

Hydrocarbon pollutants shape bacterial community assembly of harbor sediments

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Abstract

Petroleum pollution results in co-contamination by different classes of molecules, entailing the occurrence of marine sediments difficult to remediate, as in the case of the Ancona harbor (Mediterranean Sea, Italy). Autochthonous bioaugmentation (ABA), by exploiting the indigenous microbes of the environment to be treated, could represent a successful bioremediation strategy. In this perspective we aimed to i) identify the main drivers of the bacterial communities' richness in the sediments, ii) establish enrichment cultures with different hydrocarbon pollutants evaluating their effects on the bacterial communities' composition, and iii) obtain a collection of hydrocarbon degrading bacteria potentially exploitable in ABA. The correlation between the selection of different specialized bacterial populations and the type of pollutants was demonstrated by culture-independent analyses, and by establishing a collection of bacteria with different hydrocarbon degradation traits. Our observations indicate that pollution dictates the diversity of sediment bacterial communities and shapes the ABA potential in harbor sediments.

KEYWORDS

Oil contamination; autochthonous bioaugmentation; pyrosequencing; bacterial community; *Alcanivorax*

1. INTRODUCTION

The Mediterranean Sea is one of the most congested basins in terms of oil tanker traffic, hosting about 20% of the global traffic. Furthermore the coastlines are densely inhabited and, especially in the northern side, highly industrialized. As a consequence, Mediterranean coasts and harbors, especially the industrial ones, are constantly exposed to crude oil hydrocarbons (HC) deriving mainly from oil tankers and wastes of refineries, chemical industries and oil pipelines (Daffonchio et al., 2013, 2012). The thousands of different oil molecules have a wide range of physico-chemical properties and toxicity. The individual molecules within the four main groups of oil HC, saturated HC, aromatic HC, resins and asphaltenes, are also classified according to their molecular weight into light fractions (low molecular weight) and heavy fractions (high molecular weight). The latter includes the most recalcitrant compounds to degradation that tend to accumulate in the sediments of harbors and coastal areas nearby oil-related facilities.

The Ancona harbor (Italy) is located in the Adriatic Sea and is one of the largest Mediterranean commercial harbors, with intense ferry and merchant fluxes, and it is surrounded by shipyards and important industrial plants. High concentration of pollutant chemicals, including aliphatic HC, polycyclic aromatic HC and naphthalene have been measured in its sediments, along with high concentration of different heavy metals like Cu, Mn, Ni and Zn (Dell'Anno et al., 2009; Mei and Danovaro, 2004; Mirto and Danovaro, 2004)

Bioaugmentation is the addition of degrading bacteria to a polluted environment and it is recognized as a potential strategy for supporting the clean-up of polluted sediments (Head et al., 2006). We have recently shown that the origin of the added microorganisms is essential to predict the success of a sediment treatment through bioaugmentation and that allochthonous microorganisms present several limitations due to the adaptation to the local environmental conditions (Fodelianakis et al., 2015). In this perspective, a new approach for bioaugmentation, called autochthonous bioaugmentation (ABA), has been proposed (Hosokawa et al., 2009) and recognized as the best strategy for remediate marine oil polluted samples (Nikolopoulou et al., 2013). ABA is a site-tailored strategy based on the use of autochthonous microbial populations,

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previously enriched under laboratory conditions and re-inoculated in the polluted site to be treated, taking
advantage of their natural ability to cope with the unique environmental setting where they come from. To
set-up successful ABA strategy, it is pivotal to gain knowledge of the indigenous microbial populations, of
the occurring environmental parameters and their influence on survival and degradation rate of the selected
microorganisms.

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The present study aimed to assess i) the driving factors shaping the bacterial communities inhabiting the
Ancona harbor's sediments through culture-dependent and independent approaches and ii) the effect of
different HC pollutants on the bacterial community richness and diversity.

21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 2. MATERIALS and METHODS

2.1 - Sites description and sampling and sediment physico-chemical characterization

Sediment samples were collected from ten polluted stations in the harbor (codes: U1 - U10) and five control
pristine stations (codes: C1 - C5) 3.5 km far away from the harbor, along the dominant NW/SE current
(Table 1, Figure 1a). Sampling depths ranged from 2.5 m in the harbor area to 13 m in the control stations.

For each sample, pH and redox potential were measured. Samples for granulometric analyses (percentages of
gravel, sand and clay in the sediment), humidity and specific weight were transferred into plastic containers
and stored at +4°C. For the analyses of trace metals, HC and total polycyclic aromatic hydrocarbons (PAHs),
samples were transferred into inert HDPE containers and stored at -18°C until laboratory analyses. Samples
for the determination of total organic carbon (TOC) and molecular ecology analyses were transferred,
respectively, into Petri dishes and sterile plastic containers, and stored at -20°C. For the determination of
total bacteria, sediment slurries were prepared using sterile buffered formaldehyde (4% final concentration),
while for bacteria isolation sediment sub-samples were collected using a sterile spoon and stored at +4°C.

TOC was determined by weight loss on combustion technique after removal of carbonate with dilute (IN)
HCl. A portion of sediments was weighed into a porcelain crucible and ignited in a muffle furnace at 550°C
for two hours. The crucible was cooled in a desiccator, re-weighed and the TOC was calculated as the weight
loss in percentage (Gaudette E., Flight R., Toner L., 1974). The presence and abundance of different HC -

1 C>12 and PAHs- was estimated by chromatographic techniques. The HC concentration was analyzed by
2 Agilent 7890 -USA. A HP-5 capillary chromatographic column (30 m x 0.32 mm I.D.) and a capillary
3 column (30 m x 0.25 mm I.D.) were used for GC-FID and GC-MS analyses, respectively. Nitrogen was the
4 carrier gas with 3 ml/min. Injector and detector temperatures were maintained at 300 °C and 320 °C,
5 respectively. The identification of n-paraffin peaks was established using a reference mixture of n-paraffin of
6 known composition.
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12 To determine the total content of heavy metals (Cu, Zn, Cr, Ni, Cd, Pb, Hg, V and As) in sediments, samples
13 (0.1 g) were digested with HNO₃/HClO₄ (4:1, v/v) in a microwave oven (CEM, MARS5). After digestion,
14 the volume of each sample was adjusted to 20 mL using deionized water. Heavy metals and As content was
15 determined by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS, Agilent Technologies, Santa
16 Clara, CA, USA). Standards of heavy metals and of As for concentrations ranging from 0 to 1 mg/L were
17 prepared from multi-element calibration standard-2A solution (Agilent Technologies) and from sodium
18 arsenite solution (NaAsO₂) (Sigma-Aldrich, St Louis, MO, USA) respectively. For all the measures by ICP-
19 MS an aliquot of a 2 mg/L of an internal standard solution (45Sc, 89Y, 159Tb, Agilent Technologies) was
20 added both to samples and a calibration curve to give a final concentration of 20 µg/L. The instrument was
21 tuned daily with a multi-element tuning solution for optimized signal-to-noise ratio.
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38 **2.2 - Total prokaryote and hydrocarbonoclastic bacteria abundance**

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42 Total prokaryotic abundance was determined on sediment collected in the surface layer (0-1 cm). The
43 samples were suspended in pyrophosphate solution (final concentration 5 mM) and sonicated (60 W, one
44 minute for three consecutive times, with 30-seconds intervals). The supernatant was then sub-sampled and
45 filtered into sterile 0.2 µm pore-size filters. Filters were then stained with SYBR Green I and cells counted
46 by means of Epifluorescence Microscope (EFM) at 1000X magnification.
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51 The number of HC-degrading bacteria in the U5 and U7 sites was evaluated through the Most Probable
52 Number (MPN) method, modifying the previously described method (Cappello et al., 2006). Serial dilutions
53 of the sediments were performed and inoculated 1:10 in triplicates in ONR7a mineral medium (Dyksterhouse
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1 et al., 1995) added with crude oil (1% v/v) as unique carbon source and cycloheximide (0.01% w/v) to
2 inhibit eukaryotic growth. Bacterial growth was evaluated after 28 days static incubation at 25 °C.
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6 **2.3 - Bacterial ARISA fingerprinting and community 16S rRNA gene profiling and sequencing**

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10 Total DNA was extracted from 0.5 g of sediment using the “Power Soil” kit (MoBio Laboratories Inc.,
11 Carlsbad, CA, USA) following the manufacturer’s instructions. DNA was quantified using a NanoDrop 1000
12 spectrophotometer (Thermo Scientific, Waltham, MA, USA).
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16 ARISA-PCR was conducted on 20 ng of DNA template on each sample by using the primer set ITSf, 5’-
17 GTC GTA ACA AGG TAG GCC GTA-3’ and ITSr, 5’-GCC AAG GCA TCC ACC 3’, as previously
18 described (Cardinale et al., 2004). ARISA fragments were separated by using the ABI3730XL genetic
19 analyzer applying the internal standard 1200-LIZ. The output peak matrix was transferred to Microsoft Excel
20 for the following analysis. Peaks showing height value < 50 were removed from the output peak matrix
21 before statistical analyses. Each polymorphic ARISA peak is defined as a different OTU. Richness was
22 defined as the number of OTUs present in each sample.
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32 Barcoded pyrosequencing assays on the 16S rRNA gene were carried out using bacterial universal primers
33 (27 F mod 5’ – AGRGTTTGATCMTGGCTCAG – 3’; 519 R mod bio 5’ – GTNTTACNGCGGCKGCTG –
34 3’) targeting the variable regions of 16S rRNA V1-V3 and amplifying a fragment of approximately 400 bp.
35 The amplified 16S rRNA regions contain enough nucleotide variability to be useful in identification of
36 bacterial species (Chakravorty et al., 2007; Van de Peer et al., 1996). PCR reactions and next generation
37 pyrosequencing were performed by the company MR DNA (Shallowater, TX – U.S.) as previously described
38 (Bargiela et al., 2015a). A total of 66365 raw, barcoded amplicons of the V1–V3 region of the 16S rRNA
39 gene, were obtained. The reads were trimmed to remove pyrosequencing adaptors, low quality base calls
40 (<30 Phred score) and size-selected (between 350 and 500 bp) using the QIIME (Caporaso et al., 2010)
41 pipeline filtering scripts. High quality sequence reads that were not flagged as chimeras after screening with
42 Chimeras layer were clustered into operational taxonomic units (OTUs), based on a sequence identity
43 threshold of 97%, using Uclust (Edgar, 2010); drawing one sequence for each OTU, as representative, and
44 then aligned to Greengenes (<http://greengenes.lbl.gov/>) using PyNast (Caporaso et al., 2010). Sequences
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1 representative of each OTU were taxonomically classified by BLASTn-based comparisons to the
2 Greengenes and Silva databases within QIIME.

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4 Bacterial 16S rRNA gene fragments (~550 bp) were PCR amplified using primers 907R (3'-
5 CCGTCAATTCCTTTGAGTTT- 5') and GC-357F (3'-CCTACGGGAGGCAGCAG- 5' with a 5'-end GC-
6 clamp) targeting a portion of the 16S rRNA gene that includes the hypervariable V3-V5 regions (Muyzer et
7 al., 1993). PCR reactions were performed as previously described (Mapelli et al., 2013a). PCR amplification
8 of *alkB* gene, encoding for the alkane monooxygenase, was performed on the total DNA extracted from
9 Ancona harbor enrichments (U5-CO, U5-DB, U5-DS, U7-CO, U7-DB, U7-DS) and from U5 and U7
10 sediments as previously reported (Amer et al., 2015).

11 For Denaturing Gradient Gel Electrophoresis (DGGE), PCR products (~100 ng) were loaded in a 0.7 mm
12 polyacrylamide gel (7% w/v acrylamide-bisacrylamide, 37.5:1) containing 38% to 63% and 40% to 70%
13 urea-formamide denaturing gradient (100% corresponds to 7M urea and 40% v/v formamide), respectively
14 for 16S rRNA and *alkB* genes. The gels were run for 17 h at 60 °C by applying a constant voltage of 90 V in
15 1X Tris-acetate-EDTA (TAE) buffer. After electrophoresis, the gels were stained for 15 min in 1X TAE
16 buffer containing 1X SYBR Green (Molecular Probes, Leiden, the Netherlands) according to manufacturer's
17 instructions and rinsed three times for 10 min with distilled water. Gels images were captured using a Gel
18 Doc 2000 apparatus (Bio-Rad, Milan, Italy). The band patterns of the DGGE gel were analyzed using Image
19 J software and Microsoft Excel XLSTAT software (Addinsoft Inc., New York, NY, USA) to perform a
20 cluster analysis as previously described (Marasco et al., 2012). DGGE bands were excised from the gels of
21 *alkB* gene with a sterile scalpel and eluted in 50 µl of sterile Milli-Q water at 37 °C for 3 h. The eluted DNA
22 was amplified by PCR using primers DalkF and DalkR and positive amplifications were sequenced by
23 MacroGen Inc., South Korea.

24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 **2.4 - Microcosm Enrichments**

52 Different enrichment cultures were established using polluted sediments as inocula and different HC
53 molecules as the sole carbon sources. One g of sediment collected either from sampling site U5 or U7 was
54 inoculated in minimal mineral media ONR7a supplemented by 1% v/v crude oil (CO), 1% v/v diesel oil
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1 (DB) or 1% w/v naphthalene (N). Cycloheximide (0.01% w/v) was added to all the microcosms to avoid
2 eukaryote growth. The microcosms were incubated at 30°C with constant shaking at 150 rpm until turbidity
3 appeared. In the case of diesel oil added microcosms, 1 mL of liquid culture collected at the interface
4 between the diesel oil layer and the growth media aqueous phase was re-inoculated in fresh ONR7a medium
5 aiming to enrich those bacteria adhering on the diesel droplets floating on the medium surface: this newly
6 established microcosm was defined as “diesel surface” (DS). Total DNA was extracted from 10 mL of each
7 enrichments from the cells retained on 0.2 µM pore size filters, accordingly to previously described methods
8 (Mapelli et al., 2013b).
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20 **2.5 - Bacteria isolation and identification and evaluation of metabolic and biotechnological traits**

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24 Bacterial isolates have been obtained in pure cultures exclusively from crude oil and diesel oil enrichments,
25 by plating on ONR7a solid medium ten-fold dilutions of the positive enrichments, supplementing by the
26 same HC source. After purification, the isolates were streaked with and without HC compounds and those
27 able to grow exclusively in presence of the HC were selected for further characterization.
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33 The bacteria collection was dereplicated through Internal Transcribed Spacer (ITS) PCR-fingerprinting. ITS-
34 fingerprinting was performed as previously described (Mapelli et al., 2013a) using the primers ITS-F (3’-
35 GTCGTAACAAGGTAGCCGTA-5) and ITS-R (3’-CTACGGCTAC CTTGTTACGA-5’) (Daffonchio et
36 al., 1998). One isolate for each ITS group, established basing on identical ITS-fingerprinting, was identified
37 by partial 16S rRNA sequencing and subsequent alignment of the sequence in NCBI database. The
38 amplification of the bacterial 16S rRNA gene was performed using the universal primer 27F (3’-AG
39 AGTTTGATCMTGGCTCAG-5’) and 1492R (3’-CTACGGCTACCTTGTTACGA-5’). The PCR
40 amplification conditions and thermal protocol were set up as previously described (Mapelli et al., 2013a)
41 providing a PCR amplicon of approximately 1400 bp that was partially sequenced by Macrogen Inc., South
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30°C, than the test was performed in 96 micro-wells plates. The wells were coated with 7 µL of crude oil and left to equilibrate for 24 h at room temperature. Twenty µL of samples were added in the center of the wells and after 1 min the drops were visually examined and compared with the positive controls (SDS 1%) and negative controls (deionized water and sterile growth media). Both the cells culture and the supernatant were tested.

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Emulsification test was performed modifying the procedure previously described (Batista et al., 2006; Das et al., 1998). Cells were grown in 20 mL of ONR7a medium added with 1% of crude oil as the sole carbon source, at 30°C and with constant shaking. After 1 week of incubation cells were pelleted (4500 rpm, 10 min, 4°C) and the supernatant was used to perform the test. Three mL of *p*-Xylene and the same volume of supernatant of the cell culture were vortexed together for 2 min and the solution was left to equilibrate at room temperature for 24 h. The height of the emulsion layer was than measured and the emulsification activity was calculated as follows: $EV_{24h} (\%) = (\text{height of the emulsion layer}/\text{total height}) \times 100$. To evaluate emulsification, the height of the emulsification layer obtained from each lysate was compared with positive (SDS 1%) and negative (sterile growth media) samples, while to evaluate the stability of the emulsion the emulsification activity value at 24 h was compared with the emulsification valued calculated after 48 h (EV_{48h}).

