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UNIVERSITY OF CALIFORNIA,
IRVINE

Biodegradation of Microcystins by Bacterial Consortia

THESIS

submitted in partial satisfaction of the requirements
for the degree of

MASTER OF SCIENCE

in Engineering
with a specialization in Environmental Engineering

by

Yuen Ming Cheung

Thesis Committee:
Professor Sunny Jiang, Ph.D, Chair
Professor Betty Olson, Ph.D
Professor Diego Rosso, Ph.D

2015

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This thesis is dedicated to God almighty. Thank you for giving me the strength and courage to pull through. I could not have done it without you

ABSTRACT OF THE THESIS

Biodegradation of Microcystins by Bacterial Consortia

By

Yuen Ming Cheung

Master of Science in Engineering

With a Concentration in Environmental Engineering

University of California, Irvine, 2015

Professor Sunny Jiang, Chair

Increases in frequency and intensity of freshwater cyanobacterial blooms (cyanoHABs) place significant challenges to drinking water plants for human health protection from exposure to algal toxins. Specifically, microcystins (MC), the hepatotoxin produced by cyanobacteria, is not effectively removed by the current water treatment processes. The goal of this study is to isolate and characterize bacteria that are capable of degrading MC in order to develop biofilters that can effectively remove the toxin. Water and sediment samples from a drinking water reservoir and a freshwater pond were collected and enriched for MC degrading bacteria. Among nearly 100 fast growing bacterial isolates were obtained from the enrichment culture and identified by gene sequencing, none were MC degraders due to the carryover of the trace amount of ethanol in the MC stock that were used by fast growing non-degraders. On the contrary, the bacterial consortia obtained from enrichment cultures can efficiently degrade MC. Although both consortia enriched in the presence or absence of ethanol can perform MC degradation, the degradation rates were improved for most of the consortia when the ethanol was removed from the enrichment culture in spite of lower cell growth rate. This indicates that the fast growing bacteria in the enrichment culture are not the key players in the degradation of MC. Bacterial community analysis also confirmed the higher diversity in consortia associated with ethanol. However, there is not a clear separation of bacterial diversity between the MC degrading and non-degrading consortia, indicating the complexity of consortia in MC degradation.

1. INTRODUCTION

1.1 Background

Cyanobacterial harmful algal bloom (cyanoHABs) in freshwater is a global problem facing by water authorities worldwide. The toxins produced by toxigenic cyanobacteria pose a health risk to the public. Cyanotoxins are currently not regulated by EPA drinking water regulations, but they are likely subject to monitoring under Unregulated Contaminant Monitoring Rule which is expected to begin in 2018 [1].

The Metropolitan Water District of Southern California (MWDSC) has drinking water reservoirs which have suffered from algal problems of planktonic blooms and benthic proliferations for years. Some blooms were composed of toxigenic cyanobacteria, *Microcystis* and benthic *Phormidium sp*, which produce hepatotoxic microcystin (MC) [2]. After the severe toxic algal bloom happened in two reservoirs, Lake Skinner and Silverwood Lake in 2001, MWDSC began monitoring the cyanotoxin in their system [2, 3]. The concentration of MC in the water samples collected from MWDSC's system ranged from 0.116 μg/L to 55.27 μg/L [2]. Some are much higher than 1 μg/L of microcystin-LR (MC-LR) under WHO drinking water guideline [4] and 0.8 μg/L of microcystin under California recreational water guideline [5].

MC cannot be effectively removed by conventional water treatment alone. The rapid algal growths in the reservoir are officially controlled with algicide copper sulfate [6]. The drawback of this approach is that algicide may lyse the cyanobacterial cell to release MC rapidly and cause secondary contamination of copper to aquatic biota [7]. Previous studies have shown that biodegradation is a viable method to eliminate MC in effective manner without causing harmful byproduct [8,9,10].

Lake with prolonged record of algal bloom containing microcystin may have endemic bacteria which are able to degrade the toxin [11], such that water and sediments samples for this study were collected at Lake Skinner, which has experienced algal blooms and developed benthic algal mats [2]. It is one of the reservoirs in Riverside County operated by MWDSC, locating at south and west of Los Angeles. As illustrated in Figure 1.1, Lake Skinner receives water from northern California rivers and streams under California State Water

Project and Colorado River. It provides water for San Diego region and its southern region [2,6]. Besides, it also provides recreational area for the public to fishing and sailing [3]. When toxic cyanobacterial bloom happened in 2001, the MC concentration of two surface bloom material sample was 304μg/L in average [3]. It is hypothesized that the bacteria originated from such lake may have capability of MC degradation.

