Genome analysis of a plastisphere-associated *Oceanimonas* sp. NSJ1 sequenced on Nanopore MinION platform

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Abstract

*Oceanimonas* sp. NSJ1 was isolated from macroplastic debris collected previously from Junput, an intertidal beach, facing the northeast coastal Bay of Bengal of the Northern Indian Ocean. The genome of this isolate is closely related to *Oceanimonas doudoroffii* with a genome size of 3.56 Mbp. The genome annotation confirmed the presence of 5919 total genes, out of which 5809 were CDSs (coding sequences) and all are protein-coding. The genome codes for 110 RNA with 25 rRNA, 84 tRNA (transfer RNA), and one tmRNA (transfer-messenger RNA). Analyses of the annotated genome of *Oceanimonas* sp. NSJ1 revealed the presence of enzymes involved in the degradation of polycyclic aromatic hydrocarbons. The presence of phthalate 4,5-dioxygenase oxygenase reductase subunit pht2 within the genome also highlights the novelty of this isolate and future functional potential for studying phthalate degradation in marine environment.

Introduction

Plastic waste is a rapidly growing global issue, with almost 370 million tonnes of global plastic production reported in the year 2019 (Plastics 2021). About 21% of the generated plastics has been recycled or incinerated, and the rest of plastic wastes are released in some form or other into the environment (Yuan et al 2020). The most commonly used plastic polymers which constitute around 80% of the total plastic are polyethylene, polypropylene, polyvinyl chloride, polystyrene and polyethylene terephthalate. Under *in situ* environmental conditions, degradation of these synthetic polymers is very slow. However, physico-chemical and ecological factors including physical abrasions, exposure to sunlight, and microbial metabolisms can speed up the rate of degradation. Along with the damage caused by plastics, the complex additives significantly harm when broken down and subsequently get released in the environment leading to long-term toxicity (Hermabessiere et al 2017). For more than a decade, additives such as polybrominated diphenyl ethers (PBDEs), bisphenol A, and some phthalates have been extensively studied (Hermabessiere et al 2017). These studies have found linkages with carcinogenicity, neurotoxicity, obesity, and endocrine disruptions (Llorca and Farré 2021). Among phthalate plasticizers, benzyl butyl phthalate (BBP), Di-2-ethylhexyl phthalate (DEHP) and dibutyl phthalate (DBP) are categorized as poisonous and known to have long-lasting impacts on marine ecosystems as well as across organisinal groups (Kaplan et al 2013). Therefore, there is a need to develop eco-friendly solutions for plastic management as part of circular economy and to achieve healthy and sustainable coastal ocean. The conventional methods of plastic waste management such as burning or dumping in landfill sites can release harmful by-products, and there are limitations with current recycling practices. Recent efforts to explore the plastic degrading potential of microbial communities can ultimately prove to be very cost-effective from global context of achieving healthy ecosystems (Ru et al 2020; Joshi et al 2022).
The draft genome of *Oceanimonas* sp. NSJ1 consisted of 3567689 bases which assembled into 16 contigs (figure 1). The GC content was 60.32%. Genome analysis indicated the presence of 5919 total genes out of which 5809 were CDSs. A total of 5809 coding genes with 5809 protein-coding CDSs were found. The genome codes closest related genomes is shown in figure 1. The whole-genome sequence-based phylogeny (figure S1) showed closest affiliation to *Oceanimonas doudoroffii*. The branches with bootstrap value equal to 100 is shown. AAI analysis showed maximum amino acid identity with *Oceanimonas doudoroffii* with average AAI value of 0.794. The average AAI values of other related genomes are shown in table 1.

### Material and methods

#### Isolation and genomic DNA extraction

The bacterium *Oceanimonas* sp. NSJ1 was isolated from macroplastic debris collected in June 2021 from Junput as part of CBoBTS. The isolate was grown in LB medium with salinity of 15 and pH 8.47. Genomic DNA (gDNA) was extracted by using modified published protocol (Boström et al 2004) as detailed earlier (Samanta and Bhadury 2018). The gDNA was run on 1% agarose gel and quantified using a Nanodrop 2000c Spectrophotometer (Thermo Fisher Scientific, USA).

