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Diversity and DMS(P)-related Genes in Culturable Bacterial Communities in Malaysian Coastal Waters

(Kepelbagaian dan Gen berkaitan-DMS(P) dalam Komuniti Kultur Bakteria di Perairan Pantai Malaysia)

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ABSTRACT

*Little is known about the diversity and roles of microbial communities in the South China Sea, especially the eastern region. This study aimed to expand our knowledge on the diversity of these communities in Malaysian waters, as well as their potential involvement in the breakdown or osmoregulation of dimethylsulphoniopropionate (DMSP). Water samples were collected during local cruises (Kuching, Kota Kinabalu, and Semporna) from the SHIVA expedition and the diversity of bacterial communities were analysed through the isolation and identification of 176 strains of cultured bacteria. The bacteria were further screened for the existence of two key genes (*dmdA, dddP*) which were involved in competing, enzymatically-mediated DMSP degradation pathways. The composition of bacterial communities in the three areas varied and changes were mirrored in physico-chemical parameters. Riverine input was highest in Kuching, which was mirrored by dominance of potentially pathogenic* Vibrio *sp., whereas the Kota Kinabalu community was more indicative of an open ocean environment. Isolates obtained from Kota Kinabalu and Semporna showed that the communities in these areas have potential roles in bioremediation, nitrogen fixing and sulphate reduction. Bacteria isolated from Kuching displayed the highest abundance (44%) of both DMSP-degrading genes, while the bacterial community in Kota Kinabalu had the highest percentage (28%) of* dmdA *gene occurrence and the* dddP *gene responsible for DMS production was most abundant (33%) within the community in Semporna. To the best of our knowledge, this is the first study looking at the diversity of culturable bacteria in coastal waters of East Malaysia and also their potential roles in the DMS(P) cycle.*

Keywords: Culturable bacterial communities; dimethylsulphide; dimethylsulphoniopropionate; diversity

ABSTRAK

*Kepelbagaian dan peranan yang dimainkan oleh komuniti mikrob di Laut China Selatan, khususnya di Wilayah Timur, adalah kurang diketahui. Kajian ini bertujuan untuk mengembangkan pengetahuan tentang kepelbagaian komuniti ini di perairan Malaysia, serta potensi penglibatan mereka dalam penguraian atau pengawalan osmosis dimetilsulfoniopropionat (DMSP). Sampel air diperoleh semasa pelayaran tempatan (Kuching, Kota Kinabalu dan Semporna) daripada ekspedisi SHIVA dan kepelbagaian komuniti bakteria telah dianalisis melalui pengasingan dan pengenalpastian 176 strain kultur bakteria. Bakteria ini seterusnya disaring untuk menentukan kehadiran dua gen utama (*dmdA, dddP*) yang terlibat dalam dua laluan bersaingan degradasi DMSP secara berenzim. Komposisi komuniti bakteria dalam tiga kawasan ini berbeza dan perbezaan ini boleh dilihat dalam parameter fisiko-kimia. Input sungai paling tinggi di Kuching dan ini ditunjukkan melalui dominasi* Vibrio *sp., yang berpotensi untuk menjadi patogenik, manakala komuniti Kota Kinabalu adalah petunjuk untuk persekitaran lautan terbuka. Bakteria yang diasingkan dari Kota Kinabalu dan Semporna menunjukkan bahawa komuniti dalam dua kawasan ini berpotensi memainkan peranan dalam bioremediasi, pengikatan nitrogen dan penurunan sulfat. Bakteria yang diasingkan dari Kuching menunjukkan kebanyakan tertinggi (44%) untuk kedua-dua gen pengurai DMSP, manakala komuniti bakteria di Kota Kinabalu menunjukkan peratusan tertinggi (28%) kejadian gen dmdA dan gen dddP yang bertanggungjawab untuk pengeluaran DMS adalah paling banyak (33%) dalam komuniti di Semporna. Sepanjang pengetahuan kami, ini merupakan penyelidikan pertama yang melihat kepelbagaian bakteria yang boleh dikultur di perairan pantai Malaysia timur dan juga potensi penglibatan mereka dalam kitaran DMS(P).*

Kata kunci: Dimetilsulfida; dimetilsulfoniopropionat; kepelbagaian; komuniti kultur bakteria

INTRODUCTION

The South China Sea is a marginal sea that is part of the Pacific Ocean, encompassing an area from the Karimata Straits in the south, to the Straits of Taiwan and Luzon in the North (Morton & Blackmore 2001). The Celebes Sea is connected to the South China Sea through the Sulu Sea (Yoshida et al. 2007). While the bacterial communities in the Celebes and Sulu Seas have been reported to display some similarities (Yoshida et al. 2007), not much is known about the diversity and function of the microbial communities in South China Sea, especially regarding the eastern region (Kuching and Kota Kinabalu). Most studies about bacterial communities focused on regions near China (Jiang et al. 2007; Liao et al. 2009; Li et

al. 2006; Tao et al. 2008; Zhu et al. 2013), with the exceptions of Kuek et al. (2015), Lee et al. (2009) and Song et al. (in preparation), all of whom sampled from the coasts of Malaysia. Aside from Kuek et al. (2015) and Lee et al. (2009), all the other cited studies used culture-independent techniques to show the community structure and diversity of the predominant bacteria at the sampling environment. The studies by Jiang et al. (2007) and Tao et al. (2008) showed that most lineages within the Proteobacteria represented uncultured microorganisms, suggesting that a vast amount of microbial resources in the South China Sea are unknown and unexplored. Song et al. (in preparation) found that Proteobacteria (Alpha- and Gamma-) and Cyanobacteria (*Synechococcus* sp. and *Prochlorococcus* sp.) dominated at all study sites and that the highest proportion of Gammaproteobacteria was found in Sarawak. Similarly, Lee et al. (2009) discovered that Gram-negative bacteria dominated their study of cultured bacteria, with the most prevalent class belonging to the Gammaproteobacteria.

The ocean is a major source of sulphur (Andreae 1986) and microorganisms residing in the ocean have the ability to metabolise organic and inorganic sulphur (Sievert et al. 2007). Dimethylsulphoniopropionate (DMSP) represents a major carrier for sulphur transfer through microbial food webs and organic sulphur cycling in the ocean as it is an abundant component in many phytoplankton taxa and prone to microbial degradation (Kiene et al. 2000). The *Roseobacter* which are part of the Alphaproteobacteria lineage are mainly responsible for the degradation of DMSP into methanethiol (MeSH) and have been found in different regions of the world, ranging from the Sargasso Sea to the Black Sea (González et al. 2000, 1999). A competing metabolic pathway results in the production of dimethylsulphide (DMS) from DMSP (González et al. 1999; Johnston et al. 2008). Due to highly efficient bacterial DMSP demethylation and DMS consumption processes, only a small percentage (1-2%) of DMSP produced by marine phytoplankton is ventilated to the atmosphere as DMS (Levine et al. 2012). Despite the low percentage, DMS does, however, represent a major source of biogenic sulphur to the atmosphere, where oxidation products form cloud condensation nuclei and ultimately influence radiative backscatter (Andreae & Crutzen 1997; Lovelock et al. 1972; Simó 2001). The DMSP demethylase gene (*dmdA*) which encodes the first step in the demethylation pathway, is taxonomically diverse and highly abundant, present in over 50% of marine bacterioplankton (Howard et al. 2008). In comparison to *dmdA*, the genes involved in DMS production (*dddD*, *dddL*, *dddP*, *dddQ*, *dddY* and *dddW*; all of which mediate the same step of DMSP cleavage) are present in less than 10% of bacteria based on marine metagenomic surveys (Curson et al. 2011a, 2008; Howard et al. 2008; Todd et al. 2012, 2011, 2007). *dddP* is one of the most abundant occurring *ddd* genes (Levine et al. 2012; Todd et al. 2009; Varaljay et al. 2012).

The present study tries to expand our knowledge on culturable microbial communities in the eastern South China Sea and identify potential key players in the local DMS(P) cycle.

Materials and Methods

Study sites, sample collection and initial isolation

In conjunction with European and Malaysian research partners, the SHIVA (Stratospheric ozone: Halogen Impacts in a Varying Atmosphere, EU call ENV.2008.1.1.2.1) Western Pacific field campaign was performed in the fall of 2011. The core field campaign took place in the South China Sea and along the coastline of Peninsular Malaysia and Borneo using the German Research Vessel (RV) Sonne during a cruise leading from Singapore to Manila, Philippines. Local cruises took place in Kuching on November 19, 2011, Kota Kinabalu on November 23, 2011 and Semporna on November 26, 2011 (Table 1) to provide additional data for coastal input. Samples for this study were collected during the local cruises.

Physico-chemical parameters (depth, temperature, pH, salinity, nitrate, phosphate, nitrite and silicate) were quantified using a QuAAtro auto-analyser (SEAL Analytical, UK) following protocols provided in the SEAL analytical operation manual and methods published in Grashoff et al. (1999).