Figure 1.1 Location of Lake Skinner and MWDSC service area

1.2 Research Objective

The intent of this study is to obtain a better understanding of biodegradation by novel microcystin degrading bacteria and consortia explored from lakes with toxic algal bloom record. The specific objectives are as the followings:

1. To isolate and identify bacteria that are able to degrade MC, and determine their MC removal efficiency
2. To evaluate the biodegradation performance of different consortia using enzyme linked immunosorbent assays (ELISA) in terms of:
   - degradation rate
   - substrate specificity
   - consistency under environment with and without organic compound
3. To characterize MC degrading consortia and MC non-degrading consortia and to reveal any selective response of their bacterial community to MC or linking between the community and their MC degradation capability

2. LITERATURE REVIEW

2.1 Cyanobacterial Harmful Algal Blooms (cyanoHABs)

Cyanobacterial harmful algal blooms are overgrowth of toxin-producing cyanobacteria. Cyanobacteria are called blue green algae and known to be photosynthetic bacteria. They ubiquitously exist in diverse habitats, freshwater and marine. They have many ecological functions, such as nitrogen fixation and cycling of nutrients in the food chain [12].

Due to sewage from failing sewage systems, urban runoff or agriculture discharge with abundance nutrient, especially phosphorous but also nitrogen, low flow induced by urbanization and climate change, the eutrophic freshwater resources with optimal water temperatures 15-30°C, and pH 6-9 favor the proliferation of cyanobacteria [4]. The frequency of harmful algal bloom increased worldwide [13].

More than 40% of lakes and reservoirs in America, Europe and Asia are now eutrophic and provide favorable conditions for massive cyanobacterial bloom [14]. It is reported that at least one third of known cyanobacteria can produce toxins and 50-70% cyanobacteria blooms are toxic [15]. The cyanotoxins are classified into four categories: Neurotoxins, Hepatotoxins, General Cytotoxins and Endotoxins.

2.2. Microcystin

Microcystin (MC) is considered as the cyanotoxin with most concern in the United States [13]. It is the cyanotoxin which is the most widespread and the best known from both its health impacts and available detection methods [16]. Concern of the detrimental health impact of consuming water contaminated by MCs has been raised after the first recorded 50 deaths of human attributed to MCs happened in Brazil in 1996. In the assessment of blue-green algae toxins in drinking water conducted by Carmichael in 2001, it was demonstrated that 80% samples of utility source waters in the United States contain MCs [17]. MWDSC, whose operated freshwater reservoir is the sampling area of this study, was a participator in this assessment. Low levels of MCs were detected in its plant influents and even some effluents [2].

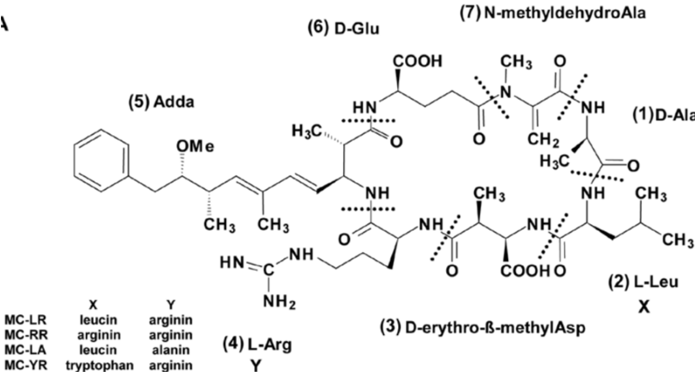


Figure 2.1 Structure of Microcystin-LR [18]