#### Whole-genome sequencing

After quality check, DNA concentration was determined with Qubit 1X dsDNA HS (High sensitivity) Assay kit (Thermo Fisher Scientific, USA). Library preparation was performed using ligation sequencing kit (SQK-LSK109) following published protocol (Oxford Nanopore Technologies, UK). The AMPure XP magnetic beads (Beckman Coulter Life Sciences, USA) was used for purification and the ligation library was run in MinION platform (Flow cell R9.4.1; Oxford Nanopore Technologies, UK). Basecalling of generated Fastq files were undertaken using Guppy (version 5.1.12) and output DNA reads with Q > 8 was subsequently considered for downstream analyses. The sequence data was checked using FastQC and adapters were trimmed using Porechop (v0.2.0) (Wick 2018). The accession number for submitted genome data is JAKNTD010000000.

#### Whole-genome sequence annotation and comparisons

The quality checked pair-end reads were assembled into contigs using Unicycler (Wick et al 2017, Wick 2018). The genome of *Oceanimonas* sp. NSJ1 was aligned into a circular map using CGView server (http://stothard.afns.ualberta.ca/cgi-bin/cgview_server/cgview_server.pl; Grant and Stothard, 2008). The whole-genome sequence-based phylogeny was performed in the Type (Strain) Genome Server (TYGS) (https://tygs.dsmz.de) (Meier-Kolthoff 2019). The genome data was compared using MASH algorithm (Ondov et al 2016). The genome distances were calculated using Genome BLAST Distance Phylogeny (GBDP) approach. Genomic relatedness with closest relatives was determined using OrthoANIu algorithm (http://www.ezbiocloud.net/tools/ani; Yoon et al 2017). Digital DDH values were calculated using genome-genome distance calculator (GGDC 2.1) applying Formula 2 (identities/HSP length) (Meier-Kolthoff et al 2013). The genome sequences used for GGDC and OrthoANIu analyses were *Oceanimonas doudoroffii*, *O. baumannii*, *O. smirnovii* and *Oceanimonas marisflavi*. Average amino acid index (AAI) was determined using AAI-profiler (http://ekhidna2.biocenter.helsinki.fi/AAI/; Medlar et al 2018). *In silico* phenotyping was performed using Treatar (https://github.com/hzi-bifo/treatar; Weimann et al 2016). Genomic annotation was carried out using Prokka and revalidated using the Prokaryotic Genome Annotation Pipeline (PGAP) (https://www.ncbi.nlm.nih.gov/genome/annotation_prok/) (Li et al 2021). Genomic islands were predicted using IslandViewer 4 (Bertelli et al 2017). The resulting protein profile was viewed by plotting the data in a circular map using GVView (server.gview.ca; Petkau et al 2010).

### Results and discussion

The coastal Bay of Bengal of the Northern Indian Ocean is home to a large number of biotopes including mangroves, estuaries, lagoons and intertidal beaches. Plastic pollution in the coastal water of Bay of Bengal (BoB) surrounding Indian coastlines have increased significantly over the years (Sunitha et al 2021). Junput, a sandy clay intertidal beach located in the state of West Bengal, India facing the northeast coastal Bay of Bengal is frequented by tourists and plastic pollution on the beach is rampant. This site is also part of the seasonal ecological monitoring program for coastal BoB- Coastal BoB Time Series (CBoBTS) (Ghosh et al 2022). In the present study, draft genome of *Oceanimonas* sp. NSJ1 isolated from plastic debris collected earlier from Junput intertidal beach has been described.
GGDC (%) and orthoANIu (%) between *Oceanimonas* sp. NSJ1 with *Oceanimonas doudorofii*, *O. baumannii*, *O. marisflavi*, and *O. smirnovii* are listed in Table 1. It can be observed that *Oceanimonas* sp. NSJ1 showed maximum orthoANIu (%) value of 98.46 and minimum GGDC (%) value of 0.0146 with *Oceanimonas doudorofii*. In silico phenotyping indicated the bacterium to be aerobic, motile and Gram-negative. The isolate is susceptible to bile and produces enzymes including arginine dihydrolase, alkaline phosphatase, oxidase, catalase, lipase, urease and DNase. The isolate can use glycerol, acetate, L-arabinose and mucate. Growth utilizing carbon sources including sucrose and D-mannose were found. It can convert nitrate to nitrite. The isolate can grow on MacConkey agar in presence of high NaCl concentration. The results of in silico phenotyping have been summarized in Figure S2. As observed earlier by field emission scanning electron microscopy, genome data also indicates evidence of motility. Proteins including flagellar motor switch protein (FliG, FliN), flagellar hook protein (FlgE), flagellar basal-body rod protein (FlgG, FlgF), flagellar hook-basal body complex (FlIE) and flagellar biosynthesis protein (FlhA, FlhF) are encoded by the genome. Genome annotation confirmed the presence of genes involved in photosynthesis (oxidative C₂ cycle). The presence of urea transporter and urease cluster ureDEFG were encountered in *Oceanimonas* sp. NS1. This indicates the metabolic capability of this isolate to utilize urea as a source of nitrogen for growth. Genome annotation has also revealed the presence of PhaC (pHA synthase). PHAs or Polyhydroalkanoates are considered as good candidates in replacing commercial petrochemical plastics since these are biodegradable (Neoh et al. 2022).

