Insight into the genome sequence of a sediment-adapted marine
bacterium *Neptunomonas antarctica* S3-22^T from Antarctica

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Abstract

Neptunomonas antarctica S3-22^T was isolated from marine sediment of the Nella
Fjord, Antarctica. Here we report the draft genome sequence of *Neptunomonas*
antarctica, which comprises 4,568,828 bp with a mean G + C content of 45.7%. We
found numerous genes related to resistance, motility and chemotaxis, nitrogen
metabolism, aromatic compounds metabolism and stress response. These metabolic
features and related genes revealed genetic basis for the adaptation to the marine
sediment environment in Antarctica. The genome sequence of *Neptunomonas*
antarctica S3-22^T may also provide further insights into the ecological role of the
genus *Neptunomonas*.

Keywords: *Neptunomonas Antarctica*; marine sediment; Antarctica; metabolic features

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1. Introduction

The genus *Neptunomonas* represents a Gram-negative, flagellated group of marine bacteria and currently contains 7 species (Hedlund et al., 1999). Species of the genus *Neptunomonas* were mostly isolated from marine sediments. *Neptunomonas naphthovorans* NAG-2N-126^T was isolated from creosote-contaminated sediment of Puget Sound and is a polycyclic aromatic hydrocarbon-degrading bacterium (Hedlund et al., 1999). *Neptunomonas japonica* JAMM 0745^T was isolated from the sediment adjacent to a sperm whale carcass off Kagoshima, Japan (Miyazaki et al., 2008) and is currently the only whole-genome sequenced species of the genus *Neptunomonas* (Tatusova et al., 2014).

We previously isolated *Neptunomonas antarctica* S3-22^T (S3-22^T) from marine sediment of the Nella Fjord, Antarctica at a water depth of 20 m (Zhang et al., 2010). S3-22^T is facultatively aerobic and motile with single polar flagella (Zhang et al., 2010). The strain has been deposited under the accession number CCTCC AB 209086^T (= KACC 14056^T). In this study, we report the draft genome sequence of S3-22^T and aim to uncover its genetic basis for adapting to Antarctic marine sediment. The study may also provide further insights into sediment-associated adaptive evolution for the genus *Neptunomonas*.

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2. Data description

S3-22^T genome was sequenced using Illumina Hiseq2000 platform (Table 1). A 500 bp paired-end library was constructed and a total of 500 Mb clean data (read length 90 bp) were obtained representing a 109.4-fold coverage. Reads were assembled into 55 contigs (N50 contig length = 183,668 bp, largest contig length = 977,173 bp) with SOAPdenovo version 1.05 (Li et al., 2010). The final assembly has a size of 4,568,828 bp and a mean G + C content of 45.7% (Table 2). Gene calling and annotation were performed by Rapid Annotation using Subsystem Technology (RAST) version 2.0 (Aziz et al., 2008). A total of 4288 protein-coding sequences (CDSs) were determined and occupied 87.8% of the genome. 3328 CDSs (77.6%) were annotated with defined functions and 960 CDSs (22.4%) were annotated as hypothetical proteins. 60 tRNA genes, 1 incomplete 16S rRNA gene, 2 incomplete 23S rRNA genes and 1 incomplete 5S rRNA gene were annotated. The predicted genes were also annotated by similarity searches against National Center for Biotechnology Information (NCBI) nr database with E-value cutoff 1E-5 and identity cutoff 30%. Metabolic pathways were reconstructed by Kyoto Encyclopedia of Genes and Genomes (KEGG) (Kanehisa and Goto, 2000).

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Table 1. General features of *Neptunomonas antarctica* S3-22^T and MIGS mandatory information.

Item	Description
MIGS data	
Investigation_type	Bacteria_archaea
Collected_by	Xi-Ying Zhang
Collection_date	12-Jan-07
Lat_lon	69.36 S 76.37 E
Depth	20 m
Country	Antarctica
Ref_biomaterial	PMID: 19801396
Biotic_relationship	Free living

Trophic_level	Heterotroph
Rel_to_oxygen	Facultatively aerobic
Isol_growth_condt	PMID: 19801396
Sequencing_meth	Illumina Hiseq 2000 platform
Num_replicons	NA
Assembly	SOAPdenovo v.1.05
Finishing_strategy	Draft
Annot_source	RAST
Estimated_size	4,568,828
Environment (biome)	Sea coast [ENVO: 00000303]
Environment (feature)	Cold temperature habitat [ENVO:00002026]
Environment (material)	Marine sediment [ENVO: 00002113]
Environmental package	Sediment [ENVO: 00002007]
Geo_loc_name	Antarctica:Nella Fjord
Source_mat_id	CCTCC AB 209086 ^T ; KACC 14056 ^T
Temp	4–25 deg C (optimum 15 deg C)
Salinity	0.5 to 5% (optimum 2 to 3%)
pH range	6.0 to 8.0 (optimum 6.5 to 7.0)
Motility	Yes
Genome assembly data	
Assembly method	SOAPdenovo v.1.05
Assembly name	Nant_v1
Genome coverage	109.4 ×
Sequencing technology	Illumina Hiseq 2000 platform

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65 Table 2. Comparison of genome features of *Neptunomonas antarctica* S3-22^T and
66 *Neptunomonas japonica* JAMM 0745^T.