Sea water samples were streaked on marine agar at half strength (Difco, 2.76% solution, dissolved in purified water) and incubated under aerobic conditions at 30°C. Bacterial colonies were isolated based on their morphological differences. Colonies were picked and purified by repeated streaking on plates. Pure cultures were preserved as a glycerol suspension (20%, w/v) at -80°C.

Diversity indices

Several ecological diversity indices frequently applied to microbial community profile data were used in order to compare diversity among microbial communities, enabling us to quantify diversity within the communities and describe their numerical structure. Taxonomic classification up to genus was used as some BLAST results could only relate the isolates to strains which have been identified up to genus level. Five representative stations were chosen per sampling site in order to standardise the sampling effort and enable us to compare among the sampling sites.

The Margalef index (D_{Mg}) is an accurate index to sample richness which utilises absolute numbers compared to a density data matrix (Gamito 2010; Magurran 2004). Meanwhile, the commonly used Shanon index (H') considers proportions, ensuring no differences when using either data set (Gamito 2010). The Shannon evenness index (J') is derived from H' which therefore makes it sensitive to changes in evenness of rare species, thereby

TABLE 1. Locations of sampling stations in Kuching, Kota Kinabalu and Semporna

Sampling stations	GPS coordinates		
	Kuching	Kota Kinabalu	Semporna
Station 1	1°39'28.81"N, 110°31'24.42"E	6° 3'4.56"N, 116° 5'54.60"E	4°35'15.96"N, 118°32'58.14"E
Station 2	1°42'44.24"N, 110°33'23.46"E	6° 3'5.82"N, 116° 4'1.45"E	N/A
Station 3	1°45'32.93"N, 110°35'16.86"E	6° 3'4.02"N, 116° 0'2.77"E	N/A
Station 4	1°48'2.16"N, 110°37'51.53"E	6° 2'49.85"N, 115°57'38.26"E	N/A
Station 5	1°50'54.15"N, 110°40'11.26"E	6° 4'23.64"N, 115°54'36.42"E	4°37'31.26"N, 118°41'5.99"E
Station 6	N/A	N/A	4°35'56.76"N, 118°43'19.14"E
Station 7	N/A	N/A	4°35'30.66"N, 118°42'17.10"E
Station 8	N/A	N/A	4°33'17.83"N, 118°39'22.57"E

possibly overestimating its true value (Hill et al. 2003). The Smith and Wilson evenness index (E_{var}), however, is known to show greater resolution in reflecting true values (Blackwood et al. 2007).

DNA EXTRACTION AND PURIFICATION OF CULTURED BACTERIA

The isolates were grown in marine broth at half strength at 30°C with shaking at 180 rpm. The cells were pelleted by centrifugation at 13,000 rpm for 5 min before re-suspension in 50 µL of TE buffer (10 mM Tris-HC pH8.0, 1 mM EDTA). Three cycles of freezing in a -80°C freezer for 3 min and thawing in an 85°C water bath for 3 min were conducted to release DNA from the microbial cells.

PCR AMPLIFICATION OF BACTERIAL 16S RRNA GENES

The bacterial DNA were amplified by polymerase chain reaction (PCR) and PCR products were purified using PureLink® PCR Purification Kit following the manufacturer's protocol (Invitrogen Life Technologies). Amplification of bacterial 16S rRNA genes was performed with broad-specificity primers 8F (Eden et al. 1991) and 519R (Lane et al. 1985). Amplification was performed by RedTaqMix (Sigma Aldrich) using instructions provided by Sigma Aldrich with the following cycling conditions: Initial denaturation at 96°C for 4 min, 40 cycles of 96°C for 1 min, 55°C for 1 min, extension at 72°C for 2 min and then a final elongation at 72°C for 4 min. The samples of extracted DNA were analysed on a 1% agarose gel containing 1 µg of ethidium bromide per mL.

SEQUENCING AND PHYLOGENETIC ANALYSIS

Sequences were analysed against the NCBI (USA) database using BLAST program packages and matched to known 16S rRNA gene sequences (Altschul et al. 1990; Zhang et al. 2000). Ambiguous sequences were checked manually by eye and further edited using MUSCLE (Edgar 2004). Sequences were aligned and phylogenetic trees reconstructed with MEGA 6 (Tamura et al. 2013) using the maximum likelihood method based on Tamura-Nei model (Tamura & Nei 1993). The nucleotide sequences obtained in the present study have been deposited in GenBank database (http://www.ncbi.nlm.nih.gov) under accession numbers KF373319 to KF373440.

PCR AMPLIFICATION OF BACTERIAL DMSP CLEAVAGE (DDDP) AND DEMETHYLATION (DMDA) GENES

The bacterial DNA were amplified by polymerase chain reaction (PCR) and PCR products were purified using PureLink® PCR Purification Kit following the manufacturer's protocol (Invitrogen Life Technologies). Amplification of *dddP* genes was performed with degenerate *dddP* primers dddP_874F and dddP_971R (Levine et al. 2012) while amplification of *dmdA* genes was performed with universal *dmdA* primers dmdAUF160 and dmdAUR697 (Varaljay et al. 2010). Amplification was performed by using RedTaqMix (Sigma Aldrich) with the following cycling conditions: Initial denaturation at 95°C for 5 min, 40 cycles of 95°C for 30 s, 41°C for 30 s, extension at 72°C for 30 s and then a final denaturation and annealing for 1 min each. The samples of extracted DNA were analysed on a 1% agarose gel containing 1 µg of ethidium bromide per mL.

RESULTS AND DISCUSSION

PHYSICO-CHEMICAL PARAMETERS

Basic physico-chemical parameters were recorded during sampling in Kuching and Kota Kinabalu (Table 2). Values for Semporna were not reported as the measuring instruments were not in working order at the time of sampling. The sampling stations at Kota Kinabalu stretched further away from the coastline and displayed average values of salinity at 31.88 ppt, pH of 8.36 and temperature of 29.65°C, all indicative of a typical ocean environment (Raven et al. 2005). The first sampling station at Kuching (KCH-1) was closer to the river mouth of the Sarawak river and displayed a visible influence by riverine water with its surface water displaying a salinity of 28.48 ppt and pH of 7.90. The riverine input at Kuching was also visible with

higher nitrate, phosphate, nitrite and silicate values closer to the river mouth (KCH-1 and KCH-2). Nutrient levels in Kuching were also generally higher than in Kota Kinabalu. To assess differences in distribution in the upper surface layers, the samples were also taken from 5 m depth (KCH-5 and KK-5). Interestingly, the samples for Kota Kinabalu showed consistent values. For Kuching however, silicate concentration dropped from 63.64 to 24.00 μM within the first 5 m, indicative of an active biological pump (Dugdale et al. 1995).

DIVERSITY OF CULTURABLE BACTERIAL COMMUNITIES

A total of 36 isolates were obtained from Kuching waters and 89% of the cultured bacteria were clustered within the Gammaproteobacteria and 11% within the Alphaproteobacteria (Figures 1 & 2). In Kota Kinabalu waters, 39 isolates were obtained and the majority (72% of the cultured bacteria) were clustered within the Gammaproteobacteria (Figures 1 & 3). The remaining isolates were members of the Firmicutes (18%) and Alphaproteobacteria (10%). In Semporna waters, 24 isolates were obtained from four phylogenetic groups. In total, 83% of the cultured bacteria were members of the Gammaproteobacteria, 9% Alphaproteobacteria and 4% each in Betaproteobacteria and Firmicutes groups (Figures 1 & 4). Our results correlated with existing records of microbial communities found in coastal and open-ocean environments (Bernard et al. 2000).

Values for sample richness using both D_{Mg} and H' indicated that the bacterial communities in Kota Kinabalu and Semporna were more diverse than the one in Kuching (D_{Mg} of 3.82 and 3.36 compared to 1.67; Table 3). Evenness values from both J' and E_{var} also indicate that the communities in Kota Kinabalu and Kuching are more evenly distributed. In the following, we discuss some highlights of the bacterial diversity found at the three sampling sites.

The cultured Alphaproteobacteria can be found across all three sampling sites and consists of representatives from Caulobacteraceae, Phyllobacteriaceae, Rhodobacteraceae and Rhodospirillaceae (Figures 2 to 4). Isolates from this group were likely to be involved in the nitrogen cycle and possibly in the degradation of hydrocarbons (Bell et al. 1992; Itoh et al. 1989; Labbé et al. 2004; Richardson et al. 1989; Zumft 1997).