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The capacity of the isolates to form biofilm was determined by the crystal violet staining assay (Antoniani et al., 2010). Bacteria were grown for 72 hours in liquid ONR7a medium added with 1% Na-pyruvate, at 30°C in 96 wells, flat bottom, polystyrene microtiter plates. After the growth, the optical density 600 nm (OD_{600nm}) of the cell culture was measured. The liquid culture was then removed, and cells attached to the microtiter plates were washed gently with PBS and stained for 10 min with 0.03% crystal violet, thoroughly washed with PBS and water, and dried. For semiquantitative determination of biofilms, crystal violet stained cells were re-suspended in 0.2 ml of 95% ethanol by pipetting. The OD_{600nm} of crystal violet stained biofilm cells was determined and normalized to OD_{600nm} of cells from the corresponding liquid cultures; this value is defined as “adhesion units”. The isolates were considered biofilm producers if they showed an OD value of at least 0.12. If the OD exceeded 0.240, they were classified as strongly adherent. Strains displaying OD values greater than 0.12 but less than 0.240 were classified as weakly adherent (Amer et al., 2015).

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2 The uricolytic activity of the isolates was tested with a modification of a previously described protocol
3 (Morales-Jiménez et al., 2013). Cells were grown in ONR7a liquid medium added with 1% Na-pyruvate as
4 the sole carbon source. After 4 days of incubation at 30°C, 10 µL of cell cultures were spotted in triplicates
5 on ONR7a (pH6.5) agar plates added with 1% Na-pyruvate as carbon sources and 0.5% uric acid and
6 incubated at 30°C. Active strains determined the formation of a clear halo around the colony. The hydrolytic
7 activity was defined as the diameter of the colony plus the clear zone divided by the diameter of the colony.
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9 Catechol 2,3 dioxygenase (C2,3O) activity was determined modifying a previously described method
10 (Margesin et al., 2003). Ten µL of a solution containing 90 mM catechol in 50 mM Tris-acetate buffer (pH
11 7.5) was added over the cells grown on agar plates. The presence of C2,3O activity resulted in the formation
12 of a green-brownish color within 2 h at room temperature.
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24 **2.6 - Nucleotide sequence accession numbers**

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28 Nucleotide sequences were edited in Chromas Lite 2.01 (<http://www.technelysium.com.au>) and subjected to
29 BLAST search (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). The partial 16S rRNA gene sequences obtained from
30 the bacterial isolates are available at ENA under accession numbers LN872945 – LN872962. All original
31 non-chimeric 16S rRNA hypervariable tag 454 sequences are archived at the EBI European Read Archive
32 under accession number PRJEB10119.
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42 **2.7 - Statistical analyses**

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46 Significant differences in i) environmental chemical-physical parameters and ii) the bacterial community
47 richness between control and polluted sediments were investigated by permutational analysis of variance
48 (PERMANOVA) (Anderson, 2001).
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53 Non-metric multidimensional scaling (nMDS) was carried out as visual information to explore similarities
54 between bacteria communities richness evaluated through ARISA fingerprinting. The same set of biotic data
55 was used, together with the environmental parameters recorded at each site, in the distance-based
56 multivariate analysis for a linear model (DistLM) (Anderson, 2002) to determine which significant
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1 environmental variables explain the observed similarity among the samples. The Akaike Information
2 Criterion (AIC) was used to select the predictor variables. The contribution of each environmental variable
3 was assessed using ‘marginal tests’ to assess the statistical significance and percentage contribution of each
4 variable. A distance-based redundancy analysis (dbRDA) was used for graphical visualization of the DistLM
5 results. All the statistical tests were performed by PRIMER v. 6.1 (Clarke and Gorley, 2006),
6 PERMANOVA+ for PRIMER routines (Anderson et al., 2008).
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15 3. RESULTS

16 3.1- Physico-chemical analyses

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24 Minor differences of physical characteristics were found between the collected sediments (Table 1). The
25 granulometric analysis of the sediments revealed the dominance of the clay fraction (on average 55%) and
26 the reduced presence of gravel, while the pH values in the samples ranged between 7.47 and 7.85 –
27 accordingly to the typical pH value of coastal marine environments, which is comprised between 7.5 and 8.4
28 (Chester and Jickells, 2012). The redox potential of the samples ranged between 28 and 247.3 mV, showing
29 that sediments were oxygenated in the surface layers.
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33 All the sediments collected in the harbor area displayed high concentrations of heavy HC (C>12), ranging
34 from 65.3 to 316.5 mg/kg while in the offshore stations the heavy HCs concentration showed a lower
35 average value of 34.98 mg/kg (Table 2). The concentration of polycyclic aromatic hydrocarbons (PAHs)
36 measured in the sediments was high in seven of the ten sites analyzed inside the harbor area, showing the
37 highest concentration at stations U1 and U4 (Table 2). In addition to HC, we also investigated the
38 concentrations of heavy metals in the sediments, which tend to precipitate and to be absorbed by sediments
39 particles. Their concentration was higher than the Italian law limits for nickel, chromium, cadmium and lead
40 (Table 2). Cadmium concentration was high in all the stations located inside the harbor, differently from
41 what observed at the offshore stations. Sediments were highly concentrated of chromium in almost all the
42 sites independently from their location, displaying low values only in three of the offshore sites, while the
43 highest values were detected at stations U2 and U3 inside the harbor. Nickel was present at high
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1 concentration in all the sampling stations while a high concentration of lead was detected only at site U1
2 (Table 2). The statistical PERMANOVA analysis was applied on the physico-chemical matrix showing a
3 significant difference ($p=0.0089$) between the sediments collected inside the harbor and those collected
4 offshore, thus allowing to define the two sites as polluted and pristine stations, respectively.
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10 **3.2 Microbial community richness**

11 Prokaryote abundance in all the sampling sites was estimated through SYBR Green staining and ranged
12 between 4.04×10^8 (U7) and 1.13×10^9 (U5) cell/g d.s. in the sediments collected from the polluted area, while
13 it decreased slightly in the offshore control sites, where prokaryote cells ranged from 9.76×10^7 (C5) to
14 7.46×10^8 (C1) cell/g d.s (Table 1).
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24 The total bacterial communities of pristine and polluted sediments were investigated through ARISA
25 fingerprinting. ARISA profiles of the polluted sediments comprised between 38 and 78 OTUs (i.e. ARISA
26 peaks) in the sites U2 and U3 respectively, while those obtained from the offshore control sites showed a
27 higher numbers of OTUs ranging between 87 (C4) and 94 (C2). This result suggests the presence of a higher
28 number of different taxa in the pristine stations respect to the polluted ones. nMDS analysis based on the
29 ARISA fingerprints (Figure 1b) showed a clear separation between the bacterial communities inhabiting
30 polluted and control sites (stress value = 0.01). PERMANOVA test indicated that the richness of the
31 sediment-dwelling bacterial communities from the pristine and polluted areas of the Ancona harbor was
32 significantly different ($p = 0.0003$).
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44 A DistLM analysis was applied to identify the environmental variables influencing the bacterial community
45 richness in the polluted and control sites of the Ancona harbor. The results of DistLM showed that the
46 variation of community's richness according to ARISA profiles was significantly related to five
47 environmental variables (Figure 1c). Among the chemical components, cadmium ($p = 0.0001$), chromium (p
48 $= 0.0001$), copper ($p = 0.0453$), HCs C>12 ($p = 0.0055$) and TOC ($p = 0.0043$) were significant, while none
49 of the measured physical properties was significantly related to the richness values. The two axes of the
50 dbRDA explained 86.0% of the total variation (Figure 1c).
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3.3 – Microbial diversity in microcosm enrichments established with different pollutants as the sole carbon source

HC-degrading bacterial cultures were enriched from the polluted sediments collected in the sites U5 and U7, characterized respectively by the highest and the lowest value of prokaryote cell abundance (Table 1). The number of HC degrading bacteria inhabiting these sediments was evaluated through the MPN method and corresponded to 7.3×10^2 cell/g d.s. in site U5 and 4.3×10^3 cell/g d.s. in site U7.

The structure of the original sediment and the microcosm enriched bacterial communities was evaluated by applying two molecular techniques targeting 16S rRNA gene, PCR-DGGE fingerprinting and barcoded pyrosequencing. PCR-DGGE analysis showed the occurrence of different and more complex band patterns in the original sediments than in the established enrichments (Figure 2a). Overall, independently from the sediment origin, the PCR-DGGE profiles were more similar when diesel or crude oil were added in the medium, while naphthalene enrichment cultures selected more specific bacterial populations, as confirmed by PCR-DGGE cluster analysis (Figure 2a-b).

Rarefaction curves of pyrosequencing data showed that all the libraries reached saturation and covered the whole bacterial diversity within the samples (Figure S2). Both the sediment samples showed similar Shannon indexes (U5: $H' = 8.201$; U7: $H' = 8.363$), higher than those detected in the examined U5 enrichments with the different pollutants (U5-N: $H' = 4.717$; U5-CO: $H' = 4.589$; U5-DB: $H' = 3.704$). Principal coordinates analyses (PCoA), explaining 68.71% of the diversity observed (Figure 3a), showed the differential clustering of environmental and cultivated bacterial communities. Along PC1, explaining 43.52% of the diversity, it was possible to identify two groups, represented by the environmental samples U5 and U7 and diesel, crude oil and naphthalene microcosms, respectively. On the other side, along PC2, the naphthalene enrichment culture U5-N was well separated from the other enrichments. The bacterial community of site U5 was dominated by four different phyla (Proteobacteria, 52.82%, that covered more than half of the total diversity; Firmicutes, 15.98%; Bacteroidetes, 9.4% and Fusobacteria, 5.17%) while U7 was dominated only by three different phyla (Proteobacteria, 38.21%; Firmicutes, 31.9% and Bacteroidetes, 9.7%) (Figure 3c, Table S1).

Both sediments were dominated by Gammaproteobacteria and Clostridia classes (Figure 3d, Table S1), the

1 former representing 38% of the bacterial populations of sediment U5 and 23% of sediment U7, while
2 Clostridia represented respectively the 16% and 31% of the bacterial community.
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4 The established microcosms hosted a less diverse bacterial community (Figure 3, Table S1). Only four out of
5 the 20 different phyla identified in the U5 sediment were present in the enrichment U5-DB, three in U5-CO
6 and four in U5-N (Figure 3c). Diesel and crude oil microcosms were dominated by Proteobacteria (98%),
7 while the phylum Firmicutes was the most abundant (64%) in the naphthalene enrichment, followed by
8 Proteobacteria (34%). The low level of bacterial diversity occurring in the microcosms could be observed
9 also at finer phylogenetic scale (Figure 3d): Gammaproteobacteria dominated both diesel and crude oil
10 enrichments (98%), while naphthalene enrichment comprised mostly Bacilli (64.5%) and
11 Gammaproteobacteria (31%). The genera that dominated diesel and crude oil microcosms were *Alcanivorax*
12 and *Marinobacter*, which together represented about 70% of the bacterial diversity, while the naphthalene
13 enrichment was mainly dominated by *Bacillus* (55.6%) and *Halomonas* (15.5%) genera (Figure S1).
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15 PCR-DGGE profiles of the *alkB* gene in the sediments (U5 and U7) were more complex in the diesel and
16 crude oil microcosms, indicating a more diverse HC-degrading microbial community compared to the
17 communities enriched in laboratory microcosms (Figure 2c-d). All the identified *alkB* gene sequences were
18 associated to few hydrocarbonoclastic bacterial species typically spread in marine polluted ecosystems, i.e.
19 *M. hydrocarbonoclasticus*, *M. adhearence*, *Alcanivorax. hodgenensis* and *A. borkumensis*, (Table S2).
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40 **3.4 Hydrocarbon degrading strains**

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44 A collection of HC degrading bacteria was established from the U5 and U7 microcosms enriched on diesel
45 and crude oil. The isolated strains belonged to few bacterial species (Table 3). Despite the highly diverse
46 bacterial community detected by 16S rRNA gene pyrosequencing in the original sediments, only the species
47 *A. borkumenis* (26 isolates), *M. hydrocarbonoclasticus* (3) and *Pseudomonas pachastrellae* (1) were isolated
48 from the enrichments inoculated with sediment U5 whereas *A. borkumenis* (29) and *Marinobacter excellens*
49 (1) were obtained in pure cultures from enrichments inoculated with sediment U7.
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58 The isolates were clustered in different phylogenetic groups on the bases of their ITS fingerprints and at least
59 one representative of each cluster was tested for biotechnological properties related to bioremediation,
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1 including production of biosurfactants and bioemulsifiers, biofilm formation and degradation of uric acid and
2 catechol (Table 3). Drop collapse test indicated that none of the strains produced biosurfactants. Nonetheless
3 almost all of them (with the exception of U5-DS4, U7-CO6 and U7-DB8) produced emulsifying molecules
4 with different levels of activity and stability (Table 3). Among the tested strains, the relative emulsification
5 activity at 24 hours ranged between 5.2% of *A. borkumensis* strain U5-DS9 and 48.3% of *A. borkumensis*
6 U7-DS3, while the stability of the emulsification measured at 48 hours varied between 100% of *A.*
7 *borkumensis*, *M. hydrocarbonoclasticus* and *P. pachastrellae* representatives (Table 3) and 66.5% of *A.*
8 *borkumensis* U5-DB11. Most of the isolates were capable to form biofilm (Table 3), a phenotype that could
9 enhance their fitness in the sediment environment. The majority of the strains (89%) could be classified as
10 strongly adherent and among them *A. borkumensis* U5-DB1 was the best performer. Four strains (*A.*
11 *borkumensis* U7-CO14 and *M. hydrocarbonoclasticus* strains U5-DB8, U5-DB14 and U5-DB18) were also
12 able to degrade uric acid and all of the strains were positive for the catechol 2,3 dioxygenase activity (Table
13 3).

31 4. DISCUSSION

32 This study investigated the influence of pollution on the assemblage of the bacterial populations thriving in
33 natural and polluted sediments of the Ancona harbor, and provides isolated bacteria that may play role in
34 ABA. The sediment chemical analysis indicated high concentrations of crude oil-derived long chain aliphatic
35 HC (C>12), PAHs and heavy metals (Table 2). Heavy metals are of particular relevance in ABA approach
36 since they can inhibit biodegradation and bioremediation of the organic pollutants, because of their toxicity
37 toward microorganisms. In the Ancona harbor cadmium, chromium, nickel, and lead were retrieved in
38 several samples in high concentrations (Table 2). The high level of heavy HCs in the harbor area suggested
39 an input from ships and other harbor activities, while the presence of high concentration of PAHs in the
40 stations U1 and U4 is probably due to the impact of a fuel station located nearby these sites. The evaluation
41 of the total prokaryotic cell number in these sediments revealed that the contamination level did not impact
42 bacterial abundance, since the retrieved values (Table 1) are in accordance with previously published data at
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1 the sediment-water interface layers of the Adriatic sea (Molari et al., 2012) and containing high levels of
2 TOCs.

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4 Pollutants' concentrations resulted to be pivotal drivers in shaping the assembly of the sediment bacterial
5 communities as determined by ARISA fingerprinting. Five of the evaluated environmental parameters,
6 namely the concentration of cadmium, copper, chromium, HCs (C>12) and TOC were related with the
7 richness of the bacterial communities (Figure 1c) confirming that crude oil components and heavy metals are
8 among the main factors influencing the sediment bacterial communities assembly.

