), revealed the significant superiority of *Oceanimonas* sp. GK1 compared to the others in terms of biodegradation of aromatic compounds (Table 4 and Fig. 5).

This wide genome analysis has shown high potentials of the *Oceanimonas* sp. GK1 isolate for phenol biodegradation through its utilization as carbon source via the *ortho*-cleavage pathway. More specifically, in the present study, and based on the "degradation of aromatic compounds pathway" in KEGG and genome analysis, the *Oceanimonas* sp. GK1 was found capable of converting benzene to phenol, followed by its oxidization to catechol. Catechol then undergoes ring cleavage by catechol 1, 2-dioxygenase (EC 1.13.11.1) (GU3_01755) via the intradiol pathway. After several subsequent reactions, the pathway leads to acetyl-CoA and the citrate cycle, which could be up-taken as an energy source (Fig. 5).

Phenol degradation: experimental analysis

The effect of initial phenol concentration ranging from 500 to 2100 mg/l (corresponding to 1200–5092 mg COD/l, respectively) on biodegradation capabilities of *Oceanimonas* sp. GK1 in terms of COD removal efficiency was investigated. Increasing the initial phenol concentration from 500 to 1000 mg/l (1200–2540 mg COD/l, respectively) resulted in decreasing COD removal efficiency from 40.2 to 36.8 %. By further increasing the phenol concentration to 1500, 1750 and 2100 mg/l (corresponding to 5092, 4175 and 3473 mg COD/l), the decreasing trend continued and the COD removal efficiency further decreased to 24.3, 26.8 and 32.2 %, respectively. On the other hand, the cell dry weight (CDW) was found to react similarly to increasing phenol concentrations as the COD removal efficiency. Maximum CDW of 67, 51, 44, 32 and 26 g/l were achieved for initial COD concentrations of 1200, 2540, 3473, 4175 and 5092 mg COD/l, respectively. In an investigation performed by González et al., the ability of *Pseudomonas putida* ATCC 17484 to degrade phenol at concentrations ranging from 200 t 1000 mg/l in batch mode was evaluated. A maximum removal efficiency of 90 % was achieved for phenol concentration of 1000 mg/l (González et al. 2001). These results as well as the presence of multiple copies of the key genes involved in the degradation of xenobiotics compounds such as phenol would suggest *Oceanimonas* sp. GK1 as a potential platform to develop genetically-engineered strains of superior degrading capabilities.

Table 4 Comparative view of aromatic compounds biodegradation capabilities among *Tolumonas auensis* DSM 9187, *Oceanimonas* sp. GK1, *Aeromonas veronii* B565, *Aeromonas salmonicida* ssp. *salmonicida* A449 and *Aeromonas hydrophila* ssp. *hydrophila* ATCC 7966

Aromatic compounds degradation pathways	*T. auensis* DSM 9187	*O.* sp. GK1	*A.veronii* B565	*A. salmonicida* ssp. *salmonicida* A449	*A. hydrophila* ssp. *hydrophila* ATCC 7966
5-Nitroanthranilate degradation	−	+	−	−	−
Atrazine degradation I (aerobic)	−	+	−	−	−
Benzoate degradation I (aerobic)	−	+	−	−	−
Catechol degradation to β-ketoadipate	−	+	−	−	−
Chlorosalicylate degradation	−	+	−	−	−
Gentisate degradation	−	+	−	−	−
Methylsalicylate degradation	−	+	−	−	−
Phenylacetate degradation I (aerobic)	−	+	+	+	+
Protocatechuate degradation II (*ortho*-cleavage pathway)	+	+	−	+	+
Salicylate degradation I	−	+	−	−	−
Toluene degradation to protocatechuate (via *p*-cresol)	−	+	−	−	−
Shikimate degradation I	+	−	+	−	−
Protocatechuate degradation III (*para*-cleavage pathway)	−	−	+	+	+
Carbazole degradation	−	−	−	+	−