The sole Betaproteobacteria that was cultured (Figure 4) is related to *Alcaligenes faecalis* (GenBank accession number JF264463; 88% similarity) which was previously isolated from a coastal aquaculture environment. *Alcaligenes faecalis* have also been found in salt marsh and

TABLE 2. Physico-chemical parameters measured from Kuching (KCH) and Kota Kinabalu (KK) at depths of 1 and 5 m

Station	Depth (m)	Temperature (°C)	pH	Salinity (ppt)	Nitrate (μM)	Phosphate (μM)	Nitrite (μM)	Silicate (μM)
KCH-1	1	29.06	7.90	28.48	147.25	6.32	31.74	254.88
	5	29.34	8.10	30.59	BD	BD	BD	BD
KCH-2	1	28.98	8.25	30.65	32.58	3.47	13.04	81.23
	5	29.11	8.25	30.89	BD	BD	BD	BD
KCH-3	1	29.05	8.33	31.18	13.71	1.58	0.65	32.25
	5	29.16	8.30	30.53	BD	BD	BD	BD
KCH-4	1	29.00	8.33	31.07	7.90	1.05	0.00	49.09
	5	29.10	8.29	30.52	BD	BD	BD	BD
KCH-5	1	29.27	8.31	31.61	2.42	0.63	0.00	63.64
	5	29.40	8.29	31.85	2.42	0.53	0.00	24.00
KCH mean		29.15 (±0.14)	8.24 (±0.14)	30.74 (±0.92)	34.38 (±56.42)	2.26 (±2.26)	7.57 (±12.91)	84.18 (±86.16)
KK-1	1	29.80	8.44	31.85	16.77	1.58	BD	37.68
	5	29.90	8.37	32.04	BD	BD	BD	BD
KK-2	1	29.73	8.36	31.44	4.03	1.79	BD	34.86
	5	29.78	8.33	31.95	BD	BD	BD	BD
KK-3	1	29.55	8.34	31.88	3.71	1.16	BD	29.00
	5	29.54	8.33	31.87	BD	BD	BD	BD
KK-4	1	29.52	8.36	31.93	BD	BD	BD	BD
	5	29.45	8.34	31.91	BD	BD	BD	BD
KK-5	1	29.68	8.38	32.03	2.10	0.32	BD	29.76
	5	29.50	8.37	31.92	2.42	0.21	BD	30.30
KK mean		29.65 (±0.15)	8.33 (±0.03)	31.88 (±0.17)	5.81 (±6.18)	1.01 (±0.72)	BD	32.32 (±3.77)

**BD denotes values below detection limit*

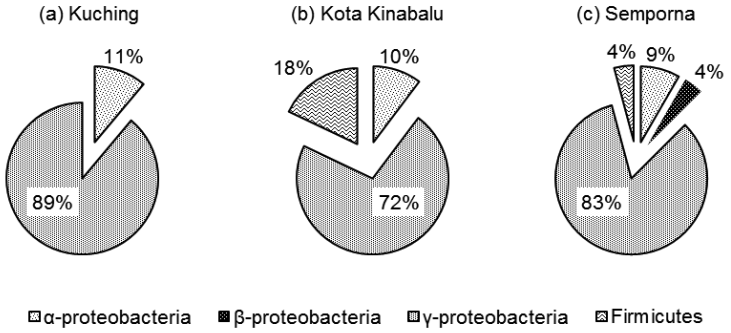

FIGURE 1. Diversity of bacterial groups based on partial 16S rRNA gene sequences from bacteria isolated from (a) Kuching, (b) Kota Kinabalu and (c) Semporna

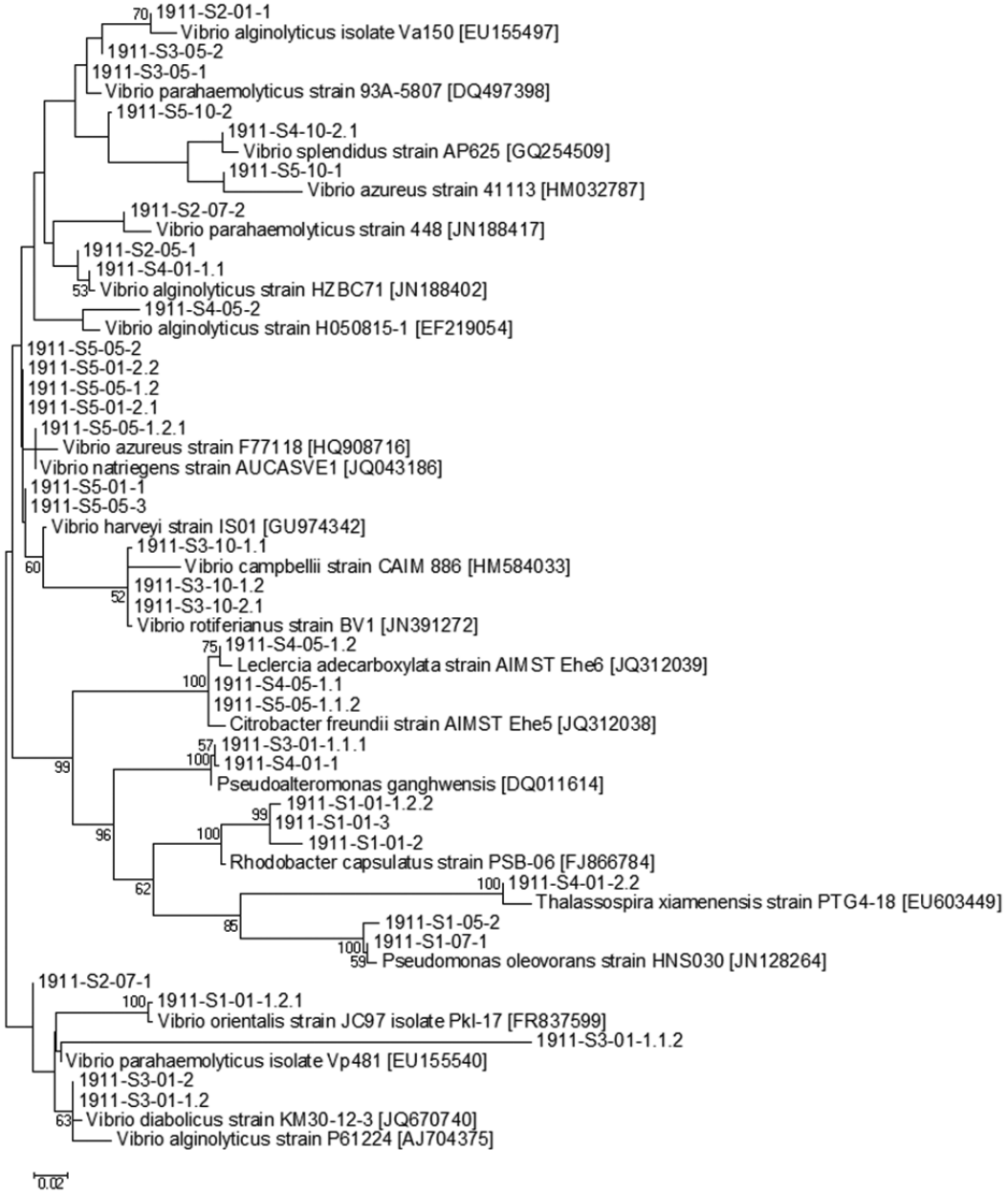

FIGURE 2. 16S rRNA gene-based phylogenetic tree representing bacterial sequences found in Kuching. The phylogenetic tree was generated with distance methods and sequence distances were estimated with the neighbour-joining method. Bootstrap values ≥50 are shown and the scale bar represents a difference of 0.02 substitution per site. Accession numbers for the reference sequences are indicated