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10 Due to their different physico-chemical parameters and microbiological composition, the sediments collected
11 at stations U5 and U7 were chosen as the starting inocula for the cultivation-based experiments. Station U7
12 showed a higher percentage of gravel and displayed higher redox potential indicating higher oxygenation
13 (Table 1). Moreover, the sediments collected at station U7 were the most polluted in term of HC (C>12)
14 concentration and, in addition, displayed a PAHs concentration about tenfold higher than in U5 (Table 2).
15 The sediments were also microbiologically different: U7 showed a lower concentration of total prokaryotic
16 cell (Table 1), but a higher amount of HC-degrading bacteria than U5. Cultivable HC degrading microbes
17 were counted by the Most Probable Number (MPN) assay, by adding crude oil as the sole carbon source,
18 thus providing to the cells the maximum set of different HC molecules. In station U7 the number of
19 cultivable HC-degrading bacteria was higher than in station U5, correlating to a higher level of HC pollution.
20 This is a further evidence that the pollution level in the sediments selects for peculiar bacterial communities,
21 able to utilize the organic pollutants as growth substrate.

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23 Both PCR-DGGE (Figure 2a, b) and pyrosequencing (Figure 3) of 16S rRNA gene confirmed the selection
24 of specific bacterial communities in the established microcosms after the addition of the different HCs and
25 indicated a high specialization toward bacterial taxa involved in their degradation. The number of phyla
26 detected through pyrosequencing decreased from 20, identified in U5 sediment, to maximum 4 in the
27 naphthalene enriched microcosm (Table S1). Proteobacteria and Firmicutes, the bacterial phyla prevailing in
28 the sediments, reached in the enrichments the quite totality of the communities, covering about 98% of the
29 total diversity. Both phyla comprise several phylogenetic groups involved in the aerobic degradation of HCs
30 (Head et al., 2006). Coherently with the high pollution level of the Ancona harbor, the sediments collected at
31 U5 and U7 sites were dominated by Gammaproteobacteria and Clostridia, nonetheless only few of the major

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genera known to be capable of HC degradation have been identified in the established 16S rRNA gene
pyrotag libraries. For instance, *Oleispira* was detected only in the sediment U7, where it represented 1% of
the total bacterial community, while members of the family *Alteromonadaceae* - including the ubiquitous
HC-degrading genus *Marinobacter* - were detected in the sediment U5 at a very low percentage (Table S1).
The hydrocarbonoclastic genus *Alcanivorax* was not detected in both the original polluted sediments
although *Alcanivorax* and *Marinobacter* genera were strongly enriched in the U5 diesel and crude oil
microcosms. This result pointed out that these genera were present in the original U5 sediment at a very low
titer and below the detection limit of the used techniques (Table S1). A high percentage of the sequences
retrieved from sediments belonged to unclassified bacterial phyla, This percentage is even higher if we
describe the bacterial community at lower phylogenetic level (e.g. genera level) (Figure 3, Table S1),
highlighting the high potential of Ancona harbor sediments to retrieve novel and yet uncultured microbial
resources. In the enrichment microcosms, nevertheless, the majority of the sequences belonged to already
described taxa, indicating that the majority of the HC degraders present in Ancona harbor sediments are
recalcitrant to cultivation.

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Alcanivorax and *Marinobacter* belong to the Gammaproteobacteria class whose dominance within the
enriched microcosms can be expected since it comprises many of the hydrocarbonoclastic bacteria
responsible for the first steps of HC degradation (Head et al., 2006; Yakimov et al., 2007). The analysis of
alkB gene diversity in the enriched microcosms (Figure 2c-d) further confirmed the more complex functional
diversity of the sediment samples than in the microcosms, and identified *Marinobacter* and *Alcanivorax* as
the genera main players of alkane degradation in the sediment of the Ancona harbor (Table S2). Naphthalene
enrichment was dominated by *Bacillus* (55.57%) and *Halomonas* (15.52%) (Table S1), both known to be
involved in the degradation of PAHs molecules (Kostka et al., 2011; McGenity et al., 2012).

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The culture conditions adopted for the isolation of HC-degrading strains from the enriched microcosms
allowed the isolation of only four species, *A. borkumensis*, *M. hydrocarbonoclasticus*, *M. excellens* and *P.*
pachastrellae (Table 3). Such a low phylogenetic diversity within the bacteria collection is presumably due
to the enrichment culture conditions, which favored the selection of few fast growing and very specialized
hydrocarbonoclastic microorganisms already reported as the first to bloom after an oil spill (Head et al., 2006
and references therein). One of the physiological traits that makes *Alcanivorax* highly competent is its

1 capability to produce biosurfactants that enhance HCs bioavailability. Members of *Marinobacter* genus are
2 able to use a wide variety of HCs (Gauthier et al., 1992; Gorshkova et al., 2003), while *Pseudomonas* spp.
3 are widespread environmental microorganisms isolated from a variety of natural sources including marine
4 sediments and seawater (Pucci et al., 2012; Velmurugan et al., 2011) and capable to use a wide range of
5 carbon sources (Kostka et al., 2011; Mahjoubi et al., 2013; Mulet et al., 2011).
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8 Surprisingly, despite the capacity to grow on HCs and the prompt growth in the different enrichment
9 cultures, none of the tested isolates was capable to produce biosurfactants in the adopted conditions (Table
10 3). However, 83% of the tested strains were able to produce bioemulsifying factors, another class of surface-
11 active compounds that reduces the interfacial tension between immiscible liquids. Indeed, while
12 biosurfactants, in most cases, have also a certain degree of emulsifying activity, bioemulsifiers are not
13 necessarily able to act as biosurfactant molecules (Karant et al., 1999), but their production is nonetheless
14 considered an advantageous trait in oil polluted environments (Batista et al., 2006).
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17 The isolates from the different enrichment cultures showed interesting biotechnological properties for
18 applications in ABA. The lack of nitrogen and phosphorus in marine environments is one of the main factors
19 negatively affecting the growth of HC-degrading bacteria in polluted sites. Recently, the use of uric acid as a
20 natural fertilizer for the bioremediation of marine oil pollution has been proposed to overcome this limitation
21 (Ron and Rosenberg, 2014) and a microcosm-scale study showed its rapid conversion to ammonium and a
22 role in the selection of microbial communities encompassing known hydrocarbonoclastic bacteria (Bargiela
23 et al., 2015b; Gertler et al., 2015). Four of the tested isolates (one *A. borkumensis* and three *M.*
24 *hydrocarbonoclasticus*) were capable to degrade uric acid suggesting their potential to be responsive to uric
25 acid amendment in bioremediation trials. Sixty six percent of the tested strains showed to be capable of
26 biofilm formation. Cells that grow as biofilm have higher chances to survive and adapt to the difficult
27 condition of polluted environments, being protected within the biofilm matrix and thanks to the beneficial
28 interactions occurring between the different microorganisms inhabiting the biofilm (Singh et al., 2006). All
29 the strains proved to be able to degrade catechol, a central metabolite in aromatic HC degradation pathways
30 (Fuentes et al., 2014). Taken together, all the above phenotypic traits render the isolated bacteria interesting
31 for application in polluted natural environments, for instance in ABA.
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1 Our results, supported by the molecular ecology analyses and the cultivation approach, indicated that in the
2 sediments of the Ancona harbor heavy metals and HCs drive the selection of specific bacterial communities
3 with HC degrading potential. The bacterial genera enriched in the sediment present interesting properties for
4 application in the ABA approach. Nonetheless, the results also remarked the need for innovative cultivation-
5 dependent strategies to bring into culture the new taxa identified in the sediments by the cultivation-
6 independent pyrosequencing analyses. This would allow to retrieve a larger spectrum of marine bacterial
7 diversity to further expand the potential for ABA approaches in marine sites with multiple contaminations.
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FIGURE CAPTIONS

Figure 1

a) Location of the study area and sampling stations. Satellite image of Ancona harbor (Italy, Mediterranean Sea) showing the position of the sampling sites. **b)** Non-metric multidimensional scaling (nMDS) analysis based on ARISA fingerprinting showing the clustering of bacterial communities of sediment collected inside the harbour area (black triangles) and offshore (white squares). **c)** dbRDA ordinations of the ARISA dataset overlaid with the correlations of the tested environmental variables explaining the clustering of polluted and pristine sediment samples.

Figure 2

DGGE analysis of the bacterial communities in U5 and U7 sediments and the established enrichments. **a)** 16S rRNA DGGE of the enrichment cultures. **b)** Cluster analyses based on the 16S rRNA DGGE profiles. **c)** *alkB*-DGGE of the U5 and U7 sediments and established enrichments. The numbers indicate the cut bands (the results of *alkB* sequencing are reported in Table S2).

Figure 3

16S rRNA pyrosequencing of U5 and U7 sediments and U5 established enrichments. **a)** PCoA represents the structural diversity between bacterial communities. **b)** Summary of the number of reads, OTUs, singleton and coverage recorded for each sample. **c)** Relative abundance of the bacterial phylum detected through 16S rRNA pyrosequencing. **d)** Relative abundance of the bacterial class detected through 16S rRNA pyrosequencing.

Figure S1

16S rRNA pyrosequencing of U5 and U7 sediments and U5 established enrichments. Relative abundance of the bacteria genera detected through 16S rRNA pyrosequencing.

Figure S2

Rarefaction analysis of the 16S rRNA pyrosequencing libraries established from the total DNA extracted from U5 and U7 sediments and U5 enrichments.

TABLES

Table 1

Physical characterization and value of total prokaryotic abundance of the surface sediments collected at the Ancona harbor.

Station name	Sampling coordinates		Physical features						Prokaryotic cell number (±SDT)
	Latitude	Longitude	Gravel (%)	Sand (%)	Silt (%)	Clay (%)	pH	Eh (mV)	
U-1	43°37'28,98" N	13°29'59,85"E	0	17,67	23,23	59,1	7,56	179,1	7,48E+08 (±2,21E+07)
U-2	43°37'25,91" N	13°30'12,21"E	3,42	15,68	18,26	62,7	7,7	197	7,16E+08 (±3,78E+07)
U-3	43°37'27,55" N	13°30'13,07"E	2,92	15,62	19,96	61,5	7,75	137,4	6,14E+08 (±8,90E+07)
U-4	43°36'47,25" N	13°30'10,17"E	0	18,51	33,1	48,4	7,76	247,3	1,10E+09 (±2,01E+08)
U-5	43°36'55,16" N	13°30'9,98"E	0	24,07	27,98	47,9	7,77	43,2	1,13E+09 (±1,70E+08)
U-6	43°37'1,63" N	13°30'10,37"E	0	25,59	25,14	49,3	7,8	161,4	8,25E+08 (±2,72E+08)
U-7	43°37'22,63" N	13°30'13,64"E	2,53	8,3	23,98	65,2	7,72	128,6	4,04E+08 (±3,68E+07)
U-8	43°36'53,25" N	13°29'36,60"E	0	12,75	24,15	63,1	7,64	121,4	7,04E+08 (±2,85E+08)
U-9	43°37'36,05" N	13°29'37,41"E	0	21,42	32,33	46,3	7,85	28	7,20E+08 (±3,81E+08)
U-10	43°37'52,16" N	13°29'51,71"E	0,16	22,24	27,2	50,4	7,5	142,2	7,00E+08 (±2,34E+08)
C-1	43°39'14,84" N	13°29'9,79"E	0	41,49	21,38	37,1	7,68	92,6	7,46E+08 (±2,11E+08)
C-2	43°39'9,52" N	13°28'5,26"E	0	16,11	31,34	52,6	7,47	28,4	1,03E+08 (±1,99E+07)
C-3	43°38'49,39" N	13°30'16,13"E	0	40,91	21,84	37,2	7,78	160,4	1,89E+08 (±3,02E+07)
C-4	43°38'3,74" N	13°32'37,44"E	0	18,9	33,22	47,9	7,72	101,3	4,65E+08 (±3,14E+07)
C-5	43°38'5,46" N	13°34'30,15"E	0	16,19	30,85	53	7,65	83,2	9,76E+07 (±2,70E+07)

1 **Table 2**

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3 Chemical composition (heavy metals, metalloids, hydrocarbons, TOC: Total Organic Carbon) of sediments
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5 collected at the Ancona harbor.
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Station name	Chemical characterization (mg/kg d.s)											
	Hg	Cd	Pb	As	Cr	Cu	Ni	Zi	V	HC: C>12.	HC: Total PAH	TOC
U-1	0,2	0,67	47,5	9,1	157,8	36,9	48,6	25	25	292	1,157	9,22
U-2	0,1	0,8	18,9	6,2	517,3	39	61,6	107	60,3	252	0,359	8,58
U-3	0,3	0,6	22,2	4,8	83,7	44	46,4	113,5	78,1	154	0,42	7,15
U-4	0,05	0,6	15,1	5,5	105,1	23,9	52,9	88,9	73,4	65,3	3,697	9,8
U-5	0,1	0,58	11,7	6,8	429,2	32,2	63,8	93,4	56,7	152	0,03	9,42
U-6	0,1	0,57	12	6,2	130,6	32,3	52,1	89,9	57,7	165,6	0,087	9,63
U-7	0,3	0,8	9,1	8,5	107,6	57,6	191,1	148,6	93,7	316,5	0,358	7,26
U-8	0,1	0,56	25,5	7,9	88,4	28,7	47	106,5	62,4	152	0,487	8,23
U-9	0,1	0,6	16	7,5	137,3	34	55,9	89,3	66,5	106	0,164	7,99
U-10	0,1	0,6	17,4	8,2	84,4	23,7	54	86,6	51,8	119	0,584	8,32
C-1	0,05	0,29	7,2	6,7	72	22,4	42,2	47,9	40,7	2,5	0,008	7,84
C-2	0,05	0,21	20,3	5	45,1	23,4	38,5	97,4	62,7	82,7	0,203	5,24
C-3	0,05	0,25	10,7	7,7	44,6	20,3	47,9	63,9	40,2	11,9	0,047	6,03
C-4	0,1	0,24	10,2	7,8	89,2	18,7	48,9	66,7	57,8	19	0,106	7,01
C-5	0,05	0,28	18,5	6,2	46,3	28,8	46,7	99,1	50,8	58,8	0,182	5,05

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Table 3

Identification of the bacterial isolates (including the classification in ITS groups), and the results of the tested activities of potential interest for bioremediation.

ITS number/N° of strains	Closest described specie (ACC. N°)	Code	Drop collapse	Emulsification			Biofilm formation (adhesion unit)	Uricolytic activity	Catechol degradation
				EV 24 h	EV 48 h	Stability %			
(1/52)	<i>Alcanivorax borkumensis</i> (AB435569)	U5-CO1	-	11,6	10,5	90,5	3,15	-	+
	<i>Alcanivorax borkumensis</i> (AB435569)	U5-CO3	-	0,0	0,0	na	0,89	-	+
	<i>Alcanivorax borkumensis</i> (AB435569)	U5-CO11	-	0,0	0,0	na	0,75	-	+
	<i>Alcanivorax borkumensis</i> (AB435569)	U5-DB1	-	9,7	14,6	100,0	3,42	-	+
	<i>Alcanivorax borkumensis</i> (NR_074890)	U5-DS4	-	20,3	19,7	97,0	2,86	-	+
	<i>Alcanivorax borkumensis</i> (NR_074890)	U5-DS8	-	9,8	11,7	100,0	2,86	-	+
(3/3)	<i>Alcanivorax borkumensis</i> (NR_074890)	U5-DB11	-	40,5	26,9	66,4	3,32	-	+
	<i>Alcanivorax borkumensis</i> (NR_074890)	U5-DS9	-	5,2	6,1	100,0	2,91	-	+
(1/52)	<i>Alcanivorax borkumensis</i> (AB435569)	U7-CO6	-	20,1	15,8	78,6	0,96	-	+
	<i>Alcanivorax borkumensis</i> (AB435569)	U7-CO14	-	25,2	21,7	86,1	1,46	1,7	+
	<i>Alcanivorax borkumensis</i> (AB435569)	U7-DB18	-	33,3	32,2	96,7	0,15	-	+
	<i>Alcanivorax borkumensis</i> (AB435569)	U7-DS3	-	48,3	48,0	99,4	0,34	-	+
	<i>Alcanivorax borkumensis</i> (AB435569)	U7-DS10	-	36,4	35,6	97,8	0,24	-	+
(7/1)	<i>Marinobacter excellens</i> (KC534446)	U7-DB8	-	29,4	27,9	94,9	1,44	-	+
(2/1)	<i>Marinobacter hydrocarbonoclasticus</i> (NR_074619)	U5-DB8	-	0,0	0,0	na	3,17	1,8	+
(4/1)	<i>Marinobacter hydrocarbonoclasticus</i> (JN160753)	U5-DB14	-	26,7	25,7	96,3	3,26	1,8	+
(5/1)	<i>Marinobacter hydrocarbonoclasticus</i> (JQ799097)	U5-DB18	-	10,0	10,0	100,0	1,25	1,8	+
(6/1)	<i>Pseudomonas pachastrellae</i> (AY880300)	U5-DS3	-	6,7	10,0	100,0	1,99	-	+
		CN (ONR7a)	-	0,0	0,0	na	0	-	-
		CP (SDS1%)	+	62,0	60,8	98,1	na	na	na