Table 5 Cazy profile of *Oceanimonas* sp. GK1 genome

Category	CAZy family	Number of sequences	*Oceanimonas* sp. GK1 locus	Reference accession no.	In silico prediction
Glycosyl hydrolases (GHs)	GH3	2	GU3_02110	AEY00179.1	β-Glucosidase
			GU3_09135	AEY01583.1	Glycoside hydrolase
	GH8	1	GU3_14400	AEY02633.1	Endo-1,4-D-glucanase
	GH23	5	GU3_06630	AEY01082.1	Membrane-bound lytic transglycosylase F
			GU3_06750	AEY01106.1	Soluble lytic murein transglycosylase
			GU3_06765	AEY01109.1	Membrane-bound lytic murein transglycosylase D
			GU3_13515	AEY02456.1	Transcriptional regulatory protein (Lytic transglycosylase)
			GU3_13925	AEY02538.1	Membrane-bound lytic murein Mtransglycosylase
	GH73	1	GU3_13535	AEY02460.1	Flagellar rod assembly protein/ muramidase FlgJ
	GH103	2	GU3_08825	AEY01521.1	Lytic murein transglycosylase
			GU3_06125	AEY00981.1	Lytic murein transglycosylase B
Glycosyl transferase family (GTs)	GT2	5	GU3_03185	AEY00394.1	Glycosyl transferase
			GU3_06865	AEY01129.1	Glycosyl transferase
			GU3_12835	AEY02322.1	Glycosyltransferase
			GU3_14410	AEY02635.1	Cellulose synthase catalytic subunit
			GU3_14775	AEY02708.1	Glycosyl transferase
	GT4	4	GU3_03225	AEY00402.1	Predicted xylanase/chitin deacetylase
			GU3_14710	AEY02695.1	Glycosyl transferase group 1
			GU3_14745	AEY02702.1	Mannosyltransferase B
			GU3_12815	AEY02318.1	Glycosyltransferase
	GT9	4	GU3_03180	AEY00393.1	ADP-heptose:LPS heptosyltransferase
			GU3_03190	AEY00395.1	ADP-heptose–LPS heptosyltransferase III
			GU3_03210	AEY00399.1	Lipopolysaccharide heptosyltransferase II
			GU3_03215	AEY00400.1	Lipooligosaccharided-glycero-D-manno-heptosyltransferase
	GT19	1	GU3_12925	AEY02338.1	Lipid-A-disaccharide synthase
	GT28	1	GU3_04370	AEY00631.1	Undecaprenyldiphospho-muramoylpentapeptide beta-N-acetylglucosaminyltransferase
	GT30	1	GU3_03205	AEY00398.1	3-Deoxy-D-manno-octulosonic-acid transferase
Glycosyl transferase family (GTs)	GT32	1	GU3_03110	AEY00379.1	Mannosyltransferase OCH1
	GT51	3	GU3_03845	AEY00526.1	Penicillin-binding protein 1B
			GU3_09545	AEY01665.1	Monofunctional biosynthetic peptidoglycan transglycosylase
			GU3_14970	AEY02747.1	Penicillin-binding protein 1A
	GTnc	1	GU3_14780	AEY02709.1	Hypothetical protein
Carbohydrate esterases (CEs)	CE4	2	GU3_03225	AEY00402.1	Predicted xylanase/chitin deacetylase
			GU3_15815	AEY02914.1	Putative urate catabolism protein
	CE9	1	GU3_11925	AEY02140.1	N-Acetylglucosamine-6-phosphate deacetylase
	CE11	1	GU3_04400	AEY00637.1	UDP-3-O-[3-hydroxymyristoyl] N-acetylglucosamine deacetylase
	CEnc	1	GU3_14835	AEY02720.1	S-Formylglutathione hydrolase
Carbohydrate binding modules (CBMs)	CBM50	6	GU3_00055	AEX99767.1	LysM domain-containing protein
			GU3_05400	AEY00837.1	N-Acetylmuramoyl-L-alanine amidase
			GU3_15095	AEY02772.1	Lipoprotein NlpD
			GU3_06765	AEY01109.1	Membrane-bound lytic murein transglycosylase D