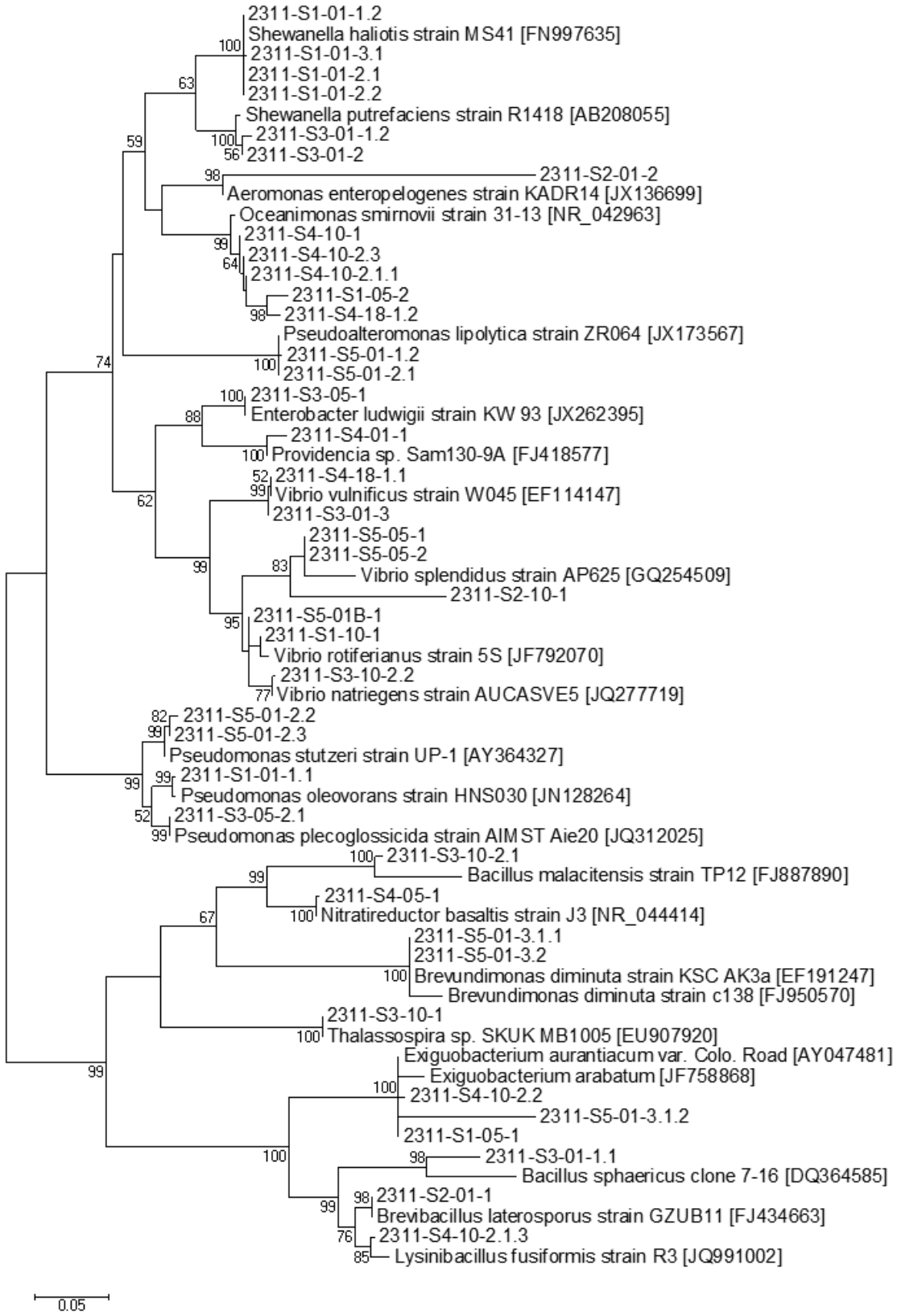

FIGURE 3. 16S rRNA gene-based phylogenetic tree representing bacterial sequences found in Kota Kinabalu. The phylogenetic tree was generated with distance methods and sequence distances were estimated with the neighbour-joining method. Bootstrap values ≥50 are shown and the scale bar represents a difference of 0.05 substitution per site. Accession numbers for the reference sequences are indicated

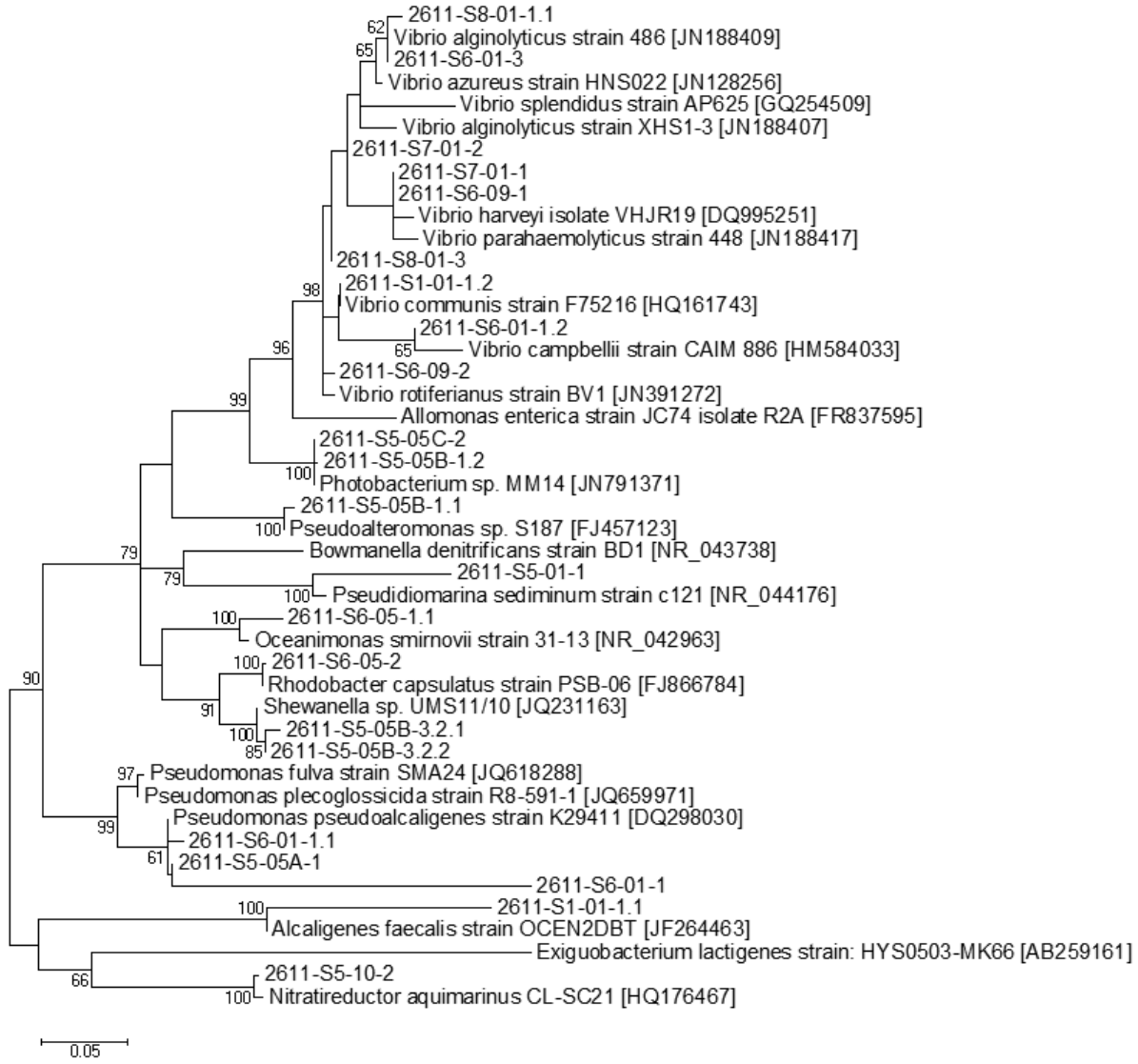

FIGURE 4. 16S rRNA gene-based phylogenetic tree representing bacterial sequences found in Semporna. The phylogenetic tree was generated with distance methods and sequence distances were estimated with the neighbour-joining method. Bootstrap values ≥50 are shown and the scale bar represents a difference of 0.05 substitution per site. Accession numbers for the reference sequences are indicated

TABLE 3. Indices used to quantify the diversity of bacterial communities at Kuching, Kota Kinabalu and Semporna

Genus	Kuching	Kota Kinabalu	Semporna
Total isolates (N)	36	39	48
Total genus (S)	7	15	14
Margalef index (D_{Mg})	1.67	3.82	3.36
Shannon index (H')	1.14	2.42	2.18
Shannon evenness (J')	0.59	0.89	0.83
Smith and Wilson evenness (E_{var})	0.49	0.69	0.59

**Formulae of diversity indices are from Margalef (1958), Shannon and Weaver (1963) and Smith and Wilson (1996)*

estuarine waters (Ansede et al. 2001) and has the potential to degrade DMSP to DMS via acrylate metabolism through the induction of β-hydroxypropionate (Ansede et al. 1999; Yoch et al. 1997).

Within the Gammaproteobacteria group, isolates from Aeromonadaceae, Pseudoalteromonadaceae, Shewanellaceae, Pseudomonadaceae and Vibronaceae can be found across all three sampling sites (Figures 2 to 4). Isolates from Kuching which were related to strains of *Pseudoalteromonas ganghwensis* (GenBank accession number DQ011614; 99% similarity) have been observed to possess the ability to form biofilms and may contribute

in part to the removal of excess proteineous matters from the sediment sludge of fish farms (Iijima et al. 2009). *Pseudoalteromonas lipolytica* (GenBank accession number JX173567) has only been recently characterised (Xu et al. 2010) and has the ability to hydrolyse lipids and reduce nitrate to nitrite. Kota Kinabalu has isolates that were closely related to this particular strain.

Members of Vibrionaceae are common in the marine environment, with species found in hydrothermal vents, deep sea, open water, estuaries and marine sediments (Eilers et al. 2000; Lee & Ruby 1994; Maruyama et al. 2000; Raguénès et al. 1997) and is the most heavily represented family within the Gammaproteobacteria. Studies have suggested that some *Vibrio* can degrade ecologically hazardous compounds, such as polycyclic aromatic hydrocarbons (Ramaiah et al. 2000) and are major decomposers of chitin in the ocean (Hedlund & Staley 2001; Nagasawa & Terazaki 1987). They have also been shown to cause potentially lethal diseases in humans and fish (Kusuda & Kawai 1998; McCarter 1999). More recently, studies have shown *Vibrio shiloi* to be a coral pathogen, producing toxins that inhibit photosynthesis and lyse zooxanthellae resulting in coral bleaching (Banin et al. 2000a, 2000b). Species such as *Vibrio parahaemolyticus* and *Vibrio vulnificus* have been shown to express virulence-related properties such as the production of *toxR* gene (Lin et al. 1993; Okuda et al. 2001) and production of phenolate siderophore (Stelma et al. 1992). *Vibrio harveyi* and *Photobacterium* sp. are luminous bacteria which often cause disease in aquaculture (Baticados et al. 1990; Prayitno & Latchford 1995). While most *Vibrio* sp. isolated from Kuching appeared to be related to pathogenic strains, many of the isolates from Kota Kinabalu and Semporna have potential roles in bioremediation, nitrogen fixing and sulphate reduction.