Table S1

Identification and relative abundance of the OTUs (97% similarity) detected by 16S rRNA pyrosequencing in U5 and U7 sediments and U5 enrichment cultures.

	phylum	class	order	family	genus	U5	U7	U5-DB	U5-CO	U5-N
1	Bacteria	Uncl. Bacteria	Uncl. Bacteria	Uncl. Bacteria	Uncl. Bacteria	7,64973558	13,27566754	0,29459648	0,216727416	0,905947441
2	Bacteria	Acidobacteria	Uncl. Acidobacteria	Uncl. Acidobacteria	Uncl. Acidobacteria	0,036895831	0,131628432	0	0	0
3	Bacteria	Acidobacteria	AT-s2-57	Uncl. Acidobacteria	Uncl. Acidobacteria	0	0,047010154	0	0	0
4	Bacteria	Acidobacteria	Acidobacteria-6	BPC015	Uncl. Acidobacteria	0	0,018804062	0	0	0
5	Bacteria	Acidobacteria	Acidobacteria-6	CCU21	Uncl. Acidobacteria	0,01229861	0,009402031	0	0	0
6	Bacteria	Acidobacteria	Acidobacteria-6	iii1-15	Uncl. Acidobacteria	0	0,018804062	0	0	0
7	Bacteria	Acidobacteria	Acidobacteriia	Acidobacteriales	Koribacteraceae	0	0,009402031	0	0	0
8	Bacteria	Acidobacteria	BPC102	B110	Uncl. Acidobacteria	0,381256918	0,253854833	0	0	0
9	Bacteria	Acidobacteria	BPC102	MVS-40	Uncl. Acidobacteria	0	0,018804062	0	0	0
10	Bacteria	Acidobacteria	OS-K	Uncl. Acidobacteria	Uncl. Acidobacteria	1,488131841	0,545317789	0	0	0
11	Bacteria	Acidobacteria	RB25	Uncl. Acidobacteria	Uncl. Acidobacteria	0,196777764	0,11282437	0	0	0
12	Bacteria	Acidobacteria	Sva0725	Sva0725	Uncl. Acidobacteria	0,221374985	0,244452802	0	0	0,006915629
13	Bacteria	Acidobacteria	iii1-8	32-20	Uncl. Acidobacteria	0	0,009402031	0	0	0
14	Bacteria	Actinobacteria	Uncl. Actinobacteria	Uncl. Actinobacteria	Uncl. Actinobacteria	0,01229861	0	0	0	0
15	Bacteria	Actinobacteria	Acidimicrobiia	Acidimicrobiales	Uncl. Acidimicrobiales	0	0,037608123	0	0	0
16	Bacteria	Actinobacteria	Acidimicrobiia	Acidimicrobiales	Uncl. Acidimicrobiales	0	0,028206093	0	0	0
17	Bacteria	Actinobacteria	Acidimicrobiia	Acidimicrobiales	C111	0	0,056412185	0	0	0,006915629
18	Bacteria	Actinobacteria	Acidimicrobiia	Acidimicrobiales	EB1017	0	0,018804062	0	0	0
19	Bacteria	Actinobacteria	Acidimicrobiia	Acidimicrobiales	koll13	0,172180544	0,347875141	0	0	0,020746888
20	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Uncl. Actinomycetales	0	0,009402031	0	0	0
21	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Bogoriellaceae	0	0,037608123	0	0	0
22	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Geodermatophilaceae	0	0,009402031	0	0	0
23	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Intrasporangiaceae	0	0,018804062	0	0	0
24	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	0	0,018804062	0	0	0
25	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	0	0	0	0	0,006915629
26	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Microbacterium	0	0,009402031	0	0	0

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	phylum	class	order	family	genus	U5	U7	U5-DB	U5-CO	U5-N
Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Mycobacteriaceae	Mycobacterium	0	0,009402031	0	0	0
Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Pseudonocardiaceae	Uncl. Pseudonocardiaceae	0	0,018804062	0	0	0
Bacteria	Actinobacteria	Actinobacteria	WCHB1-81	At425_EubF1	Uncl. Actinobacteria	0	0,009402031	0	0	0
Bacteria	Actinobacteria	OPB41	Uncl. Actinobacteria	Uncl. Actinobacteria	Uncl. Actinobacteria	0,024597221	0,065814216	0	0	0
Bacteria	Actinobacteria	Thermoleophilia	Uncl. Thermoleophilia	Uncl. Thermoleophilia	Uncl. Thermoleophilia	0,024597221	0	0	0	0
Bacteria	Actinobacteria	Thermoleophilia	Gaiellales	Gaiellaceae	Uncl. Gaiellaceae	0	0,122226401	0	0	0
Bacteria	Actinobacteria	Thermoleophilia	Solirubrobacterales	Other	Other	0	0,009402031	0	0	0
Bacteria	Actinobacteria	Thermoleophilia	Solirubrobacterales	Uncl. Solirubrobacterales	Uncl. Solirubrobacterales	0	0,018804062	0	0	0
Bacteria	Actinobacteria	Thermoleophilia	Solirubrobacterales	Solirubrobacteraceae	Uncl. Solirubrobacteraceae	0	0,009402031	0	0	0
Bacteria	BRC1	NPL-UPA2	Uncl. Bacteria	Uncl. Bacteria	Uncl. Bacteria	0,01229861	0,018804062	0	0	0
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Other	Other	0,01229861	0	0	0	0
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Uncl. Bacteroidales	Uncl. Bacteroidales	2,533513713	2,265889432	0	0	0,062240664
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Marinilabiaceae	Uncl. Marinilabiaceae	0,01229861	0,028206093	0	0	0
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	SB-1	Uncl. Bacteroidales	0,122986103	0,178638586	0	0	0
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	VC21_Bac22	Uncl. Bacteroidales	0,553437462	0,169236555	0	0	0,062240664
Bacteria	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	Uncl. Cytophagaceae	0	0,009402031	0	0	0
Bacteria	Bacteroidetes	Cytophagia	Cytophagales	Flammeovirgaceae	Uncl. Flammeovirgaceae	0,135284713	0,197442648	0	0	0
Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Cryomorphaceae	Fluviicola	0,01229861	0,009402031	0	0	0
Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Other	0,061493051	0,178638586	0	0	0
Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Uncl. Flavobacteriaceae	5,768048211	5,998495675	1,302426545	0,866909664	0,096818811
Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Aequorivita	0	0	0	0,088661216	0
Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Bizionia	0	0,037608123	0,007752539	0,049256231	0
Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Flavobacterium	0	0,037608123	0	0	0
Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Lutimonas	0,01229861	0,009402031	0	0	0
Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Psychroflexus	0	0,009402031	0	0	0
Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Robiginitalea	0,147583323	0,197442648	0	0	0
Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Sediminicola	0	0,056412185	0	0	0
Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Tenacibaculum	0	0,009402031	0	0,147768693	0
Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Ulvibacter	0,01229861	0,197442648	0	0	0
Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Winogradskyella	0,01229861	0,037608123	0	0	0

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	phylum	class	order	family	genus	U5	U7	U5-DB	U5-CO	U5-N
4	Bacteria	Bacteroidetes	[Rhodothermi]	[Rhodothermales]	Rhodothermaceae	Uncl. Rhodothermaceae	0	0,018804062	0	0
5	Bacteria	Bacteroidetes	[Saprosirae]	[Saprosirales]	Chitinophagaceae	Uncl. Chitinophagaceae	0	0,028206093	0	0
6	Bacteria	Bacteroidetes	[Saprosirae]	[Saprosirales]	Saprosiraceae	Uncl. Saprosiraceae	0	0,028206093	0	0
7	Bacteria	Caldithrix	Caldithrixae	Caldithrixales	Caldithrixaceae	Uncl. Caldithrixaceae	0,036895831	0,018804062	0	0
8	Bacteria	Caldithrix	Caldithrixae	Caldithrixales	Caldithrixaceae	LCP-26	0,061493051	0,056412185	0	0
9	Bacteria	Chlorobi	Uncl. Chlorobi	Uncl. Chlorobi	Uncl. Chlorobi	Uncl. Chlorobi	0	0	0	0,013831259
10	Bacteria	Chlorobi	BSV26	PK329	Uncl. Chlorobi	Uncl. Chlorobi	0	0,037608123	0	0
11	Bacteria	Chlorobi	Ignavibacteria	Ignavibacteriales	Ignavibacteriaceae	Uncl. Ignavibacteriaceae	0,01229861	0,028206093	0	0
12	Bacteria	Chlorobi	Ignavibacteria	Ignavibacteriales	IheB3-7	Uncl. Ignavibacteriales	0,159881933	0,065814216	0	0,006915629
13	Bacteria	Chlorobi	OPB56	Uncl. Chlorobi	Uncl. Chlorobi	Uncl. Chlorobi	0,01229861	0	0	0
14	Bacteria	Chloroflexi	Anaerolineae	Uncl. Anaerolineae	Uncl. Anaerolineae	Uncl. Anaerolineae	0,024597221	0	0	0
15	Bacteria	Chloroflexi	Anaerolineae	Anaerolineales	Anaerolinaceae	C1_B004	0,049194441	0,056412185	0	0
16	Bacteria	Chloroflexi	Anaerolineae	Anaerolineales	Anaerolinaceae	T78	0,270569426	0,065814216	0	0
17	Bacteria	Chloroflexi	Anaerolineae	Caldilineales	Caldilineaceae	Uncl. Caldilineaceae	0,024597221	0	0	0,006915629
18	Bacteria	Chloroflexi	Anaerolineae	Caldilineales	Caldilineaceae	Caldilinea	0	0,009402031	0	0
19	Bacteria	Chloroflexi	Anaerolineae	DRC31	Uncl. Anaerolineae	Uncl. Anaerolineae	0,098388882	0	0	0
20	Bacteria	Chloroflexi	Anaerolineae	GCA004	Uncl. Anaerolineae	Uncl. Anaerolineae	0,4796458	0,366679203	0	0
21	Bacteria	Chloroflexi	Anaerolineae	MSB-1E9	Uncl. Anaerolineae	Uncl. Anaerolineae	0,024597221	0	0	0
22	Bacteria	Chloroflexi	Anaerolineae	O4D2Z37	Uncl. Anaerolineae	Uncl. Anaerolineae	0,110687492	0,009402031	0	0
23	Bacteria	Chloroflexi	Anaerolineae	OPB11	Uncl. Anaerolineae	Uncl. Anaerolineae	0	0,018804062	0	0
24	Bacteria	Chloroflexi	Anaerolineae	S0208	Uncl. Anaerolineae	Uncl. Anaerolineae	0,01229861	0	0	0
25	Bacteria	Chloroflexi	Anaerolineae	SB-34	Uncl. Anaerolineae	Uncl. Anaerolineae	0,024597221	0,131628432	0	0
26	Bacteria	Chloroflexi	Anaerolineae	SBR1031	SHA-31	Uncl. Anaerolineae	0	0,009402031	0	0
27	Bacteria	Chloroflexi	Anaerolineae	SHA-20	Uncl. Anaerolineae	Uncl. Anaerolineae	0,01229861	0,018804062	0	0
28	Bacteria	Chloroflexi	Dehalococcoidetes	Uncl. Dehalococcoidetes	Uncl. Dehalococcoidetes	Uncl. Dehalococcoidetes	0,073791662	0,188040617	0	0
29	Bacteria	Chloroflexi	Dehalococcoidetes	Dehalococcoidales	Dehalococcoidaceae	Uncl. Dehalococcoidaceae	0,061493051	0	0	0,006915629
30	Bacteria	Chloroflexi	Dehalococcoidetes	FS117-23B-02	Uncl. Dehalococcoidetes	Uncl. Dehalococcoidetes	0	0,037608123	0	0
31	Bacteria	Chloroflexi	Dehalococcoidetes	GIF9	Uncl. Dehalococcoidetes	Uncl. Dehalococcoidetes	0,024597221	0,047010154	0	0
32	Bacteria	Chloroflexi	Ellin6529	Uncl. Chloroflexi	Uncl. Chloroflexi	Uncl. Chloroflexi	0,01229861	0,22564874	0	0,027662517
33	Bacteria	Chloroflexi	Thermomicrobia	JG30-KF-CM45	Uncl. Thermomicrobia	Uncl. Thermomicrobia	0	0,018804062	0	0

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	phylum	class	order	family	genus	U5	U7	U5-DB	U5-CO	U5-N
Bacteria	Cyanobacteria	Chloroplast	Chlorophyta	Mamiellaceae	Uncl. Mamiellaceae	0	0,009402031	0	0	0
Bacteria	Cyanobacteria	Chloroplast	Stramenopiles	Uncl. Stramenopiles	Uncl. Stramenopiles	0,024597221	0,047010154	0	0	0
Bacteria	Firmicutes	Bacilli	Bacillales	Bacillaceae	Other	0	0	0,023257617	0	4,204702628
Bacteria	Firmicutes	Bacilli	Bacillales	Bacillaceae	Uncl. Bacillaceae	0	0,009402031	0	0	0,006915629
Bacteria	Firmicutes	Bacilli	Bacillales	Bacillaceae	Bacillus	0	0,131628432	0	0	55,77455048
Bacteria	Firmicutes	Bacilli	Bacillales	Bacillaceae	Geobacillus	0	0	0	0	0,006915629
Bacteria	Firmicutes	Bacilli	Bacillales	Bacillaceae	Gracilibacillus	0	0	0	0	0,041493776
Bacteria	Firmicutes	Bacilli	Bacillales	Paenibacillaceae	Paenibacillus	0,01229861	0	0	0	0
Bacteria	Firmicutes	Bacilli	Bacillales	Planococcaceae	Other	0	0	0	0	0,013831259
Bacteria	Firmicutes	Bacilli	Bacillales	Planococcaceae	Paenisporosarcina	0	0,037608123	0	0	0
Bacteria	Firmicutes	Bacilli	Bacillales	Planococcaceae	Planococcus	0	0,037608123	0	0	4,349930844
Bacteria	Firmicutes	Bacilli	Bacillales	[Exiguobacteraceae]	Exiguobacterium	0	0	0	0	0,131396957
Bacteria	Firmicutes	Bacilli	Haloplasmatales	Haloplasmataceae	Uncl. Haloplasmataceae	0	0,197442648	0	0	0
Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	0	0	0	0	0,013831259
Bacteria	Firmicutes	Bacilli	Turicibacterales	Turicibacteraceae	Turicibacter	0	0,009402031	0	0	0
Bacteria	Firmicutes	Clostridia	Clostridiales	Other	Other	0,073791662	0,206844678	0	0	0
Bacteria	Firmicutes	Clostridia	Clostridiales	Uncl. Clostridiales	Uncl. Clostridiales	0,036895831	0,33847311	0	0	0
Bacteria	Firmicutes	Clostridia	Clostridiales	Christensenellaceae	Uncl. Christensenellaceae	0,049194441	0,028206093	0	0	0
Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Other	8,14167999	6,440391124	0	0	0
Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Uncl. Clostridiaceae	1,205263805	1,551335088	0	0	0
Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Caldanaerocella	0,061493051	0,169236555	0	0	0
Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Caloramator	0,01229861	0	0	0	0
Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridiisalibacter	0	0	0	0	0,006915629
Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium	4,046242775	14,54494171	0	0	0
Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Geosporobacter_Thermotalea	0,307465256	0,639338097	0	0	0
Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Proteiniclasticum	0	0,075216247	0	0	0,193637621
Bacteria	Firmicutes	Clostridia	Clostridiales	Dehalobacteriaceae	Dehalobacterium	0	0,009402031	0	0	0
Bacteria	Firmicutes	Clostridia	Clostridiales	JTB215	Uncl. Clostridiales	0	0	0,108535545	0	0
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Uncl. Lachnospiraceae	0,01229861	1,400902595	0	0	0
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Coproccoccus	0	0,028206093	0	0	0