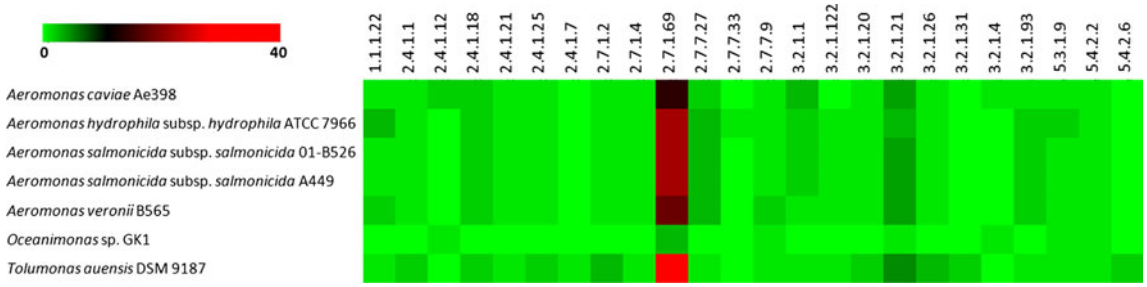

Fig. 6 Heatmap analysis of starch metabolism pathway of the investigated genomes. *Oceanimonas* sp. GK1 possesses just 7 enzymes of the 24 enzymes generally existing in the starch metabolism pathways while retaining the cellulose synthase (EC 2.4.1.12) and cellulase (EC 3.2.1.4)-encoding genes

Carbohydrate metabolism

Another feature of *Oceanimonas* sp. GK1 is its abilities and disabilities in metabolizing carbohydrates. According to the carbohydrate-active enzyme (CAZy) analysis, *Oceanimonas* sp. GK1 is predicted to possess 39 genes (1.36 % of all genes) encoding carbohydrate-degrading enzymes. These enzymes include glycosyl hydrolases (GHs)(5 families), carbohydrate binding modules (CBMs) (1 family), carbohydrate esterases (CEs) (4 families), and glycosyl transferase (GTs) (9 families) (Table 5). Some of these enzymes fall into more than one CAZy type category (i.e., both GHs and CBMs). Therefore, a total of 44 CAZymes were identified using this ontology.

As a case in point, in comparison with the other studied genomes, the enzymes involved in the starch metabolism pathways are very limited in *Oceanimonas* sp. GK1. The resulting heatmap obtained by the PATRIC comparative pathway tool (Wattam et al. 2014) for this comparison showed that *Oceanimonas* sp. GK1 possesses just 7 enzymes of the 24 enzymes generally existing in the starch metabolism pathways (Fig. 6). Interestingly, the *Oceanimonas* sp. GK1 retains the cellulose synthase (EC 2.4.1.12) and cellulase (EC 3.2.1.4)-encoding genes. Nevertheless, with the exception of *Aeromonas caviae* Ae398, the other genomes lacked both enzymes and such a capability would make *Oceanimonas* sp. GK1 a good candidate to be considered for further cellulolytic activity analyses for applications in biotechnology and industry.

Conclusion

In the present study, the biochemical and physiological properties of a hypersaline *Oceanimonas* sp. GK1 were investigated both experimentally and in silico at the genome scale. The microbe's capabilities in degrading aromatic compounds, specially phenol, and utilizing its degradation products in the citrate cycle (despite its enzymatic limitations in the carbohydrate metabolism pathway) represents the high level of adaptation exhibiting by this bacterium to the extreme life style and utilization of unusual carbon and energy sources. Finally, the investigation and analysis of the other adaptation features and genetical potentials of *Oceanimonas* sp. GK1 make it highly recommended.

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