Members of the cultured Firmicutes group consisted of members of the Bacillaceae, Bacillaceae Family XII. incertae sedis and Paenibacillaceae. Isolates from Bacillaceae were mostly related to *Bacillus* spp. and *Lysinibacillus* spp. and are unique to each sampling site. Isolates from the Bacillaceae Family XII. incertae sedis were matched with *Exiguobacterium* spp. which have previously been isolated from, or molecularly detected in, a wide range of habitats including cold and hot environments with temperatures ranging from -12 to 55°C (Vishnivetskaya et al. 2009). Interestingly, members of this family were only isolated from Kota Kinabalu and Semporna where recent temperature spikes resulted in mass coral bleaching in the region (Tan & Heron 2011). Of the three sampling sites, Sarawak was the only area with no reported bleaching events (Tun et al. 2010).

In conclusion, several species isolated from Kuching waters appear to be related to pathogenic strains, whereas many of the isolates from Kota Kinabalu and Semporna have potential roles in bioremediation, nitrogen fixing and sulphate reduction.

BACTERIAL STRAINS WITH POTENTIAL TO METABOLISE DMS AND/OR DEMETHYLATE DMSP

To date, there are no available reports on the sulphur cycle in the region or of DMSP catabolism from bacterial communities of Kuching, Kota Kinabalu and Semporna; neither are any bioinformatics data available on the prevalence of *dmdA* and *dddP* genes in bacteria from these regions. As part of our effort to understand the importance of bacteria in the region for the local sulphur cycle, we screened our isolates for the presence of *dmdA* and *dddP* genes.

Previously reported bacteria with the ability to demethylate DMSP and/or metabolise DMS which we also managed to isolate and culture include *Rhodobacter* and *Roseovarius* within the Alphaproteobacteria (Curson et al. 2008; González et al. 2003; Johnston et al. 2008; Kirkwood et al. 2010; Moran et al. 2007; Todd et al. 2009); the aforementioned *Alcaligenes faecalis* within the Betaproteobacteria; *Oceanimonas*, *Pseudomonas*, *Shewanella* and *Vibrio* within the Gammaproteobacteria (Ansede et al. 1999; de Souza & Yoch 1995; Johnston et al. 2008; Moran et al. 2007; Raina et al. 2010, 2009; Sievert et al. 2007; Yoch 2002; Yoch et al. 1997); and *Bacillus* within the Firmicutes (Todd et al. 2009).

Bacteria isolated from Kuching displayed the highest abundance of both DMSP-degrading genes (44% of all isolates from Kuching) compared to communities isolated from Kota Kinabalu and Semporna (with 13 and 21%, respectively). The bacterial community in Kota Kinabalu has the highest percentage of *dmdA* gene occurrence (28% of all isolates from Kota Kinabalu) while the *dddP* gene responsible for DMS production appears to be most abundant (33%) within the bacterial community in Semporna (Figure 5). Stefels (2000) has previously hypothesized that DMSP production is an overflow mechanism for when growth is unbalanced by lack of nutrients and the need to release excess energy and excess reduced sulphur. These carbon-energy overflow substances might evolve through natural selection to be useful in the cell (through auxiliary structures or defence mechanisms) (Hill et al. 1998). Based on our findings, it seems likely that at low nutrient conditions, the distribution of *dmdA* and *dddP* genes within the bacterial community becomes more specific (more *dmdA* in KK and more *dddP* in Semporna) to adapt to a preferred pathway to degrade DMSP. This is discussed as follows.

The sampling locations at Kuching and Kota Kinabalu were observed to have heavy shipping traffic which may influence the sulphur concentration in the area. Ship plumes emit large amounts of anthropogenic nitrogen and sulphur into the atmosphere, particularly within potential transport distance of land regions (Corbett et al. 1999) which may influence the algal production of DMSP (Malin & Erst 1997). The waters of Kota Kinabalu are known for having seasonal phytoplankton blooms (Adam et al. 2011). The relative production of DMSP was suggested to depend on nitrogen availability (Andreae 1986). Small haptophytes (e.g. coccolithophorids) and many small dinoflagellates are

typical of more nitrogen-deficient conditions, so they have evolved to produce more DMSP, implying the probability of finding higher levels of DMSP is greater under conditions of nitrogen depletion during phytoplankton blooms (Simó 2001). Nitrate and nitrite concentrations at Kota Kinabalu are low (5.81 μM on average and not detectable, respectively; Table 2), especially in comparison with Kuching (up to 147.25 and 31.74 μM nearest to the coast), indicating a low nutrient environment and suggesting the likelihood of high concentration of DMSP in the area especially in the event of phytoplankton blooms. The bacterial community in the area have possibly evolved to adapt to these conditions and preferred the demethylation pathway as the occurrence of *dmdA* genes is the highest within the community at Kota Kinabalu (28% of all isolates; Figure 5).

Due to riverine input, the waters of Kuching showed higher nutrient concentrations compared to Kota Kinabalu and Semporna and it is possible that the high nutrient environment at Kuching forces the bacterial community in the area to be more metabolically flexible. This is highlighted by the fact that the dominant genus, *Vibrio*, emerged as the key group in these waters with high occurrences of both *dmdA* and *dddP* genes (representing 100% of all isolates within the Gammaproteobacteria with both genes; Figure 6) and they seem well-adapted to the variable environmental conditions. Other key players involved in DMS production seem to be members of the *Pseudomonas* and *Pseudoalteromonas*, whereas *Citrobacter* seem to be involved in DMSP assimilation in Kuching waters.

Vibrio is also potential key players in samples from Kota Kinabalu but seem to serve a slightly different role. Occurrence of DMSP assimilation genes within the Gammaproteobacteria is again dominated by *Vibrio*; however, *Shewanella*-related isolates are more likely to possess both genes (Figure 7). This may be due to the different *Vibrio* species that form the majority in Kuching and Kota Kinabalu communities (*Vibrio parahaemolyticus* and *Vibrio splendidus* respectively; Figures 2 and 3). The higher diversity of the community at Kota Kinabalu shows a possible correlation with flexibility of DMSP degradation pathways with a more even distribution of groups possessing both *dmdA* and *dddP* genes compared to communities in Kuching and Semporna (Figures 6 & 8, respectively).

The sampling stations at Semporna were observed to be surrounded by seaweed farms. Micro- and macroalgae and halophytic plants are abundant sources of DMSP in the marine environment (Yoch 2002) and past studies (Scarratt

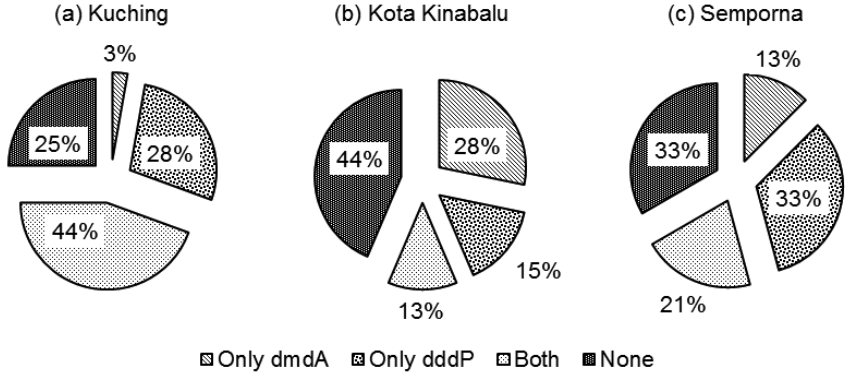

FIGURE 5. Relative abundance of *dmdA* and *dddP* genes in cultured bacterial communities from the waters of (a) Kuching, (b) Kota Kinabalu and (c) Semporna