**Resistance to toxic compounds and stress**

Analysis of annotated genome sequence of *Oceanimonas* sp. NSJ1 revealed the presence of protein-coding genes that offer resistance against heavy metals and toxic-compounds such as copper resistance protein (CopB), transcriptional activator protein (CopR) linked to copper homeostasis, cobalt-zinc-cadmium resistance protein (CzcA) and nickel transport system permease protein (NiKb). The identification of these genes suggest the potential of this isolate to thrive in presence of metals and other toxic compounds. Arsenic resistance genes or genes involved in arsenic detoxification were also found including arsenate reductase, arsenical-resistance protein (Acr3) and arsenic resistance transcriptional regulator (ArsR1).

**Figure 1.** Genome map of *Oceanimonas* sp. NSJ1 in comparison with the closest relatives. The circular map also shows the GC content and GC skew (+/−). The gap portions show no overlapping regions with the closest neighbours.

**Table 1.** Comparison of *Oceanimonas* sp. NSJ1 with closest relatives.

| Organism               | OrthoANIu value (%) | Average AA1 | GGDC (%) |
|------------------------|---------------------|-------------|----------|
| *Oceanimonas doudorofii* | 98.46               | 0.794       | 0.0146   |
| *Oceanimonas baumannii*  | 81.89               | 0.742       | 0.1497   |
| *Oceanimonas smirnovii*  | 82.35               | 0.1428      |          |
| *Oceanimonas marisflavi* | 87.19               | 0.1089      |          |
The choline and betaine uptake as well as betaine biosynthesis systems which play an imperative role in bacterial osmoregulation and stress tolerance (Sleator and Hill 2002) have been identified in the genome. This system includes genes for L-proline/glycine/betaine transporter protein, one gene for high-affinity choline uptake protein BetT, genes for choline dehydrogenase (gbsB and betA), and two genes for betaine-aldehyde dehydrogenase. Two genes, trkA and trkH have been found in the genome that encodes for components of the Trk system for potassium uptake in response to osmotic stress. The rps gene family is known to play an important role in acclimation of translational machinery during cold stress and signatures for RpsD, RpsG, RpsL and RpsU proteins were encountered in the genome. Polycyclic aromatic hydrocarbon degradation potential

Aromatic hydrocarbons like phenol and its compounds are the most common pollutants originating majorly from petrochemical-based industries. Apart from use in dyes and chemical industries, phenol is condensed with aldehydes to make resinous compounds. Phenol methanal resin is used to make thermosetting plastics such as melamine and bakelite. The investigation of genome sequences showed the presence of enzymes involved in pathways linked to degradation of phenol, benzoate, fluorobenzoate, toluene, styrene and naphthalene such as Catechol 1,2-dioxygenase, Phenol hydroxylase P1 protein, Phenol hydroxylase P2 protein, Phenol hydroxylase P5 protein, Phenol regulator (MopR) as well as Naphthalene 1,2-dioxygenase. Catechol 1, 2-dioxygenase is a crucial enzyme through which aerobic microorganisms convert aromatic compounds into intermediates of the tricarboxylic acid cycle (table 2). It catalyses the addition of molecular oxygen into catechol with subsequent cleavage of the aromatic rings. These properties make Catechol 1, 2-dioxygenase a promising biocatalyst for the biodegradation of aromatic compounds present in any environment (Li et al 2021). The genome analyses also confirmed the presence of arylesterases, a class of enzymes that catalyse hydrolysis of a number of aromatic carboxylic acid esters (e.g., phenylacetate) and also confer resistance to organophosphate toxicity.