*dashed line indicated the bonding between different amino-acids

MC is monocyclic hepatotoxic peptide produced by several cyanobacteria genera, including *Microcystis*, *Anabaena*, *Nostoc*, *Oscillatoria* and *Planktothrix*. Its natural physiological or ecological function are still unclear [19]. It was suggested that it functions as metal ion carrier to transport iron across the cell membrane and acts as infochemical for cyanobacteria to signal their population when they are under stress conditions [20]. It is the secondary metabolite constituent by 7 non-ribosomal amino acid (5 constant non-protein amino acid and 2 variable protein amino acid). The general structure of MC is cyclo-D-alanine-X-erythro-β-methyl-D-aspartic acid (iso)-Y-Adda-D-glutamic acid (iso)-N-methyldehydroalanine with two non-coserved sites X and Y in the ring structure (Figure 2.1). Adda is 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyl-deca-4,6-dienoic acid. Molecular weight of MCs is from 909 to 1115 [21]. This metabolite exists in significant amount of cyanobacterial biomass (up to 1% of dry weight) [21]. Mostly, MCs exist intracellularly. They are released when the MC-producing cyanobacteria dies and the cell membrane ruptures (Table 2.2) [22].

MC has more than 90 variants [19]. Microcystin-LR (MC-LR) is the most representative variant which generally comprises 50-100% of the total MC in environmental samples [13]. The structure of MC-LR is cyclo-D-alanine-L-leucine-erythro-β-methyl-D-aspartic acid (iso)-L-arginine-Adda-D-glutamic acid (iso)-N-methyldehydroalanine. MC-LR is the only variant, which a drinking water guideline has been set by the World Health Organization (WHO) in 1998 [4]. For human health risks assessment purposes, WHO derived the

value of tolerably daily intake (TDI) for MC-LR in 0.04 mg/kg of body weight, based on liver pathology observed in a 13-week study in mice and applying an uncertainty factor of 1000 [23]. Based on 80% TDI, average adult body weight of 60 kg and an average water intake for adults of 2 litres per day, WHO recommended maximal acceptable concentration of MC-LR is 1 μg/L in drinking water [4]. The calculation was as follows:

80% x 0.04 μg microcystin/kg body weight/day x 60 kg person / 2 L water/day = 0.96 μg/L ~1 μg/L

MCs not only pose a threat to our drinking water system, but also have risk to human through bioaccumulation. It is documented that MC accumulates in aquatic organism, including aquatic macrophytes and fauna [24,25]

Table 2.1: Nominated cyanotoxins in USEPA candidate contaminant list – Microcystin

Number of known variants	**Primary organ affected**	**Health Effects***	**Responsible Cyanobacteria**
80-90	Liver	Abdominal pain Vomiting and diarrhea Liver inflammation and hemorrhage Acute pneumonia Acute dermatitis Kidney damage Potential tumor growth promotion	*Microcystis* *Anabaena* *Planktothrix* *Anabaenopsis* *Aphanizomenon*

*Source: Harmful Algal Research and Response National Environmental Science Strategy (HARRNESS)

Table 2.2: Distribution of MCs during laboratory culture of *Microcystis aeruginosa* [22]

Age of culture	**Distribution of toxins (%)**	
	Cells	**Water**
Young		
Slowly-growing cells	100	0
Rapidly-growing cells	75-90	10-25
Old		
Slowly-growing intact cells	70-80	20-30
Decaying cells (leaking cell contents)	30-40	60-70

2.4 Toxicity of Microcystin

Microcystin-LR is an extremely acute toxin. Liver is the target organ of microcystin. Its toxicity mainly induced by irreversible inhibition of protein phosphatases PP1 and PP2A, which are key regulatory enzymes in catalyzing dephosphorylation of serine/threonine residues in various phosphoproteins. The inhibition of these enzyme results in loss of cytoskeletal integrity and apoptosis of hepatocytes [26]. The other adverse impact of microcystin suggested by epideminology study includes causing oxidative stress and promoting liver tumor formation [21]. Liver damage caused by MCs may ultimately lead to lethal in mammals.

Methyl-dehydroalanine (Mdha) and 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyl-deca-4,6-dienoic acid (Adda) moiety are integral to binding of microcystins to protein phosphatases in organisms [27] Both the cyclic structure and Adda are necessary for causing toxicity of microcystin. The linearised MC is more than 100 times less toxic than cyclic MC and the other degraded MC products, including the tetrapeptide and intact Adda were found essentially non-toxic [14, 28]. The MC variants used in this study, MC-LR and MC-LA was found having similar toxicological effect [29]. The toxicity of MC and its enzymatic degraded products are summarized in Table 2.3.