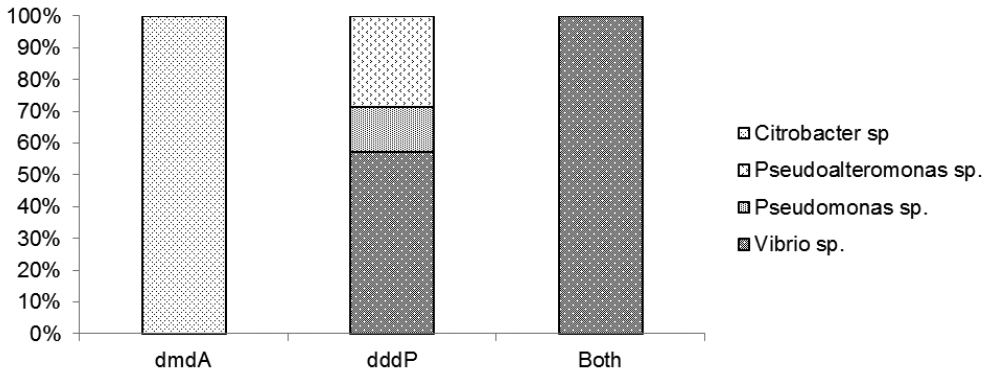

FIGURE 6. Relative abundance of *dmdA* and *dddP* genes in isolated Gammaproteobacteria from Kuching

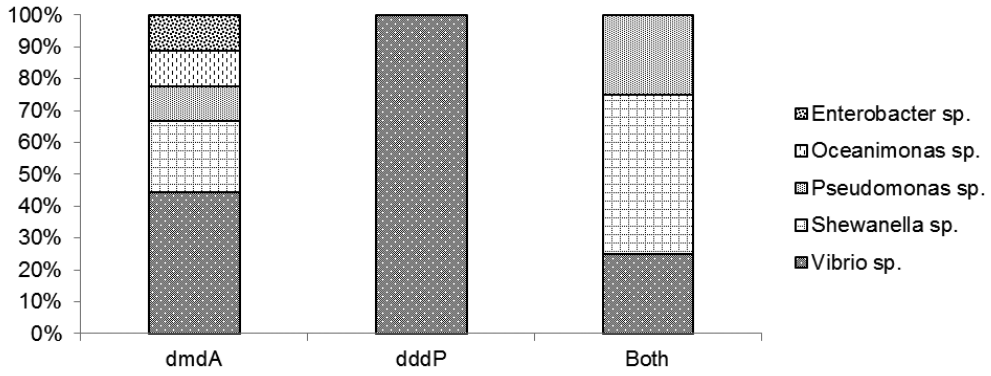

FIGURE 7. Relative abundance of *dmdA* and *dddP* genes in isolated Gammaproteobacteria from Kota Kinabalu

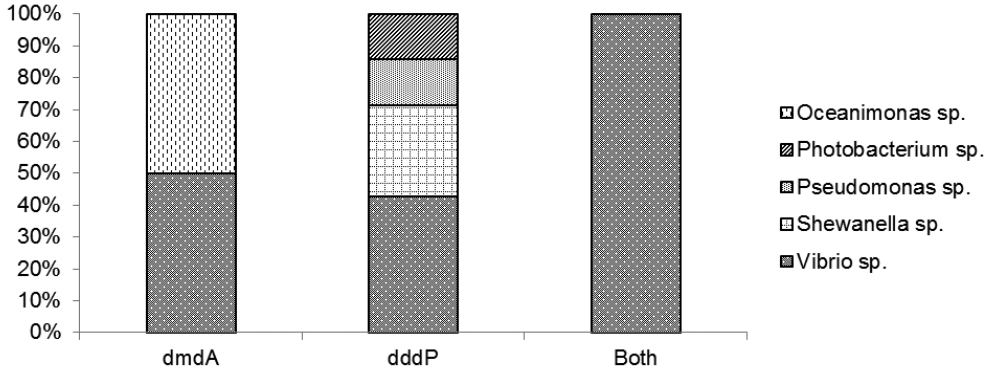

FIGURE 8. Relative abundance of *dmdA* and *dddP* genes in isolated Gammaproteobacteria from Semporna

et al. 2000) suggested that bacteria growing near algal cells might be exposed to high local levels of DMSP, which would lead to DMS yields that are higher than those inferred from bulk seawater measurements. Our results supported this as the *dddP* gene which was responsible for DMS production was most abundant in the bacterial community at Semporna (33% of all isolates; Figure 5). The *Alcaligenes faecalis*-related isolate was obtained from Semporna (Figure 4) and showed positive results for both *dmdA* and *dddP* genes. Its colonies displayed yellow pigmentation indicative of its potential to produce DMS.

Oceanimonas sp. is one of the earliest Gammaproteobacteria to have been studied biochemically for multiple DMSP-degrading genes including dddP (Yoch 2002) and isolates related to them were isolated from Kuching and Semporna (Figures 2 & 4). Studies have indicated that *Oceanimonas* sp. have multiple DMSP-degrading genes, allowing them to play a role in the sulphur cycle (Curson et al. 2011b). The availability of different *ddd* genes in *Oceanimonas* sp. implies that DMSP may be a key substrate for this bacteria genus, enabling them to produce DMS from DMSP (Ledyard et al. 1993). They also have a cytoplasmic DMSP lyase (de Souza & Yoch 1995; Yoch et al. 1997) resembling the periplasmic *dddY* of *Alcaligenes faecalis* (de Souza et al. 1996). Our results showed an interesting feature with *Oceanimonas* isolates from Kota Kinabalu and Semporna possessing *dmdA* genes (Figures 7 & 8).

Based on our preliminary observations, we believe that many of our isolates have the ability to undergo both DMSP-degradation processes depending on current environmental conditions. Considering the observed conditions of the sampling sites, our data supports the hypothesis of a 'bacterial switch'. Further studies which include DMS and DMSP measurements as well as a further look into other factors controlling bacterial activity (e.g. UV radiation and dissolved organic matter) (Kirchmann 2000) in these areas are recommended to better understand the role of these bacterial communities in DMSP cycling in the area.

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APPENDIX

16S rRNA gene sequence analysis of bacterial cultures based on BLAST analysis

Sequence	GenBank accession number	Closest match	Identities	Phylogenetic division
1911-S1-01-1.2.1	KF373319	*Vibrio orientalis* strain JC97, isolate Pkl-17 [FR837599]	465/468 (99%)	Gammaproteobacteria; Vibrionales; Vibrionaceae; Vibrio
1911-S1-01-1.2.2	KF373320	*Rhodobacter capsulatus* strain PSB-06 [FJ866784]	434/440 (99%)	Alphaproteobacteria; Rhodobacterales; Rhodobacteraceae; Rhodobacter
1911-S1-01-2	KF373321	*Rhodobacter capsulatus* strain PSB-06 [FJ866784]	440/455 (97%)	Alphaproteobacteria; Rhodobacterales; Rhodobacteraceae; Rhodobacter
1911-S1-01-3	KF373322	*Rhodobacter capsulatus* strain PSB-06 [FJ866784]	433/440 (98%)	Alphaproteobacteria; Rhodobacterales; Rhodobacteraceae; Rhodobacter
1911-S1-05-2	KF373323	*Pseudomonas oleovorans* strain HNS030 [JN128264]	456/457 (99%)	Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; Pseudomonas; Pseudomonas
1911-S1-07-1	KF373324	*Pseudomonas oleovorans* strain HNS030 [JN128264]	459/460 (99%)	Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; Pseudomonas; Pseudomonas
1911-S2-01-1	KF373325	*Vibrio alginolyticus* isolate Va150 [EU155497]	476/478 (99%)	Gammaproteobacteria; Vibrionales; Vibrionaceae; Vibrio
1911-S2-05-1	KF373326	*Vibrio alginolyticus* strain HZBC71 [JN188402]	471/474 (99%)	Gammaproteobacteria; Vibrionales; Vibrionaceae; Vibrio
1911-S2-07-1	KF373327	*Vibrio alginolyticus* strain HZBC71 [JN188402]	473/475 (99%)	Gammaproteobacteria; Vibrionales; Vibrionaceae; Vibrio
1911-S2-07-2	KF373328	*Vibrio parahaemolyticus* isolate Vp481 [EU155540]	471/473 (99%)	Gammaproteobacteria; Vibrionales; Vibrionaceae; Vibrio
1911-S3-01-1.1.1	KF373329	*Pseudoalteromonas ganghwensis* [DQ011614]	464/465 (99%)	Gammaproteobacteria; Alteromonadales; Pseudoalteromonadaceae; Pseudoalteromonas
1911-S3-01-1.1.2	KF373330	*Vibrio parahaemolyticus* strain VPMP55 [JQ663925]	319/402 (79%)	Gammaproteobacteria; Vibrionales; Vibrionaceae; Vibrio
1911-S3-01-1.2	KF373331	*Vibrio alginolyticus* strain P61224 [AJ704375]	474/475 (99%)	Gammaproteobacteria; Vibrionales; Vibrionaceae; Vibrio
1911-S3-01-2	KF373332	*Vibrio diabolicus* strain KM30-12-3 [JQ670740]	475/478 (99%)	Gammaproteobacteria; Vibrionales; Vibrionaceae; Vibrio
1911-S3-05-1	KF373333	*Vibrio parahaemolyticus* strain 93A-5807 [DQ497398]	474/476 (99%)	Gammaproteobacteria; Vibrionales; Vibrionaceae; Vibrio
1911-S3-05-2	KF373334	*Vibrio parahaemolyticus* strain 93A-5807 [DQ497398]	470/473 (99%)	Gammaproteobacteria; Vibrionales; Vibrionaceae; Vibrio
1911-S3-10-1.1	KF373335	*Vibrio harveyi* strain IS01 [GU974342]	473/474 (99%)	Gammaproteobacteria; Vibrionales; Vibrionaceae; Vibrio
1911-S3-10-1.2	KF373336	*Vibrio campbellii* strain CAIM 886 [HM584033]	473/475 (99%)	Gammaproteobacteria; Vibrionales; Vibrionaceae; Vibrio
1911-S3-10-2.1	KF373337	*Vibrio rotiferianus* strain BV1 [JN391272]	475/478 (99%)	Gammaproteobacteria; Vibrionales; Vibrionaceae; Vibrio
1911-S4-01-1	KF373338	*Pseudoalteromonas ganghwensis* [DQ011614]	462/463 (99%)	Gammaproteobacteria; Alteromonadales; Pseudoalteromonadaceae; Pseudoalteromonas
1911-S4-01-1.1	KF373339	*Vibrio alginolyticus* strain H050815-1 [EF219054]	474/475 (99%)	Gammaproteobacteria; Vibrionales; Vibrionaceae; Vibrio
1911-S4-01-2.2	KF373340	*Thalassospira xiamenensis* strain PTG4-18 [EU603449]	411/416 (99%)	Alphaproteobacteria; Rhodospirillales; Rhodospirillaceae; Thalassospira
1911-S4-05-1.1	KF373341	*Citrobacter freundii* strain AIMST Ehe5 [JQ312038]	461/462 (99%)	Gammaproteobacteria; Enterobacteriales; Enterobacteriaceae; Citrobacter
1911-S4-05-1.2	KF373342	*Leclercia adecarboxylata* strain AIMST Ehe6 [JQ312039]	461/462 (99%)	Gammaproteobacteria; Enterobacteriales; Enterobacteriaceae; Leclercia
1911-S4-05-2	KF373343	*Vibrio azureus* strain 41113 [HM032787]	452/468 (97%)	Gammaproteobacteria; Vibrionales; Vibrionaceae; Vibrio