The genome sequencing and subsequent analyses also revealed presence of enzymes involved in bisphenol degradation. Bisphenol A, a representative of this group, is commonly used as an additive in producing plastic materials such as polystyrene and polycrylates. Styrene, an alkylbenzene is an unsaturated aromatic monomer that occur naturally and can be also of anthropogenic origin. Anthropogenic sources include by-products of polystyrene, styrene-butadiene, and styrene-based resin synthesis (Mooney et al 2006). Oceanimonas sp. NSJ1 showed the presence of enzymes involved in styrene degradation pathways (Hou and Majumder 2021). The presence of phthalate 4,5-dioxygenase oxygenase reductase subunit pht2 was detected in this isolate although the same has not been reported previously in the genus Oceanimonas. The finding indicates potential role of this

| Gene name | Activity | EC.Number |
|-----------|----------|-----------|
| acnA      | Aconitate hydratase A | EC:4.2.1.3 |
| benC      | Benzoate 1,2-dioxygenase electron transfer compound | |
| catA      | Catechol 1,2-dioxygenase | EC:1.13.11.1 |
| catB      | Muconate cycloisomerase | EC:5.5.1.1 |
| catC      | Muconolactone D-isomerase | EC:5.3.3.4 |
| fadA      | Acetyl-CoA-acyltransferase | EC:2.3.1.16 |
| fadB      | Fatty acid oxidative complex subunit alpha | |
| fadJ      | 3-hydroxybutyl-CoA epimerase | EC:5.1.2.3 |
| frmA      | S-(hydroxymethyl)glutathione dehydrogenase | EC:1.1.1284 |
| gst       | Glutathione transferase | EC:2.5.1.18 |
| icd       | Isocitrate dehydrogenase | EC:1.1.1.42 |
| dmpM      | Phenol 2-monoxygenase | EC:1.14.13.7 |
| mphP      | Phenol hydroxylase P5 protein | EC:1.14.13.7 |
| mphL      | Phenol hydroxylase P1 protein | |
| mphD      | 2-oxopent-4-enoate hydratase | |
| paaJ      | 3-oxoadipyl-CoA thiolase | EC:2.3.1.174 |
| ubiH      | 2-octaprenyl-6-methoxyphenol hydroxylase | |
| xylH      | 2-hydroxy muconate tautomerase | EC:5.3.2.6 |

### Table 2. Annotation of genes in Oceanimonas sp. NSJ1 genome which are involved in pathways linked to degradation of aromatic compounds.
bacterial isolate in phthalate degradation (Boll et al. 2020). Phthalates are a group of chemicals that are used to make plastics which are more durable and often called as plasticizers.

**Other special genes**
The genes possibly acquired by horizontal gene transfer (HGT) as deduced by IslandViewer 4 are enlisted in table S1. These include genes involved in coding for integrative host factor (ihfB), which controls the virulence genes (Stonehouse et al. 2008); pasT gene which is part of type II toxin-antitoxin system; yhhw-1 involved in the degradation of quercetin (an antioxidant and anti-inflammatory agent). The phage shock protein (Psp) may play a significant role in the competition for survival under nutrient- or energy-limited conditions and involved in maintenance of membrane integrity, efficient translocation and proton motive force. The norR regulate transcription of genes involved in detoxifying nitric oxide under anaerobic conditions. The fixL (putative oxygen sensor) regulates nitrogen fixation genes. The circular genome map shows possible locations of genomic islands (figure 2).

**Conclusion**
*Oceanimonas* sp. NSJ1 isolated from plastic from the northeast coastal Bay of Bengal is closely related to *Oceanimonas duodoroiffi* based on genome analyses. The analyses revealed presence of enzymes involved in degradation of aromatic compounds including phenol, styrene and various benzoate compounds. The genome of *Oceanimonas* sp. NSJ1 contain genes that can confer metabolic adaptability to heavy metals and toxic-compounds. The presence of phthalate 4,5-dioxygenase oxygenase reductase subunit pht2 within the genome also highlights the novelty of this isolate and future functional potential for studying phthalate degradation. Moreover, in the genome a number of genes linked to aromatic ring hydroxylase highlights significance for studying polystyrene degradation. The genome also contain genomic islands acquired by HGT and harbor genes coding for unique proteins like phage shock proteins, pasT and integrative host factors. Overall, the genome of *Oceanimonas* sp. NSJ1 exhibit unique functional features with potential for application towards tackling plastic pollution in coastal water.

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Data availability statement

All data that support the findings of this study are included within the article (and any supplementary files).

Repositories

Nucleotide sequence data reported is available in GenBank under accession number-JAKNTD010000000

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