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	phylum	class	order	family	genus	U5	U7	U5-DB	U5-CO	U5-N
Bacteria	Firmicutes	Clostridia	Clostridiales	Peptococcaceae	Desulfonispora	0	0,009402031	0	0	0
Bacteria	Firmicutes	Clostridia	Clostridiales	Peptostreptococcaceae	Uncl. Peptostreptococcaceae	0	0,009402031	0	0	0
Bacteria	Firmicutes	Clostridia	Clostridiales	Peptostreptococcaceae	Tepidibacter	0,122986103	0,11282437	0	0	0
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Uncl. Ruminococcaceae	0,086090272	0,065814216	0	0	0
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Oscillospira	0,01229861	0,009402031	0	0	0
Bacteria	Firmicutes	Clostridia	Clostridiales	[Acidaminobacteraceae]	Uncl. Clostridiales	0,45504858	2,999247838	0	0	0
Bacteria	Firmicutes	Clostridia	Clostridiales	[Acidaminobacteraceae]	Fusibacter	0,147583323	0,291462956	0,015505078	0	0
Bacteria	Firmicutes	Clostridia	Clostridiales	[Acidaminobacteraceae]	WH1-8	1,094576313	1,664159458	0	0	0,193637621
Bacteria	Firmicutes	Clostridia	Clostridiales	[Mogibacteriaceae]	Other	0,01229861	0	0	0	0
Bacteria	Firmicutes	Clostridia	Clostridiales	[Mogibacteriaceae]	Uncl. Clostridiales	0	0,009402031	0	0	0
Bacteria	Firmicutes	Clostridia	Clostridiales	[Mogibacteriaceae]	Anaerovorax	0,01229861	0,065814216	0	0	0
Bacteria	Firmicutes	Clostridia	Clostridiales	[Tissierellaceae]	Dethiosulfatibacter	0,036895831	0	0	0	0
Bacteria	Firmicutes	Clostridia	Clostridiales	[Tissierellaceae]	Sedimentibacter	0,036895831	0	0	0	0
Bacteria	Fusobacteria	Fusobacteriia	Fusobacteriales	Fusobacteriaceae	Psychrilyobacter	5,165416308	0,141030463	0	0	0,048409405
Bacteria	GN02	3BR-5F	Uncl. Bacteria	Uncl. Bacteria	Uncl. Bacteria	0,049194441	0,159834524	0	0	0
Bacteria	GN02	BB34	Uncl. Bacteria	Uncl. Bacteria	Uncl. Bacteria	0,159881933	0,075216247	0	0	0
Bacteria	GN02	GN10	Uncl. Bacteria	Uncl. Bacteria	Uncl. Bacteria	0,024597221	0	0	0	0
Bacteria	GN04	Uncl. Bacteria	Uncl. Bacteria	Uncl. Bacteria	Uncl. Bacteria	0	0,037608123	0	0	0
Bacteria	GN04	GN15	Uncl. Bacteria	Uncl. Bacteria	Uncl. Bacteria	0,578034682	0,150432493	0	0	0
Bacteria	Gemmatimonadetes	Uncl. Gemmatimonadetes	Uncl. Gemmatimonadetes	Uncl. Gemmatimonadetes	Uncl. Gemmatimonadetes	0	0,009402031	0	0	0
Bacteria	Gemmatimonadetes	Gemm-1	Uncl. Gemmatimonadetes	Uncl. Gemmatimonadetes	Uncl. Gemmatimonadetes	0	0,047010154	0	0	0
Bacteria	Gemmatimonadetes	Gemm-2	Uncl. Gemmatimonadetes	Uncl. Gemmatimonadetes	Uncl. Gemmatimonadetes	0,01229861	0,028206093	0	0	0
Bacteria	Gemmatimonadetes	Gemm-4	Uncl. Gemmatimonadetes	Uncl. Gemmatimonadetes	Uncl. Gemmatimonadetes	0,110687492	0,244452802	0	0	0,006915629
Bacteria	Gemmatimonadetes	Gemmatimonadetes	Uncl. Gemmatimonadetes	Uncl. Gemmatimonadetes	Uncl. Gemmatimonadetes	0	0,065814216	0	0	0
Bacteria	Gemmatimonadetes	JL-ETNP-Z39	Uncl. Gemmatimonadetes	Uncl. Gemmatimonadetes	Uncl. Gemmatimonadetes	0,01229861	0	0	0	0
Bacteria	Hyd24-12	WM88	Uncl. Bacteria	Uncl. Bacteria	Uncl. Bacteria	0,086090272	0,028206093	0	0	0
Bacteria	Lentisphaerae	[Lentisphaeria]	Z20	Uncl. Z20	Uncl. Z20	0	0,018804062	0	0	0
Bacteria	NKB19	Uncl. Bacteria	Uncl. Bacteria	Uncl. Bacteria	Uncl. Bacteria	0	0,056412185	0	0	0
Bacteria	Nitrospirae	Nitrospira	Nitrospirales	[Thermodesulfobivibrionaceae]	LCP-6	0	0,056412185	0	0	0
Bacteria	OD1	Uncl. Bacteria	Uncl. Bacteria	Uncl. Bacteria	Uncl. Bacteria	0,01229861	0	0	0	0,013831259

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	phylum	class	order	family	genus	U5	U7	U5-DB	U5-CO	U5-N
Bacteria	OD1	ABY1	Uncl. Bacteria	Uncl. Bacteria	Uncl. Bacteria	0,135284713	0,028206093	0	0	0
Bacteria	OP11	OP11-3	Uncl. Bacteria	Uncl. Bacteria	Uncl. Bacteria	0	0,009402031	0	0	0
Bacteria	OP3	BD4-9	Uncl. Bacteria	Uncl. Bacteria	Uncl. Bacteria	0,061493051	0	0	0	0
Bacteria	OP3	koll11	Uncl. Bacteria	Uncl. Bacteria	Uncl. Bacteria	0	0,018804062	0	0	0
Bacteria	OP8	OP8_1	HMMVPog-54	Uncl. Bacteria	Uncl. Bacteria	0,061493051	0,075216247	0	0	0
Bacteria	OP8	OP8_2	Uncl. Bacteria	Uncl. Bacteria	Uncl. Bacteria	0	0,047010154	0	0	0
Bacteria	OP8	SAW1_B6	Uncl. Bacteria	Uncl. Bacteria	Uncl. Bacteria	0,122986103	0,300864987	0	0	0
Bacteria	OP9	JS1	SB-45	Uncl. Bacteria	Uncl. Bacteria	0,024597221	0	0	0	0
Bacteria	Planctomycetes	Uncl. Planctomycetes	Uncl. Planctomycetes	Uncl. Planctomycetes	Uncl. Planctomycetes	0,024597221	0,018804062	0	0	0
Bacteria	Planctomycetes	028H05-P-BN-P5	Uncl. Planctomycetes	Uncl. Planctomycetes	Uncl. Planctomycetes	0	0,009402031	0	0	0
Bacteria	Planctomycetes	C6	Ucm1571	Uncl. Planctomycetes	Uncl. Planctomycetes	0	0,018804062	0	0	0
Bacteria	Planctomycetes	C6	d113	Uncl. Planctomycetes	Uncl. Planctomycetes	0,01229861	0	0	0	0
Bacteria	Planctomycetes	OM190	agg27	Uncl. Planctomycetes	Uncl. Planctomycetes	0	0,009402031	0	0	0
Bacteria	Planctomycetes	Phycisphaerae	AKAU3564	Uncl. Phycisphaerae	Uncl. Phycisphaerae	0,01229861	0,037608123	0	0	0,006915629
Bacteria	Planctomycetes	Phycisphaerae	C86	Uncl. Phycisphaerae	Uncl. Phycisphaerae	0	0,037608123	0	0	0
Bacteria	Planctomycetes	Phycisphaerae	CCM11a	Uncl. Phycisphaerae	Uncl. Phycisphaerae	0	0,009402031	0	0	0
Bacteria	Planctomycetes	Phycisphaerae	MSBL9	Uncl. Phycisphaerae	Uncl. Phycisphaerae	0,147583323	0,131628432	0	0	0,020746888
Bacteria	Planctomycetes	Phycisphaerae	Phycisphaerales	Uncl. Phycisphaerales	Uncl. Phycisphaerales	0,049194441	0,018804062	0	0	0
Bacteria	Planctomycetes	Phycisphaerae	Pla1	Uncl. Phycisphaerae	Uncl. Phycisphaerae	0	0,009402031	0	0	0
Bacteria	Planctomycetes	Phycisphaerae	SHA-43	Uncl. Phycisphaerae	Uncl. Phycisphaerae	0	0,028206093	0	0	0
Bacteria	Planctomycetes	Phycisphaerae	WD2101	Uncl. Phycisphaerae	Uncl. Phycisphaerae	0	0,009402031	0	0	0
Bacteria	Planctomycetes	Phycisphaerae	mle1-8	Uncl. Phycisphaerae	Uncl. Phycisphaerae	0,098388882	0,028206093	0	0	0
Bacteria	Planctomycetes	Pla3	Uncl. Planctomycetes	Uncl. Planctomycetes	Uncl. Planctomycetes	0,01229861	0	0	0	0
Bacteria	Planctomycetes	Planctomycetia	Pirellulales	Pirellulaceae	Uncl. Pirellulaceae	0,135284713	0,404287326	0	0	0,006915629
Bacteria	Planctomycetes	Planctomycetia	Pirellulales	Pirellulaceae	A17	0	0	0	0	0,006915629
Bacteria	Planctomycetes	Planctomycetia	Planctomycetales	Planctomycetaceae	Planctomyces	0	0,009402031	0	0	0
Bacteria	Proteobacteria	Other	Other	Other	Other	0	0,009402031	0	0	0
Bacteria	Proteobacteria	Alphaproteobacteria	Uncl. Alphaproteobacteria	Uncl. Alphaproteobacteria	Uncl. Alphaproteobacteria	0,024597221	0,037608123	0	0	0
Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Other	Other	0	0,018804062	0	0	0
Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Uncl. Rhizobiales	Uncl. Rhizobiales	0	0,075216247	0	0	0

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	phylum	class	order	family	genus	U5	U7	U5-DB	U5-CO	U5-N
Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Uncl. Beijerinckiaceae	0	0,009402031	0	0	0
Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	Balneimonas	0	0,037608123	0	0	0
Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	Uncl. Hyphomicrobiaceae	0,036895831	0,319669049	0	0	0
Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	Devosia	0	0,009402031	0	0	0
Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	Hyphomicrobium	0,061493051	0,028206093	0	0	0
Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	Pedomicrobium	0	0,094020308	0	0	0
Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	Rhodoplanes	0	0,047010154	0	0	0,013831259
Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylobacteriaceae	Uncl. Methylobacteriaceae	0,01229861	0	0	0	0
Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Phyllobacteriaceae	Uncl. Phyllobacteriaceae	0	0,150432493	0	0	0
Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhodobiaceae	Afifella	0	0,009402031	0	0	0
Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	Uncl. Xanthobacteraceae	0	0,009402031	0	0	0
Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Hyphomonadaceae	Uncl. Hyphomonadaceae	0	0,009402031	0	0	0
Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Other	0,172180544	0,432493419	0,046515234	4,561126983	0,027662517
Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Uncl. Rhodobacteraceae	0,233673595	2,106054908	0,031010156	0,374347355	0,020746888
Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Anaerospora	0,098388882	0	0	0,029553739	0
Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Dinoroseobacter	0	0,018804062	0	0	0
Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Loktanella	0,024597221	0,723956375	0	0	0
Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Marivita	0	0,009402031	0	0	0
Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Octadecabacter	0,073791662	0,460699511	0	0	0
Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Phaeobacter	0,024597221	0,122226401	0	0,039404985	0
Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Pseudoruegeria	0	0,018804062	0	0	0
Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Rhodobacter	0	0,028206093	0,015505078	0,1280662	0
Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Ruegeria	0	0,037608123	0	0	0
Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Sulfitobacter	0,01229861	0,084618278	0	0,019702492	0
Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	Uncl. Rhodospirillaceae	0	0,075216247	0	0	0
Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	Skermanella	0	0,009402031	0	0	0
Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales	Uncl. Rickettsiales	Uncl. Rickettsiales	0,024597221	0	0	0	0
Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Erythrobacteraceae	Other	0,01229861	0,018804062	0	0	0
Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Erythrobacteraceae	Uncl. Erythrobacteraceae	0	0,028206093	0	0	0
Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Uncl. Sphingomonadaceae	0	0,065814216	0	0	0

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	phylum	class	order	family	genus	U5	U7	U5-DB	U5-CO	U5-N
Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	0	0	0	0	0,006915629
Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Alcaligenaceae	Uncl. Alcaligenaceae	0	0,028206093	0	0	0
Bacteria	Proteobacteria	Betaproteobacteria	Hydrogenophilales	Hydrogenophilaceae	Thiobacillus	0	0,028206093	0	0	0
Bacteria	Proteobacteria	Betaproteobacteria	MND1	Uncl. Betaproteobacteria	Uncl. Betaproteobacteria	0	0,009402031	0	0	0
Bacteria	Proteobacteria	Deltaproteobacteria	Other	Other	Other	0,024597221	0	0	0	0
Bacteria	Proteobacteria	Deltaproteobacteria	Uncl. Deltaproteobacteria	Uncl. Deltaproteobacteria	Uncl. Deltaproteobacteria	0,332062477	0,235050771	0	0	0
Bacteria	Proteobacteria	Deltaproteobacteria	Bdellovibrionales	Bacteriovoraceae	Uncl. Bacteriovoraceae	0	0	0	0,019702492	0
Bacteria	Proteobacteria	Deltaproteobacteria	Bdellovibrionales	Bacteriovoraceae	Bacteriovorax	0,01229861	0	0	0	0
Bacteria	Proteobacteria	Deltaproteobacteria	Desulfarculales	Desulfarculaceae	Uncl. Desulfarculaceae	0,135284713	0,065814216	0	0	0
Bacteria	Proteobacteria	Deltaproteobacteria	Desulfobacterales	Desulfobacteraceae	Other	0,073791662	0	0	0	0
Bacteria	Proteobacteria	Deltaproteobacteria	Desulfobacterales	Desulfobacteraceae	Uncl. Desulfobacteraceae	0,590333292	0,103422339	0	0	0,013831259
Bacteria	Proteobacteria	Deltaproteobacteria	Desulfobacterales	Desulfobacteraceae	Desulfococcus	1,598819333	0,639338097	0	0	0,027662517
Bacteria	Proteobacteria	Deltaproteobacteria	Desulfobacterales	Desulfobacteraceae	Desulfofrigus	0,209076374	0	0	0	0
Bacteria	Proteobacteria	Deltaproteobacteria	Desulfobacterales	Desulfobacteraceae	Desulfosarcina	0,46734719	0,018804062	0	0	0
Bacteria	Proteobacteria	Deltaproteobacteria	Desulfobacterales	Desulfobulbaceae	Uncl. Desulfobulbaceae	0,356659697	3,441143287	0,007752539	0	0,006915629
Bacteria	Proteobacteria	Deltaproteobacteria	Desulfuromonadales	Desulfuromonadaceae	Other	0,01229861	0,028206093	0	0	0
Bacteria	Proteobacteria	Deltaproteobacteria	Desulfuromonadales	Desulfuromonadaceae	Uncl. Desulfuromonadaceae	0,836305497	3,986461076	0	0	0,110650069
Bacteria	Proteobacteria	Deltaproteobacteria	Desulfuromonadales	Desulfuromonadaceae	Desulfuromonas	0	0	0	0	0,013831259
Bacteria	Proteobacteria	Deltaproteobacteria	Desulfuromonadales	Pelobacteraceae	Uncl. Pelobacteraceae	0	0,009402031	0	0	0
Bacteria	Proteobacteria	Deltaproteobacteria	Myxococcales	Uncl. Myxococcales	Uncl. Myxococcales	0,122986103	0,188040617	0	0	0
Bacteria	Proteobacteria	Deltaproteobacteria	Myxococcales	OM27	Uncl. Myxococcales	0,01229861	0,009402031	0	0	0
Bacteria	Proteobacteria	Deltaproteobacteria	Myxococcales	Polyangiaceae	Other	0	0,009402031	0	0	0
Bacteria	Proteobacteria	Deltaproteobacteria	NB1-j	Uncl. Deltaproteobacteria	Uncl. Deltaproteobacteria	0	0,009402031	0	0	0
Bacteria	Proteobacteria	Deltaproteobacteria	NB1-j	MND4	Uncl. Deltaproteobacteria	0	0,018804062	0	0	0
Bacteria	Proteobacteria	Deltaproteobacteria	NB1-j	NB1-i	Uncl. Deltaproteobacteria	0	0,141030463	0	0	0
Bacteria	Proteobacteria	Deltaproteobacteria	NKB15	Uncl. Deltaproteobacteria	Uncl. Deltaproteobacteria	0	0,009402031	0	0	0
Bacteria	Proteobacteria	Deltaproteobacteria	Sva0485	Uncl. Deltaproteobacteria	Uncl. Deltaproteobacteria	0,01229861	0,009402031	0	0	0
Bacteria	Proteobacteria	Deltaproteobacteria	Syntrophobacterales	Syntrophobacteraceae	Uncl. Syntrophobacteraceae	0,01229861	0,206844678	0	0	0
Bacteria	Proteobacteria	Epsilonproteobacteria	Campylobacterales	Campylobacteraceae	Arcobacter	0,799409667	0,075216247	0	0	0,138312586
Bacteria	Proteobacteria	Epsilonproteobacteria	Campylobacterales	Campylobacteraceae	Sulfurospirillum	0,01229861	0,009402031	0	0	0