Attributes	<i>Neptunomonas antarctica</i> S3-22 ^T	<i>Neptunomonas japonica</i> JAMM 0745 ^T
Size (bp)	4,568,828	4,391,552
Contigs (≥ 300 bp)	55	39
Largest contig length(bp)	977,173	424,979
N50 contig length (bp)	183,668	195,418
GC content (%)	45.7	43.7
Coding regions (%)	87.8	88.2
CDS	4288	4032
Hypothetical proteins	960	1634
tRNAs	60	54
rRNAs	4	10

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69 To investigate sediment-associated lifestyle of S3-22^T, we analyzed metabolic
70 features related to functional categories (Table 3). Firstly, we discovered that a total
71 of 85 genes are associated with “Virulence, Disease and Defense”. These genes may
72 participate in the resistance against the deposition of sediment-associated pollutants
73 including heavy-metal ions, antibiotics and other toxic compounds. Secondly, we
74 discovered that 62 genes are involved in flagellar motility and 19 genes in bacterial
75 chemotaxis. These genes may assist S3-22^T in hunting for sediment-associated
76 nutrients and avoiding hurts from poisons and predators. Meanwhile, S3-22^T
77 possesses one set of polar flagella and the flagella may play a significant role in
78 sediment-adapted lifestyle just as (Qin et al., 2011) reported.

79 Thirdly, we discovered that 21 genes are involved in “Nitrate and nitrite
80 ammonification” (Table 3), which is carried out by transporting nitrate and nitrite into
81 the cell with respective transporters, reducing nitrate to nitrite by nitrate reductase and
82 then reducing nitrite to ammonia by nitrite reductase. The process of reduction of
83 nitrate to ammonia is also known as dissimilatory nitrate reduction to ammonium
84 (DNRA), which is distinguished from denitrification and preserve nitrogen as the
85 form of ammonia rather than export as molecular nitrogen (N₂) (Papaspyrou et al.,
86 2014). The process of DNRA is widely distributed for microorganisms in marine
87 sediments (Fernandes et al., 2012; Papaspyrou et al., 2014; Sgouridis et al., 2011).
88 Ammonia is subsequently used for the synthesis of glutamine and glutamate by
89 “Ammonia assimilation” with 25 genes involved. As to denitrification which reduces
90 oxidized forms of nitrogen to molecular nitrogen (N₂), the process is halted at the step
91 of reducing nitrite to nitric oxide due to the lack of nitrite reductase (NO-forming). In
92 addition, a total of 7 genes are related to hydrolyze cyanate, urea and nitrile and

response to nitrosative stress. Taking into account the pathway of utilizing nitrogen sources and inhibited denitrification, we inferred that S3-22^T possesses the ability to preserve sufficient available nitrogen for its survival in marine sediment.

Fourthly, we discovered that a total of 35 genes are involved in “Aromatic Compounds Metabolism” (Table 3) as the poisonous aromatic compounds are ubiquitously distributed in marine sediments due to organic pollution (Bajt, 2012; Mzoughi and Chouba, 2011; Wang et al., 2015). These genes assist S3-22^T in degradation of aromatic compounds by complex catabolism including central meta-cleavage pathway and other aromatic compounds catabolism. Meanwhile, the degradation of aromatic compounds may supply S3-22^T with carbon and energy sources just like that of *N. naphthovorans* (Hedlund et al., 1999).

Finally, S3-22^T possesses 42 genes related to “Stress Response” (Table 3). These genes may assist S3-22^T in dealing with the cold environment in Antarctica, such as dioxygenases, which release the oxidative stress due to increased solubility of oxygen at low temperature (Medigue et al., 2005; Russo et al., 2010), and cold / heat shock proteins, which protect the cell from extreme temperatures (Jones and Inouye, 1994; Matz et al., 1995; Weber and Marahiel, 2003). In summary, through the analysis of metabolic features and related genes of S3-22^T, we revealed the genetic basis of S3-22^T to adapt to the marine sediment in Antarctica.

Table 3. Metabolic features and genes related to the adaptation to Antarctic marine sediment.

Categories	Metabolic features	Genes
Virulence, Disease and Defense	Colicin V and bacteriocin production cluster	6
	Mycobacterium virulence operon	12

		Copper resistance	13
		Cobalt-zinc-cadmium resistance	15
		Zinc resistance	1
		Mercuric resistance	1
		Chromium compounds resistance	1
		Colicin E2 resistance	1
		Fluoroquinolones resistance	4
		Arsenic resistance	4
		Multidrug resistance efflux pumps	24
		Tetracycline resistance	2
		Beta-lactamase	1
	Motility and Chemotaxis	Flagellar motility	62
		Bacterial chemotaxis	19
	Nitrogen Metabolism	Nitrate and nitrite ammonification	21
		Ammonia assimilation	25
		Denitrification	14
		Denitrifying reductase gene clusters	16
		Nitrosative stress	2
		Cyanate hydrolysis	3
		Amidase clustered with urea and nitrile hydratase functions	2
	Aromatic Compounds Metabolism	Central meta-cleavage pathway of aromatic compound degradation	8
		Salicylate and gentisate catabolism	6
		Salicylate ester degradation	1
		Gentisate degradation	5
		4-Hydroxyphenylacetic acid catabolic pathway	7
		Aromatic amin catabolism	3
		Quinate degradation	1
		p-Hydroxybenzoate degradation	1
		Catechol branch of beta-ketoadipate pathway	3
	Stress Response	Oxidative stress	16
		Dioxygenases	4
		Cold shock proteins	5
		Heat shock proteins	17

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117 Nucleotide sequence accession numbers

118 The draft genome sequence of *Neptunomonas antarctica* S3-22^T has been deposited

119 in DDBJ/EMBL/GenBank under the accession number LJSI000000000. The version

120 described in this paper is the first version, with accession number LJSI010000000. The

sequence associated contextual (meta) data are MIxS compliant (Table 1) (Yilmaz et al., 2011).

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