Sequence	GenBank accession number	Closest match	Identities	Phylogenetic division
1911-S4-10-2.1	KF373344	*Vibrio alginolyticus* strain H050815-1 [EF219054]	472/473 (99%)	Gammaproteobacteria; Vibrionales; Vibrionaceae; Vibrio
1911-S5-01-1	KF373345	*Vibrio natriegens* strain AUCASVE1 [JQ043186]	471/472 (99%)	Gammaproteobacteria; Vibrionales; Vibrionaceae; Vibrio
1911-S5-01-2.1	KF373346	*Vibrio natriegens* strain AUCASVE1 [JQ043186]	474/475 (99%)	Gammaproteobacteria; Vibrionales; Vibrionaceae; Vibrio
1911-S5-01-2.2	KF373347	*Vibrio natriegens* strain AUCASVE1 [JQ043186]	472/473 (99%)	Gammaproteobacteria; Vibrionales; Vibrionaceae; Vibrio
1911-S5-05-1.1.2	KF373348	*Citrobacter freundii* strain AIMST Ehe5 [JQ312038]	462/463 (99%)	Gammaproteobacteria; Enterobacteriales; Enterobacteriaceae; Citrobacter
1911-S5-05-1.2	KF373349	*Vibrio natriegens* strain AUCASVE1 [JQ043186]	472/473 (99%)	Gammaproteobacteria; Vibrionales; Vibrionaceae; Vibrio
1911-S5-05-1.2.1	KF373350	*Vibrio azureus* strain F77118 [HQ908716]	473/473 (100%)	Gammaproteobacteria; Vibrionales; Vibrionaceae; Vibrio
1911-S5-05-2	KF373351	*Vibrio parahaemolyticus* strain 448 [JN188417]	474/475 (99%)	Gammaproteobacteria; Vibrionales; Vibrionaceae; Vibrio
1911-S5-05-3	KF373352	*Vibrio natriegens* strain AUCASVE1 [JQ043186]	471/472 (99%)	Gammaproteobacteria; Vibrionales; Vibrionaceae; Vibrio
1911-S5-10-1	KF373353	*Vibrio azureus* strain 41113 [HM032787]	471/474 (99%)	Gammaproteobacteria; Vibrionales; Vibrionaceae; Vibrio
1911-S5-10-2	KF373354	*Vibrio splendidus* strain AP625 [GQ254509]	469/471 (99%)	Gammaproteobacteria; Vibrionales; Vibrionaceae; Vibrio
2311-S1-01-1.1	KF373355	*Pseudomonas oleovorans* strain HNS030 [JN128264]	452/453 (99%)	Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; Pseudomonas; Pseudomonas
2311-S1-01-1.2	KF373356	*Shewanella haliotis* strain MS41 [FN997635]	461/461 (100%)	Gammaproteobacteria; Alteromonadales; Shewanellaceae; Shewanella
2311-S1-01-2.1	KF373357	*Shewanella haliotis* strain MS41 [FN997635]	469/469 (100%)	Gammaproteobacteria; Alteromonadales; Shewanellaceae; Shewanella
2311-S1-01-2.2	KF373358	*Shewanella haliotis* strain MS41 [FN997635]	467/467 (100%)	Gammaproteobacteria; Alteromonadales; Shewanellaceae; Shewanella
2311-S1-01-3.1	KF373359	*Shewanella haliotis* strain MS41 [FN997635]	466/466 (100%)	Gammaproteobacteria; Alteromonadales; Shewanellaceae; Shewanella
2311-S1-05-1	KF373360	*Exiguobacterium aurantiacum* var. Colo. Road [AY047481]	485/485 (100%)	Firmicutes; Bacilli; Bacillales; Bacillales Family XII. Incertae Sedis; Exiguobacterium
2311-S1-05-2	KF373361	*Oceanimonas smirnovii* strain 31-13 [NR_042963]	442/461 (96%)	Gammaproteobacteria; Aeromonadales; Aeromonadaceae; Oceanimonas
2311-S1-10-1	KF373362	*Vibrio rotiferianus* strain 5S [JF792070]	466/470 (99%)	Gammaproteobacteria; Vibrionales; Vibrionaceae; Vibrio
2311-S2-01-1	KF373363	*Brevibacillus laterosporus* strain GZUB11 [FJ434663]	472/472 (100%)	Firmicutes; Bacilli; Bacillales; Paenibacillaceae; Brevibacillus
2311-S2-10-1	KF373364	*Vibrio splendidus* strain AP625 [GQ254509]	414/453 (91%)	Gammaproteobacteria; Vibrionales; Vibrionaceae; Vibrio
2311-S3-01-1.1	KF373365	*Bacillus sphaericus* clone 7-16 [DQ364585]	431/456 (95%)	Firmicutes; Bacilli; Bacillales; Bacillaceae; Lysinibacillus
2311-S3-01-1.2	KF373366	*Shewanella putrefaciens* strain R1418 [AB208055]	455/461 (99%)	Gammaproteobacteria; Alteromonadales; Shewanellaceae; Shewanella
2311-S3-01-2	KF373367	*Shewanella putrefaciens* strain R1418 [AB208055]	459/462 (99%)	Gammaproteobacteria; Alteromonadales; Shewanellaceae; Shewanella
2311-S3-01-3	KF373368	*Vibrio vulnificus* strain W045 [EF114147]	473/473 (100%)	Gammaproteobacteria; Vibrionales; Vibrionaceae; Vibrio
2311-S3-05-1	KF373369	*Enterobacter ludwigii* strain KW 93 [JX262395]	463/463 (100%)	Gammaproteobacteria; Enterobacteriales; Enterobacteriaceae; Enterobacter
2311-S3-05-2.1	KF373370	*Pseudomonas plecoglossicida* strain AIMST Aie20 [JQ312025]	459/459 (100%)	Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; Pseudomonas