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	phylum	class	order	family	genus	U5	U7	U5-DB	U5-CO	U5-N
Bacteria	Proteobacteria	Epsilonproteobacteria	Campylobacterales	Helicobacteraceae	Uncl. Helicobacteraceae	8,338457754	2,02143663	0,015505078	0,019702492	1,341632089
Bacteria	Proteobacteria	Epsilonproteobacteria	Campylobacterales	Helicobacteraceae	Sulfurimonas	0	0,018804062	0,085277929	0,167471185	0,242047026
Bacteria	Proteobacteria	Gammaproteobacteria	Other	Other	Other	1,033083262	0,197442648	1,775331421	0,256132401	1,867219917
Bacteria	Proteobacteria	Gammaproteobacteria	Uncl. Gammaproteobacteria	Uncl. Gammaproteobacteria	Uncl. Gammaproteobacteria	0,098388882	0,11282437	0,007752539	0	0,006915629
Bacteria	Proteobacteria	Gammaproteobacteria	Aeromonadales	Aeromonadaceae	Other	0	0,065814216	0	0	0
Bacteria	Proteobacteria	Gammaproteobacteria	Aeromonadales	Aeromonadaceae	Uncl. Gammaproteobacteria	0,01229861	0,037608123	0,015505078	0	0
Bacteria	Proteobacteria	Gammaproteobacteria	Aeromonadales	Aeromonadaceae	Oceanimonas	0,01229861	0,235050771	0	0	0
Bacteria	Proteobacteria	Gammaproteobacteria	Aeromonadales	Aeromonadaceae	Zobellella	0	0,009402031	0	0	0
Bacteria	Proteobacteria	Gammaproteobacteria	Alteromonadales	Other	Other	0,528840241	0	0,271338863	0,453157324	0,193637621
Bacteria	Proteobacteria	Gammaproteobacteria	Alteromonadales	Uncl. Alteromonadales	Uncl. Alteromonadales	0,036895831	0,028206093	0	0,009851246	0,006915629
Bacteria	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	Other	0,01229861	0	0,007752539	0,059107477	0
Bacteria	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	Marinobacter	0	0	8,628575859	42,78396217	6,749654219
Bacteria	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	Microbulbifer	0	0	0,031010156	0	0
Bacteria	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	ZD0117	0	0,009402031	0	0	0
Bacteria	Proteobacteria	Gammaproteobacteria	Alteromonadales	Colwelliaceae	Uncl. Colwelliaceae	0,676423564	0,094020308	0	0	0
Bacteria	Proteobacteria	Gammaproteobacteria	Alteromonadales	Ferrimonadaceae	Ferrimonas	0	0	0	0	0,006915629
Bacteria	Proteobacteria	Gammaproteobacteria	Alteromonadales	HTCC2188	HTCC	0	0,037608123	0	0	0
Bacteria	Proteobacteria	Gammaproteobacteria	Alteromonadales	Idiomarinaceae	Other	0	0	0,023257617	0,118214954	0
Bacteria	Proteobacteria	Gammaproteobacteria	Alteromonadales	Idiomarinaceae	Idiomarina	0	0	0,038762695	0	0
Bacteria	Proteobacteria	Gammaproteobacteria	Alteromonadales	Idiomarinaceae	Pseudidiomarina	0	0	0,038762695	0	0
Bacteria	Proteobacteria	Gammaproteobacteria	Alteromonadales	OM60	Uncl. Alteromonadales	4,673471898	0,66754419	0	0	0,013831259
Bacteria	Proteobacteria	Gammaproteobacteria	Alteromonadales	OM60	Congregibacter	0	0,009402031	0	0	0
Bacteria	Proteobacteria	Gammaproteobacteria	Alteromonadales	Psychromonadaceae	Psychromonas	4,169228877	3,638585935	0	0	0
Bacteria	Proteobacteria	Gammaproteobacteria	Alteromonadales	Shewanellaceae	Shewanella	2,631902595	0,949605115	0,116288084	0,009851246	0,027662517
Bacteria	Proteobacteria	Gammaproteobacteria	Chromatiales	Other	Other	0,024597221	0	0	0	0
Bacteria	Proteobacteria	Gammaproteobacteria	Chromatiales	Uncl. Chromatiales	Uncl. Chromatiales	6,395277334	0,611132005	0	0	0,096818811
Bacteria	Proteobacteria	Gammaproteobacteria	Chromatiales	Chromatiaceae	Other	0,036895831	0	0	0	0
Bacteria	Proteobacteria	Gammaproteobacteria	Chromatiales	Chromatiaceae	Uncl. Chromatiaceae	0,356659697	0	0	0	0
Bacteria	Proteobacteria	Gammaproteobacteria	Chromatiales	Ectothiorhodospiraceae	Uncl. Ectothiorhodospiraceae	0,024597221	0	0	0	0
Bacteria	Proteobacteria	Gammaproteobacteria	HTCC2188	HTCC2089	Uncl. Gammaproteobacteria	0,024597221	0,009402031	0	0	0

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	phylum	class	order	family	genus	U5	U7	U5-DB	U5-CO	U5-N
Bacteria	Proteobacteria	Gammaproteobacteria	Legionellales	Uncl. Legionellales	Uncl. Legionellales	0	0,047010154	0	0	0
Bacteria	Proteobacteria	Gammaproteobacteria	Legionellales	Coxiellaceae	Uncl. Coxiellaceae	0,01229861	0,009402031	0	0	0
Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Other	Other	0	0	0,015505078	0	0
Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Uncl. Oceanospirillales	Uncl. Oceanospirillales	0	0	0,007752539	0	0
Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Alcanivoracaceae	Alcanivorax	0	0	58,2680828	30,38124323	0
Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Halomonadaceae	Other	0	0	0	0,019702492	1,639004149
Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Halomonadaceae	Candidatus Portiera	0,01229861	0	0	0,039404985	0
Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Halomonadaceae	Cobetia	0	0	0	0,019702492	0
Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Halomonadaceae	Halomonas	0	0	0,015505078	0,098512462	15,5186722
Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Oceanospirillaceae	Other	0	0	0,124040623	0,009851246	0
Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Oceanospirillaceae	Uncl. Oceanospirillaceae	0	0	0,093030467	0,049256231	0,013831259
Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Oceanospirillaceae	Marinobacterium	0	0	11,35746957	17,50566447	0
Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Oceanospirillaceae	Nitriicola	0	0	5,256221413	0,679735987	1,092669433
Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Oceanospirillaceae	Oleibacter	0	0	1,403209551	0,187173677	0
Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Oceanospirillaceae	Oleispira	0	1,090635577	0	0	0
Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Other	Other	0	0,159834524	0	0	0
Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Uncl. Moraxellaceae	0,024597221	0,902594961	0	0	0
Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter	0	0	0	0	0,027662517
Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Psychrobacter	0	0,009402031	0	0	0
Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Other	0	0	0,759748818	0	0
Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Uncl. Pseudomonadaceae	0	0	0,007752539	0	0
Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	0	0,084618278	8,938677417	0,039404985	0,691562932
Bacteria	Proteobacteria	Gammaproteobacteria	Thiohalorhabdales	Uncl. Thiohalorhabdales	Uncl. Thiohalorhabdales	0,528840241	0,206844678	0	0	0,006915629
Bacteria	Proteobacteria	Gammaproteobacteria	Thiotrichales	Uncl. Thiotrichales	Uncl. Thiotrichales	0,356659697	0,009402031	0	0	0,013831259
Bacteria	Proteobacteria	Gammaproteobacteria	Thiotrichales	Piscirickettsiaceae	Uncl. Piscirickettsiaceae	1,89398598	3,666792027	0	0	0
Bacteria	Proteobacteria	Gammaproteobacteria	Thiotrichales	Piscirickettsiaceae	Methylophaga	0	0	0,837274207	0,019702492	3,665283541
Bacteria	Proteobacteria	Gammaproteobacteria	Thiotrichales	Thiotrichaceae	Uncl. Thiotrichaceae	3,185340057	0,11282437	0	0	0
Bacteria	Proteobacteria	Gammaproteobacteria	Thiotrichales	Thiotrichaceae	Cocleimonas	0	0,094020308	0	0	0
Bacteria	Proteobacteria	Gammaproteobacteria	Vibrionales	Other	Other	0,024597221	0	0	0	0
Bacteria	Proteobacteria	Gammaproteobacteria	Vibrionales	Pseudoalteromonadaceae	Pseudoalteromonas	2,41052761	4,992478375	0,007752539	0	0

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	phylum	class	order	family	genus	U5	U7	U5-DB	U5-CO	U5-N
Bacteria	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	Other	0,147583323	0,009402031	0	0	0
Bacteria	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	Uncl. Vibrionaceae	2,570409544	0,047010154	0	0	0
Bacteria	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	Photobacterium	0,110687492	0	0	0	0
Bacteria	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	Vibrio	0,528840241	0	0	0,531967294	0
Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	Uncl. Sinobacteraceae	0	0,056412185	0	0	0
Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	Steroidobacter	0	0,009402031	0	0	0
Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Lysobacter	0	0,009402031	0	0	0
Bacteria	Proteobacteria	Gammaproteobacteria	[Marinicellales]	[Marinicellaceae]	Uncl. Gammaproteobacteria	5,460582954	3,525761565	0	0	0,041493776
Bacteria	Proteobacteria	TA18	CV90	Uncl. Proteobacteria	Uncl. Proteobacteria	0,01229861	0,028206093	0	0	0
Bacteria	Proteobacteria	Zetaproteobacteria	Mariprofundales	Mariprofundaceae	Mariprofundus	0	0	0	0	0,013831259
Bacteria	SAR406	AB16	Uncl. Bacteria	Uncl. Bacteria	Uncl. Bacteria	0	0	0	0	0,006915629
Bacteria	SAR406	AB16	Arctic96B-7	A714017	so4B24	0,01229861	0,056412185	0	0	0
Bacteria	SBR1093	VHS-B5-50	Uncl. Bacteria	Uncl. Bacteria	Uncl. Bacteria	0	0,047010154	0	0	0
Bacteria	Spirochaetes	Spirochaetes	Spirochaetales	Spirochaetaceae	Uncl. Spirochaetaceae	0,024597221	0,047010154	0	0	0
Bacteria	Spirochaetes	Spirochaetes	Spirochaetales	Spirochaetaceae	Spirochaeta	0,233673595	0,103422339	0	0	0
Bacteria	Spirochaetes	[Brachyspirae]	[Brachyspirales]	A0-023	Uncl. A0-023	0	0,009402031	0	0	0
Bacteria	Spirochaetes	[Leptospirae]	[Leptospirales]	Sediment-4	SJA-88	0	0,018804062	0	0	0
Bacteria	TM6	SBRH58	Uncl. Bacteria	Uncl. Bacteria	Uncl. Bacteria	0	0,009402031	0	0	0
Bacteria	TM6	SJA-4	Uncl. Bacteria	Uncl. Bacteria	Uncl. Bacteria	0,01229861	0,009402031	0	0	0
Bacteria	TPD-58	Uncl. Bacteria	Uncl. Bacteria	Uncl. Bacteria	Uncl. Bacteria	0	0,028206093	0	0	0
Bacteria	Tenericutes	Mollicutes	Uncl. Mollicutes	Uncl. Mollicutes	Uncl. Mollicutes	0	0,056412185	0	0	0,034578147
Bacteria	Tenericutes	Mollicutes	Acholeplasmatales	Acholeplasmataceae	Uncl. Acholeplasmataceae	0	0	0	0	0,006915629
Bacteria	Verrucomicrobia	Opitutae	Uncl. Mollicutes	Uncl. Mollicutes	Uncl. Mollicutes	0,01229861	0,018804062	0	0	0
Bacteria	Verrucomicrobia	Opitutae	Opitutales	Opitutaceae	Opitutus	0	0,018804062	0	0	0
Bacteria	Verrucomicrobia	Opitutae	[Pelagicoccales]	[Pelagicocccaceae]	Pelagicococcus	0	0,009402031	0	0	0
Bacteria	Verrucomicrobia	Verruco-5	Uncl. Verrucomicrobia	Uncl. Verrucomicrobia	Uncl. Verrucomicrobia	0	0,009402031	0	0	0
Bacteria	Verrucomicrobia	Verruco-5	LD1-PB3	LD1-PA34	Uncl. LD1-PA34	0	0,009402031	0	0	0
Bacteria	Verrucomicrobia	Verruco-5	MSBL3	Uncl. Verrucomicrobia	Uncl. Verrucomicrobia	0,01229861	0	0	0	0
Bacteria	Verrucomicrobia	Verruco-5	R76-B128	Uncl. Verrucomicrobia	Uncl. Verrucomicrobia	0,110687492	0,141030463	0	0	0
Bacteria	Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	Uncl. Verrucomicrobiaceae	0,024597221	0,009402031	0	0	0

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	phylum	class	order	family	genus	U5	U7	U5-DB	U5-CO	U5-N
Bacteria	Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	Haloferula	0,01229861	0	0	0	0
Bacteria	Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	Luteolibacter	0	0	0	0	0,006915629
Bacteria	Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	Persicirhabdus	0	0,094020308	0	0	0
Bacteria	Verrucomicrobia	[Pedosphaerae]	[Pedosphaerales]	Uncl. Verrucomicrobia	Uncl. Verrucomicrobia	0	0,018804062	0	0	0
Bacteria	Verrucomicrobia	[Spartobacteria]	[Chthoniobacterales]	[Chthoniobacteraceae]	DA101	0	0,018804062	0	0	0
Bacteria	WS2	Kazan-3B-09	Uncl. WS2	Uncl. WS2	Uncl. WS2	0,036895831	0,018804062	0	0	0
Bacteria	WS2	SHA-109	Uncl. WS2	Uncl. WS2	Uncl. WS2	0,024597221	0,028206093	0	0	0
Bacteria	WS3	PRR-12	Uncl. WS3	Uncl. WS3	Uncl. WS3	0,393555528	0,028206093	0	0	0
Bacteria	WS3	PRR-12	GN03	Uncl. WS3	Uncl. WS3	0,073791662	0,056412185	0	0	0
Bacteria	WS3	PRR-12	GN03	KSB4	Uncl. WS3	1,217562415	0,488905604	0	0	0
Bacteria	WS3	PRR-12	LD1-PA13	Uncl. WS3	Uncl. WS3	0,049194441	0	0	0	0
Bacteria	WS3	PRR-12	SSS58A	Uncl. WS3	Uncl. WS3	0,344361087	0	0	0	0,006915629
Bacteria	WS3	PRR-12	Sediment-1	Uncl. WS3	Uncl. WS3	0,098388882	0,150432493	0	0	0
Bacteria	WS3	PRR-12	Sediment-1	CV106	Uncl. WS3	0	0,009402031	0	0	0
Bacteria	WS3	PRR-12	Sediment-1	MSB-4E2	Uncl. WS3	0,147583323	0,018804062	0	0	0
Bacteria	WS3	PRR-12	Sediment-1	PRR-10	Uncl. WS3	0	0,028206093	0	0	0
Bacteria	WWE1	[Cloacamonae]	[Cloacamonales]	MSBL8	Uncl. WWE1	0	0,009402031	0	0	0
Bacteria	[Caldithrix]	KSB1	Uncl. Bacteria	Uncl. Bacteria	Uncl. Bacteria	0	0,009402031	0	0	0
Bacteria	[Caldithrix]	KSB1	GW-22	Uncl. Bacteria	Uncl. Bacteria	0,036895831	0	0	0	0

Table S2

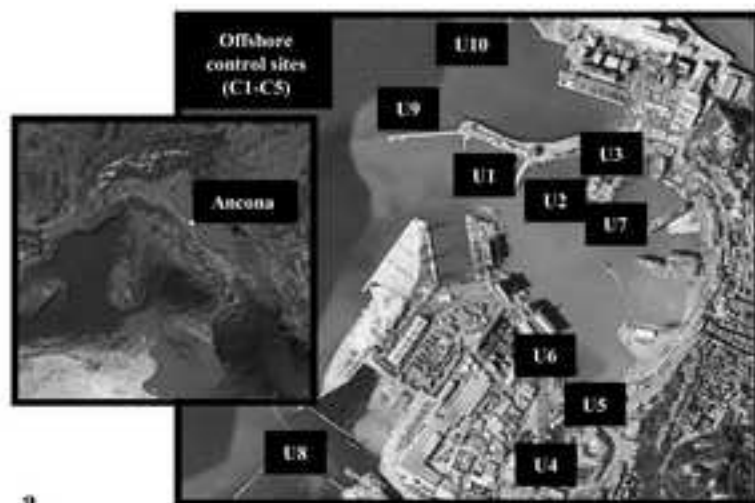
alkB DGGE bands identification. Bands excised from DGGE gels are indicated on Figure 2d.