Sequence	GenBank accession number	Closest match	Identities	Phylogenetic division
2311-S3-10-1	KF373371	*Thalassospira sp*. SKUK MB1005 [EU907920]	417/417 (100%)	Alphaproteobacteria; Rhodospirillales; Rhodospirillaceae; Thalassospira
2311-S3-10-2.1	KF373372	*Bacillus malacitensis* strain TP12 [FJ887890]	404/408 (99%)	Firmicutes; Bacilli; Bacillales; Bacillaceae; Bacillus
2311-S3-10-2.2	KF373373	*Vibrio natriegens* strain AUCASVE5 [JQ277719]	471/472 (99%)	Gammaproteobacteria; Vibrionales; Vibrionaceae; Vibrio
2311-S4-01-1	KF373374	*Providencia sp*. Sam130-9A [FJ418577]	456/460 (99%)	Gammaproteobacteria; Enterobacteriales; Enterobacteriaceae; Providencia
2311-S4-05-1	KF373375	*Nitratireductor basaltis* strain J3 [NR_044414]	409/409 (100%)	Alphaproteobacteria; Rhizobiales; Phyllobacteriaceae; Nitratireductor
2311-S4-10-1	KF373376	*Oceanimonas smirnovii* strain 31-13 [NR_042963]	463/468 (99%)	Gammaproteobacteria; Aeromonadales; Aeromonadaceae; Oceanimonas
2311-S4-10-2.1.1	KF373377	*Oceanimonas smirnovii* strain 31-13 [NR_042963]	463/468 (99%)	Gammaproteobacteria; Aeromonadales; Aeromonadaceae; Oceanimonas
2311-S4-10-2.1.3	KF373378	*Lysinibacillus fusiformis* strain R3 [JQ991002]	476/476 (100%)	Firmicutes; Bacilli; Bacillales; Bacillaceae; Lysinibacillus
2311-S4-10-2.2	KF373379	*Exiguobacterium aurantiacum* var. Colo. Road [AY047481]	489/490 (99%)	Firmicutes; Bacilli; Bacillales; Bacillales Family XII. Incertae Sedis; Exiguobacterium
2311-S4-10-2.3	KF373380	*Oceanimonas smirnovii* strain 31-13 [NR_042963]	460/465 (99%)	Gammaproteobacteria; Aeromonadales; Aeromonadaceae; Oceanimonas
2311-S4-18-1.1	KF373381	*Vibrio vulnificus* strain W045 [EF114147]	475/475 (100%)	Gammaproteobacteria; Vibrionales; Vibrionaceae; Vibrio
2311-S4-18-1.2	KF373382	*Oceanimonas smirnovii* strain 31-13 [NR_042963]	436/447 (98%)	Gammaproteobacteria; Aeromonadales; Aeromonadaceae; Oceanimonas
2311-S5-01-1.2	KF373383	*Pseudoalteromonas lipolytica* strain ZR064 [JX173567]	464/465 (99%)	Gammaproteobacteria; Alteromonadales; Pseudoalteromonadaceae; Pseudoalteromonas
2311-S5-01-2.1	KF373384	*Pseudoalteromonas lipolytica* strain ZR064 [JX173567]	463/463 (100%)	Gammaproteobacteria; Alteromonadales; Pseudoalteromonadaceae; Pseudoalteromonas
2311-S5-01-2.2	KF373385	*Pseudomonas stutzeri* strain UP-1 [AY364327]	453/454 (99%)	Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; Pseudomonas
2311-S5-01-2.3	KF373386	*Pseudomonas stutzeri* strain UP-1 [AY364327]	458/459 (99%)	Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; Pseudomonas
2311-S5-01-3.1.1	KF373387	*Brevundimonas diminuta* strain c138 [FJ950570]	405/406 (99%)	Alphaproteobacteria; Caulobacterales; Caulobacteraceae; Brevundimonas
2311-S5-01-3.1.2	KF373388	*Exiguobacterium arabatum* [JF758868]	438/479 (91%)	Firmicutes; Bacilli; Bacillales; Bacillales Family XII. Incertae Sedis; Exiguobacterium
2311-S5-01-3.2	KF373389	*Brevundimonas diminuta* strain KSC_ AK3a [EF191247]	407/407 (100%)	Alphaproteobacteria; Caulobacterales; Caulobacteraceae; Brevundimonas
2311-S5-01B-1	KF373390	*Vibrio natriegens* strain AUCASVE5 [JQ277719]	472/472 (100%)	Gammaproteobacteria; Vibrionales; Vibrionaceae; Vibrio
2311-S5-05-1	KF373391	*Vibrio splendidus* strain AP625 [GQ254509]	472/473 (99%)	Gammaproteobacteria; Vibrionales; Vibrionaceae; Vibrio
2311-S5-05-2	KF373392	*Vibrio splendidus* strain AP625 [GQ254509]	470/472 (99%)	Gammaproteobacteria; Vibrionales; Vibrionaceae; Vibrio
2611-S1-01-1.1	KF373393	*Alcaligenes faecalis* strain OCEN2DBT [JF264463]	410/465 (88%)	Betaproteobacteria; Burkholderiales; Alcaligenaceae; Alcaligenes
2611-S1-01-1.2	KF373394	*Vibrio communis* strain F75216 [HQ161743]	472/472 (100%)	Gammaproteobacteria; Vibrionales; Vibrionaceae; Vibrio
2611-S1-05-1.1	KF373395	*Exiguobacterium lactigenes* strain: HYS0503-MK66 [AB259161]	483/483 (100%)	Firmicutes; Bacilli; Bacillales; Bacillales Family XII. Incertae Sedis; Exiguobacterium
2611-S1-05-1.2	KF373396	*Oceanimonas smirnovii* strain 31-13 [NR_042963]	463/468 (99%)	Gammaproteobacteria; Aeromonadales; Aeromonadaceae; Oceanimonas
2611-S5-01-1	KF373421	*Pseudidiomarina sediminum* strain c121 [NR_044176]	423/463 (91%)	Gammaproteobacteria; Alteromonadales; Idiomarinaceae; Idiomarina

Sequence	GenBank accession number	Closest match	Identities	Phylogenetic division
2611-S5-05A-1	KF373422	*Pseudomonas pseudoalcaligenes* strain K29411 [DQ298030]	437/438 (99%)	Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; Pseudomonas; Pseudomonas
2611-S5-05B-1.1	KF373423	*Pseudoalteromonas sp*. S187 [FJ457123]	465/466 (99%)	Gammaproteobacteria; Alteromonadales; Pseudoalteromonadaceae; Pseudoalteromonas
2611-S5-05B-1.2	KF373424	*Photobacterium sp*. MM14 [JN791371]	473/473 (100%)	Gammaproteobacteria; Vibrionales; Vibrionaceae; Photobacterium
2611-S5-05B-3.2.1	KF373425	*Shewanella sp*. UMS11/10 [JQ231163]	460/465 (99%)	Gammaproteobacteria; Alteromonadales; Shewanellaceae; Shewanella
2611-S5-05B-3.2.2	KF373426	*Shewanella sp*. UMS11/10 [JQ231163]	464/465 (99%)	Gammaproteobacteria; Alteromonadales; Shewanellaceae; Shewanella
2611-S5-05C-2	KF373427	*Photobacterium sp*. MM14 [JN791371]	477/477 (100%)	Gammaproteobacteria; Vibrionales; Vibrionaceae; Photobacterium
2611-S5-10-2	KF373428	*Nitratireductor aquimarinus* CL-SC21 [HQ176467]	404/406 (99%)	Alphaproteobacteria; Rhizobiales; Phyllobacteriaceae; Nitratireductor
2611-S6-01-1	KF373429	*Pseudomonas pseudoalcaligenes* strain K29411 [DQ298030]	347/410 (85%)	Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; Pseudomonas; Pseudomonas
2611-S6-01-1.1	KF373430	*Pseudomonas pseudoalcaligenes* strain K29411 [DQ298030]	450/450 (100%)	Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; Pseudomonas; Pseudomonas
2611-S6-01-1.2	KF373431	*Vibrio campbellii* strain CAIM 886 [HM584033]	463/467 (99%)	Gammaproteobacteria; Vibrionales; Vibrionaceae; Vibrio
2611-S6-01-3	KF373432	*Vibrio alginolyticus* strain 486 [JN188409]	475/475 (100%)	Gammaproteobacteria; Vibrionales; Vibrionaceae; Vibrio
2611-S6-05-1.1	KF373433	*Oceanimonas smirnovii* strain 31-13 [NR_042963]	445/449 (99%)	Gammaproteobacteria; Aeromonadales; Aeromonadaceae; Oceanimonas
2611-S6-05-2	KF373434	*Rhodobacter capsulatus* strain PSB-06 [FJ866784]	468/468 (100%)	Alphaproteobacteria; Rhodobacterales; Rhodobacteraceae; Rhodobacter
2611-S6-09-1	KF373435	*Vibrio parahaemolyticus* strain 448 [JN188417]	474/475 (99%)	Gammaproteobacteria; Vibrionales; Vibrionaceae; Vibrio
2611-S6-09-2	KF373436	*Vibrio parahaemolyticus* strain 448 [JN188417]	471/472 (99%)	Gammaproteobacteria; Vibrionales; Vibrionaceae; Vibrio
2611-S7-01-1	KF373437	*Vibrio parahaemolyticus* strain 448 [JN188417]	475/476 (99%)	Gammaproteobacteria; Vibrionales; Vibrionaceae; Vibrio
2611-S7-01-2	KF373438	*Vibrio parahaemolyticus* strain S9-891-B0919354-5-8F [KC520577]	475/475 (100%)	Gammaproteobacteria; Vibrionales; Vibrionaceae; Vibrio
2611-S8-01-1.1	KF373439	*Vibrio alginolyticus* strain 486 [JN188409]	471/472 (99%)	Gammaproteobacteria; Vibrionales; Vibrionaceae; Vibrio
2611-S8-01-3	KF373440	*Vibrio communis* strain F75216 [HQ161743]	474/474 (100%)	Gammaproteobacteria; Vibrionales; Vibrionaceae; Vibrio