BAND	SAMPLE	CLOSEST RELATIVE	GENE	ACC N	LENGHT	%	CLOSEST DESCRIBED SPECIE	GENE	ACC N	LENGHT	%
1	U7-CO	Uncultured bacterium	alkane hydroxylase B (<i>alkB</i>) gene	JQ279687	369/453	81	<i>Marinobacter adhaerens</i>	complete genome	CP001978	364/449	81
2	U7-CO	Uncultured bacterium	alkane hydroxylase B (<i>alkB</i>) gene	JQ279687	364/443	82	<i>Marinobacter adhaerens</i>	complete genome	CP001978	359/441	81
3	U7-CO	Uncultured bacterium	alkane hydroxylase (<i>alkB</i>) gene	JN986869	364/434	84	<i>Marinobacter adhaerens</i>	complete genome	CP001978	357/428	83
4	U7-CO	Uncultured bacterium	alkane hydroxylase (<i>alkB</i>) gene	JN986869	384/452	85	<i>Marinobacter adhaerens</i>	complete genome	CP001978	375/451	83
5	U7-DB	Uncultured bacterium	alkane monooxygenase (<i>alkB</i>) gene	GQ184418	328/431	76	<i>Simidua agarivorans</i>	complete genome	CP003746	134/173	77
6	U7-DS	Uncultured bacterium	alkane monooxygenase (<i>alkB</i>) gene	JX276499	316/343	92	<i>Marinobacter hydrocarbonoclasticus</i>	alkane hydroxylase (<i>alkB</i>) gene	EU853368	281/344	82
7	U7-CO	Uncultured bacterium	alkane monooxygenase (<i>alkB</i>) gene	JQ279687	368/449	82	<i>Marinobacter adhaerens</i>	complete genome	CP001978	364/449	81
8	U7-DB	Uncultured bacterium	alkane monooxygenase (<i>alkB</i>) gene	JQ279687	366/449	82	<i>Marinobacter adhaerens</i>	complete genome	CP001978	355/437	81
9	U7-CO	Uncultured bacterium	putative alkane monooxygenase (<i>alkB</i>) gene	JQ437623	235/281	84	<i>Alcanivorax borkumensis</i>	alkane hydroxylase (<i>alkB</i>) gene	EU853322	97/123	79
10	U7-CO	Uncultured bacterium	alkane hydroxylase B (<i>alkB</i>) gene	JQ279685	381/451	84	<i>Marinobacter adhaerens</i>	complete genome	CP001978	376/449	84
11	U7-DS	Uncultured bacterium	alkane hydroxylase B (<i>alkB</i>) gene	JQ279687	372/455	82	<i>Marinobacter adhaerens</i>	complete genome	CP001978	363/449	81
12	U7-DS	Uncultured bacterium	alkane hydroxylase B (<i>alkB</i>) gene	JQ279687	383/470	81	<i>Marinobacter adhaerens</i>	complete genome	CP001978	379/466	82
13	U5-CO	Uncultured bacterium	alkane hydroxylase (<i>alkB</i>) gene	JN986861	295/344	86	<i>Alcanivorax hongdengensis</i>	alkane hydroxylase (<i>alkB</i>) gene	EU438898	186/230	81
14	U5-CO	Uncultured bacterium	alkane hydroxylase (<i>alkB</i>) gene	JN986869	358/381	94	<i>Marinobacter adhaerens</i>	complete genome	CP001978	323/377	86
15	U5-CO	Uncultured bacterium	alkane hydroxylase (<i>alkB</i>) gene	JQ279685	352/374	94	<i>Marinobacter adhaerens</i>	complete genome	CP001978	319/375	85
16	U5-DB	<i>Marinobacter adhaerens</i>	complete genome	CP001978	250/316	79	<i>Marinobacter adhaerens</i>	complete genome	CP001978	250/316	79
17	U5-CO	Uncultured bacterium	alkane hydroxylase (<i>alkB</i>) gene	JQ279685	402/418	96	<i>Marinobacter adhaerens</i>	complete genome	CP001978	362/416	87
18	U5-DS	Uncultured bacterium	alkane hydroxylase (<i>alkB</i>) gene	JQ279685	405/418	97	<i>Marinobacter adhaerens</i>	complete genome	CP001978	364/416	88
19	U5-DB	Uncultured bacterium	alkane hydroxylase (<i>alkB</i>) gene	JN986869	417/434	96	<i>Marinobacter adhaerens</i>	complete genome	CP001978	372/428	87
20	U5-DS	Uncultured bacterium	alkane hydroxylase (<i>alkB</i>) gene	JQ279685	403/418	96	<i>Marinobacter adhaerens</i>	complete genome	CP001978	360/416	87
21	U5-DB	Uncultured bacterium	alkane hydroxylase (<i>alkB</i>) gene	JQ279685	408/427	96	<i>Marinobacter adhaerens</i>	complete genome	CP001978	366/427	86
22	U5-CO	Uncultured bacterium	alkane hydroxylase (<i>alkB</i>) gene	JQ279685	294/333	88	<i>Marinobacter hydrocarbonoclasticus</i>	alkane hydroxylase (<i>alkB</i>) gene	EU853368	281/335	84
23	U5-CO	Uncultured bacterium	alkane hydroxylase (<i>alkB</i>) gene	JN986869	409/434	94	<i>Marinobacter adhaerens</i>	complete genome	CP001978	367/428	86
24	U5-CO	Uncultured bacterium	alkane hydroxylase (<i>alkB</i>) gene	JQ279685	408/431	95	<i>Marinobacter adhaerens</i>	complete genome	CP001978	368/429	86
25	U5-DB	Uncultured bacterium	alkane hydroxylase (<i>alkB</i>) gene	JQ279687	342/424	81	<i>Marinobacter hydrocarbonoclasticus</i>	alkane hydroxylase (<i>alkB</i>) gene	EU853368	331/415	80
26	U5-DB	<i>Marinobacter</i> sp. EPR21	hydroxylase (<i>alkB</i>) gene	KC610508	370/403	92	<i>Alcanivorax hongdengensis</i>	alkane hydroxylase (<i>alkB</i>) gene	EU438898	243/295	82
27	U5-DS	Uncultured bacterium	alkane hydroxylase (<i>alkB</i>) gene	JX276502	383/468	82	<i>Marinobacter adhaerens</i>	complete genome	CP001978	381/476	80
28	U5-DB	Uncultured bacterium	alkane hydroxylase (<i>alkB</i>) gene	JQ279687	377/455	83	<i>Marinobacter adhaerens</i>	complete genome	CP001978	365/449	81
29	U5-DB	Uncultured bacterium	alkane hydroxylase B (<i>alkB</i>) gene	JQ279687	390/472	83	<i>Marinobacter adhaerens</i>	complete genome	CP001978	379/466	81
30	U5-CO	Uncultured bacterium	alkane hydroxylase B (<i>alkB</i>) gene	JQ279687	385/472	81	<i>Marinobacter adhaerens</i>	complete genome	CP001978	377/466	81
31	U5-CO	Uncultured bacterium	alkane hydroxylase B (<i>alkB</i>) gene	JN986869	393/435	90	<i>Marinobacter adhaerens</i>	complete genome	CP001978	360/425	85
32	U5-CO	Uncultured bacterium	alkane hydroxylase B (<i>alkB</i>) gene	JQ279685	381/419	91	<i>Marinobacter adhaerens</i>	complete genome	CP001978	349/414	84

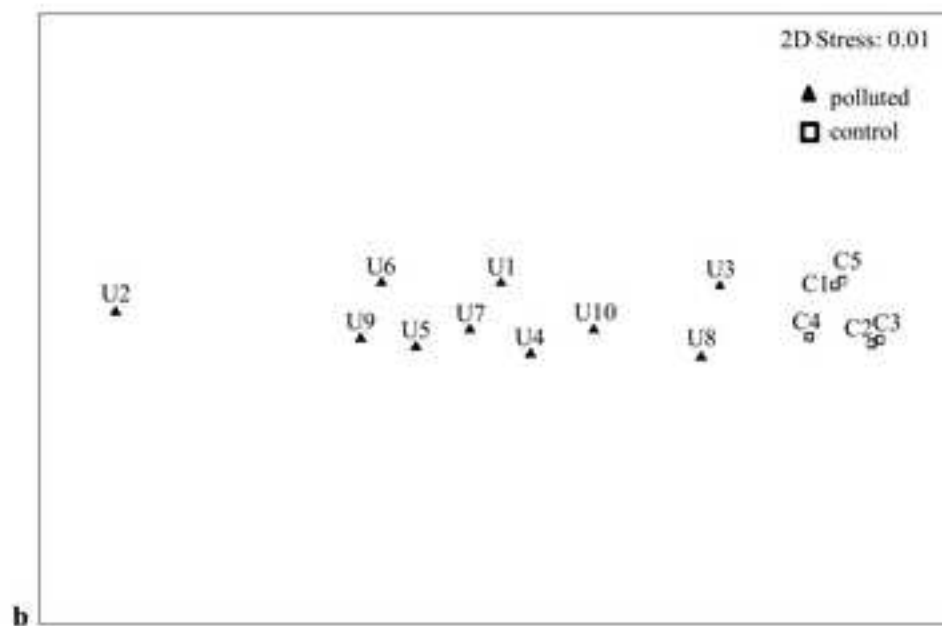
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BAND	SAMPLE	CLOSEST RELATIVE	GENE	ACC N	LENGHT	%	CLOSEST DESCRIBED SPECIE	GENE	ACC N	LENGHT	%
33	U5-CO	Uncultured bacterium	alkane hydroxylase B (alkB) gene	JN986869	357/381	94	Marinobacter adhaerens	complete genome	CP001978	321/377	85
34	U5-CO	Uncultured bacterium	alkane hydroxylase B (alkB) gene	JQ279685	364/389	94	Marinobacter adhaerens	complete genome	CP001978	333/392	85
35	U5-CO	Uncultured bacterium	alkane hydroxylase B (alkB) gene	JQ279685	358/382	94	Marinobacter adhaerens	complete genome	CP001978	321/377	85
36	U5-DS	Uncultured bacterium	alkane hydroxylase B (alkB) gene	JQ279685	461/484	95	Marinobacter adhaerens	complete genome	CP001978	420/482	87
37	U5-DS	Uncultured bacterium	alkane hydroxylase B (alkB) gene	JN986869	417/434	96	Marinobacter adhaerens	complete genome	CP001978	368/428	86
38	U5-DS	Uncultured bacterium	alkane hydroxylase B (alkB) gene	JN986869	417/434	96	Marinobacter adhaerens	complete genome	CP001978	368/428	86
39	U5-DS	Uncultured bacterium	alkane hydroxylase B (alkB) gene	JQ279685	345/367	94	Marinobacter adhaerens	complete genome	CP001978	315/367	86
40	U5-DS	Uncultured bacterium	alkane hydroxylase B (alkB) gene	JQ279685	419/436	96	Marinobacter adhaerens	complete genome	CP001978	368/428	86

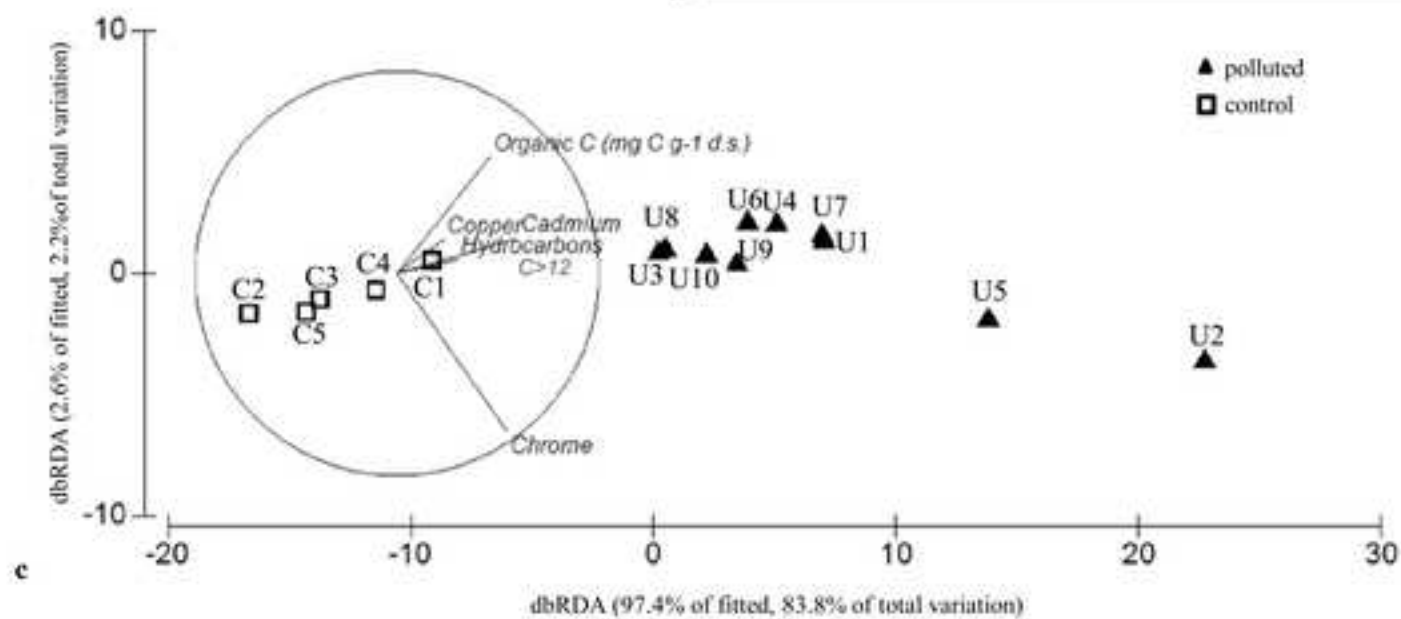
Figure 1
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Figure 2
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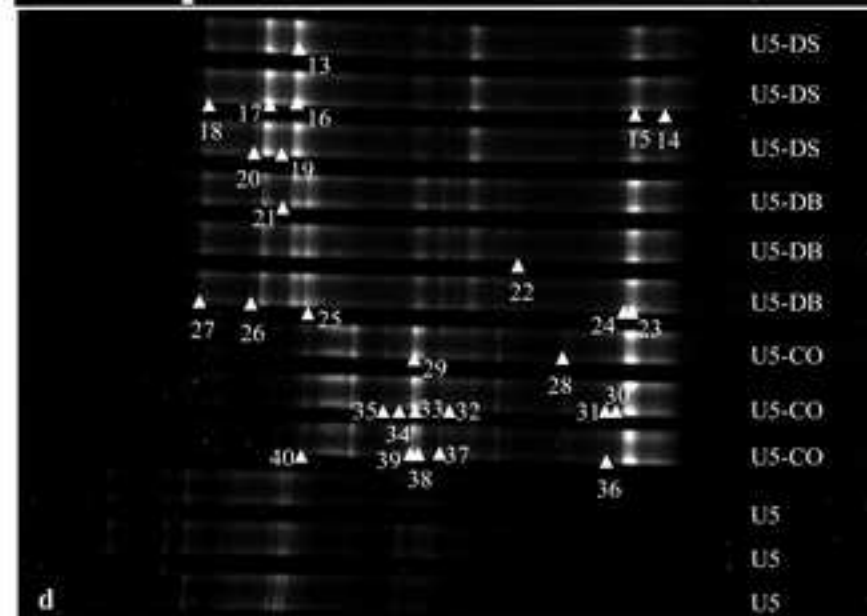
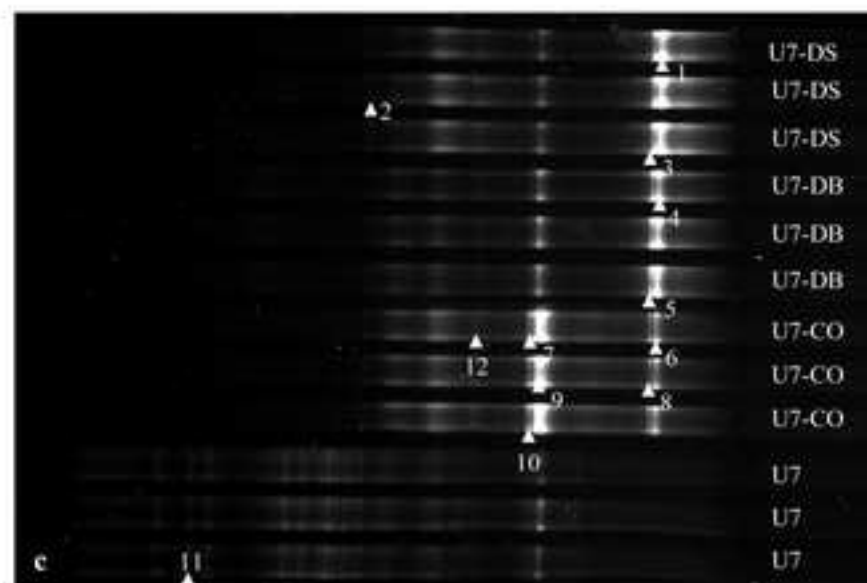
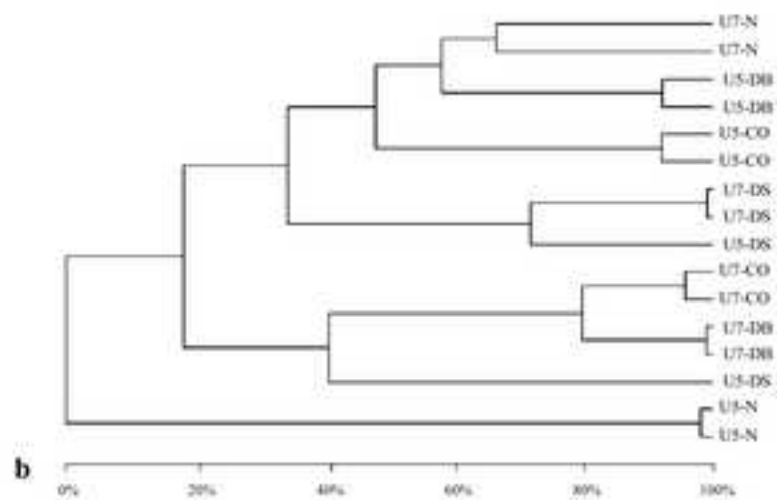
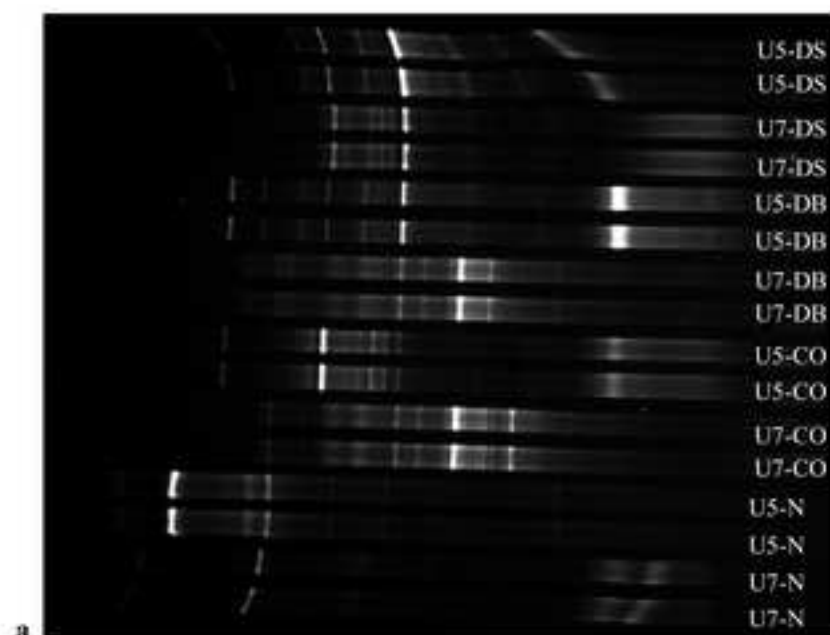
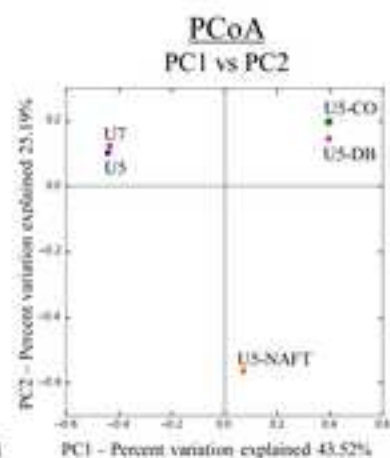


Figure 3
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b

	U7	U5	US-GB	US-CO	US-NAFT
OTU97 tot	1941	1554	429	443	525
OTU97 singleton	965	816	167	178	220
Reads tot	10636	8131	12899	10151	14460
Coverage	0.91	0.90	0.99	0.98	0.98

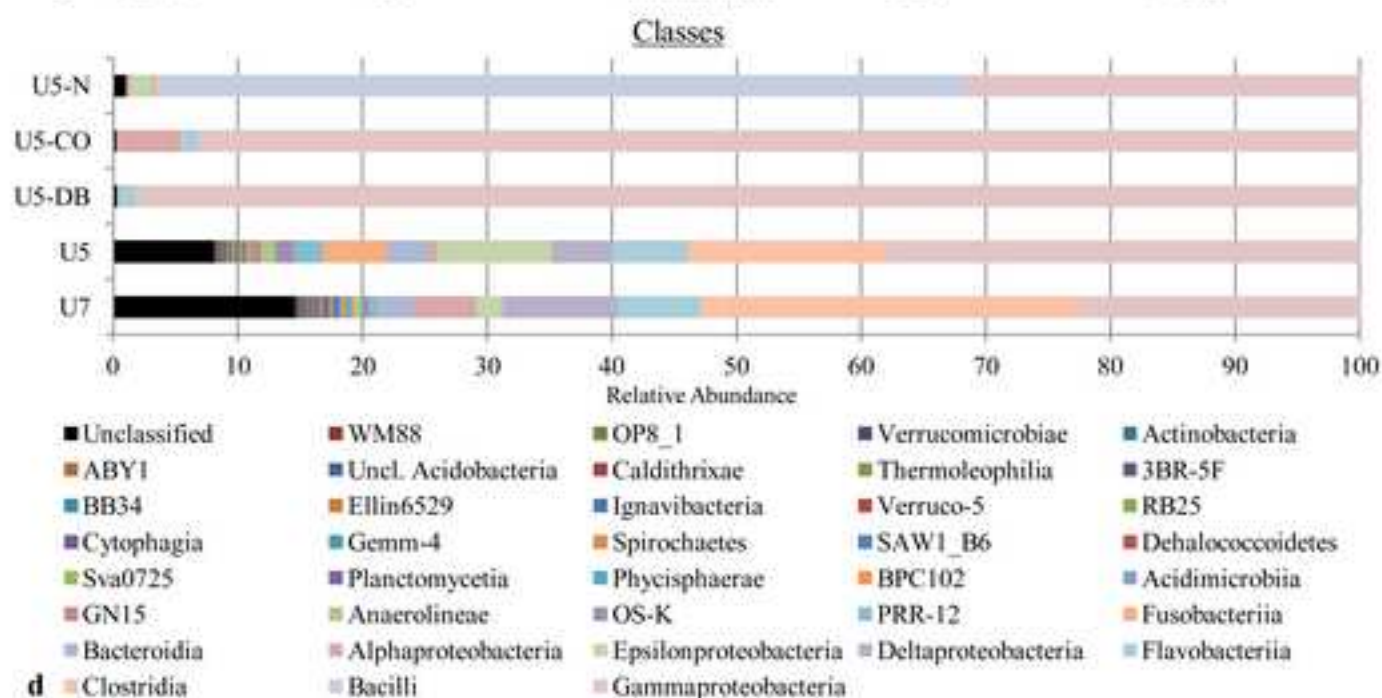
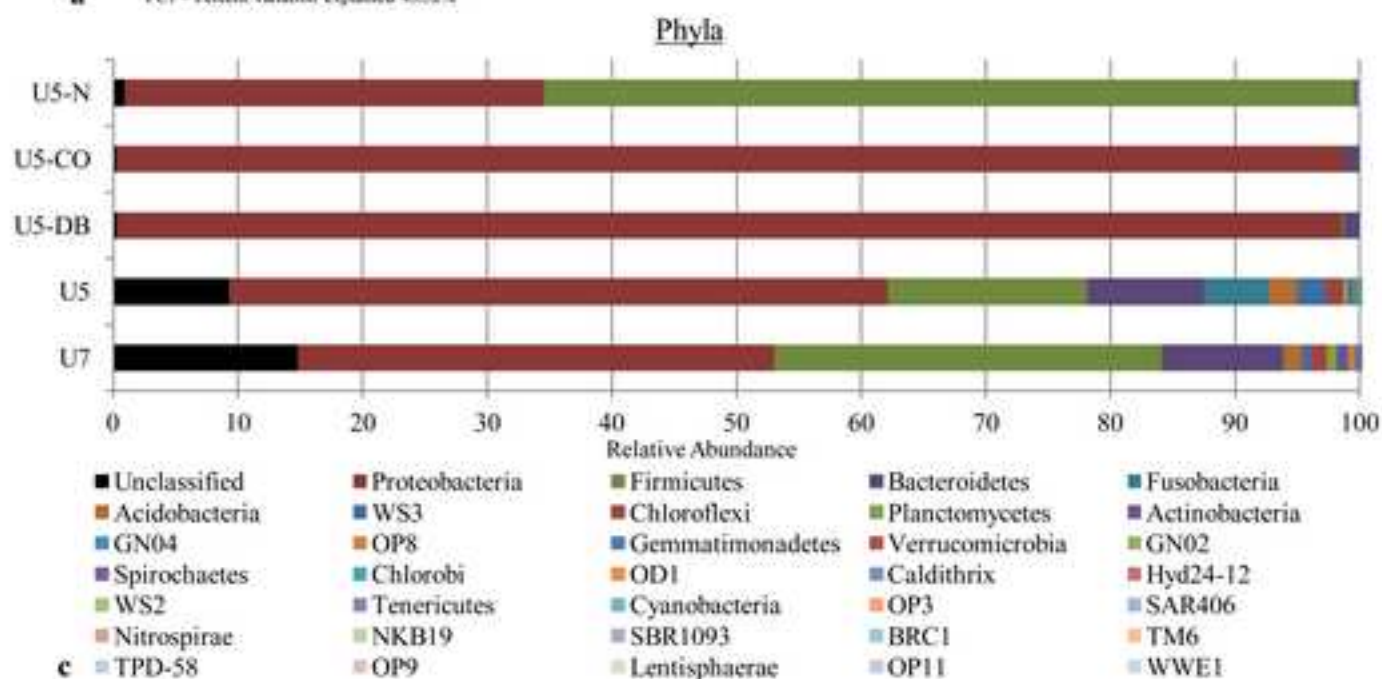
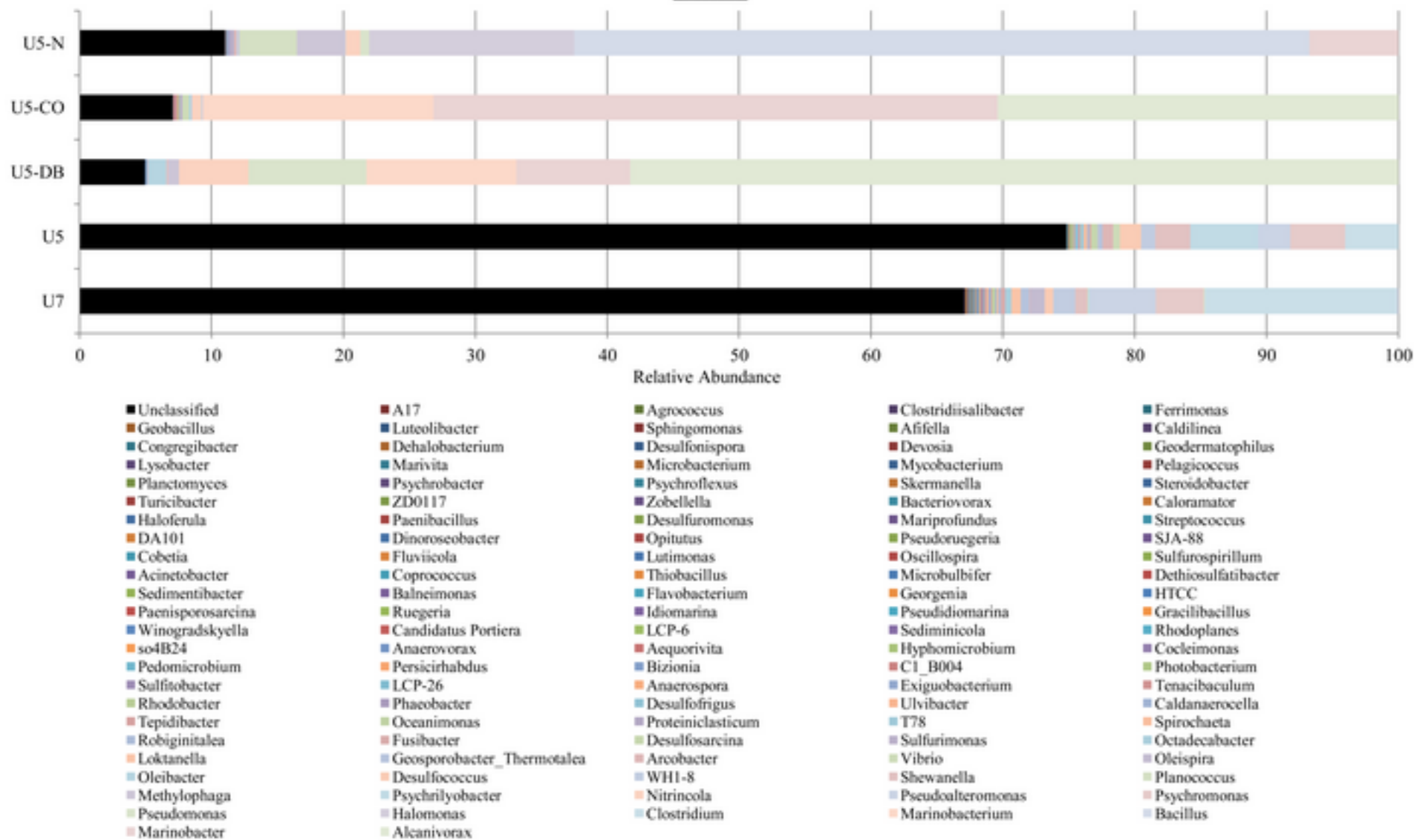


Figure S1

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Genera



Figure(s)
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