Table 2.3: Toxicity of MC and its enzymatic degradation products [30]

	Polypeptide	**IC_{50} (nM)**
Enzymatic degradation product of MC	H_2N-Adda-isoGlu-Mdha-Ala-Leu-Masp-Arg-OH	95
	H_2N-Adda-isoGlu-Mdha-Ala-OH	12
MC-LR, MC-LA, MC-YR	Cyclo-D-alanine-X-erythro-β-methyl-D-aspartic acid (iso)-Y-Adda-D-glutamic acid (iso)-N-methyldehydroalanine	0.3

** IC_{50}: Half maximal inhibitory concentration; nM: nanomolar

2.5 Analytical Methods

MC can be determined by measuring its concentration or measuring toxicity. Detection method of the toxin includes high performance liquid chromatography (HPLC) coupled with UV, photodiode array (PDA) or mass spectrometry (MS) detection following toxin extraction and concentration (Figure 2.2). The biological method includes enzyme linked immunosorbent assays (ELISAs). The toxicity assay is protein phosphatase inhibition assay (PPIA) [13, 14].

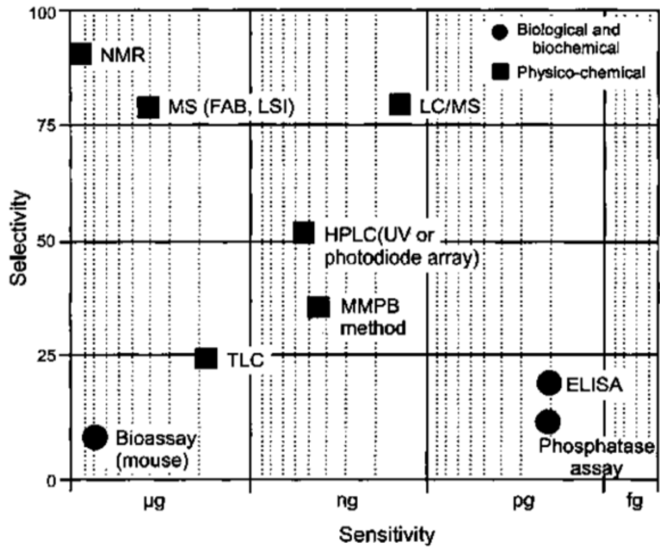


Figure 2.2 Relationship between sensitivity and selectivity of analytical methods of Microcystin [14]

2.6 ELISA Enzyme-linked immunosorbent assays

First ELISA method for practical MC quantification was developed by Chu et al. (1989) [17]. ELISA kits for determination of MC are effective in measuring MC concentration over a range of five orders of magnitude with appropriate dilutions [13]. Comparing to instrumental analytical method such as HPLC or LC/MS/MS, ELISA method has been verified as inexpensive, less time consuming, highly specific, lower detection limits and not highly skilled personnel required [13]. However, ELISA is less accurate for samples with presence of congener in different amount since standard reference MC in ELISA kits are used for quantification of all MC variants and congener (such as nodularin) [13]. In most toxic algal bloom, more than one MC variant could be present. These MC congeners may induce different cross reactivities in ELISA.

With these considerations, ELISA kits are currently utilized for screening propose of MC in freshwater samples [14].

ELISA is the most sensitive biochemical assay to detect MC (Figure 2.2). This assay is based on the specific antigen-antibody interaction. It is in microplate format. Current commercial MC ELISA kits can be divided into two types in term of target group of MC, one targets Adda moiety (Abraxis) and the other targets Leucine and Arginine in MC's cyclic structure (EnviroLogix, Beacon). Both of them are indirect competitive ELISA assay. Briefly, MCs in the samples compete with antigen (MC congener) immobilized on a microplate for the binding to the antibodies. After incubation, soluble molecules are washed away. Secondary antibody labeled with enzyme is added and reacts with the immobilized antigen. Substrates are added to react with the enzyme linked antigen and give absorbance. The resulted absorbance of the mixture reflects the amount of antigen bound with the antibody such that it is inversely related to the amount of MC in the samples. MC concentrations in the samples are calculated by comparison with a standard curve generated by the MC standards provided with the kit.

ELISA (Adda) kits utilize a polyconal congener independent antibody developed by Fischer et al. in 2001. This antibody is produced by using Adda derivatives as hapten and specifically recognizes only the Adda moiety of MC molecule. As shown in Figure 2.2, it is similarly sensitive to a number of microcystin variants so that it can reduce the problem of cross reactivity [31].

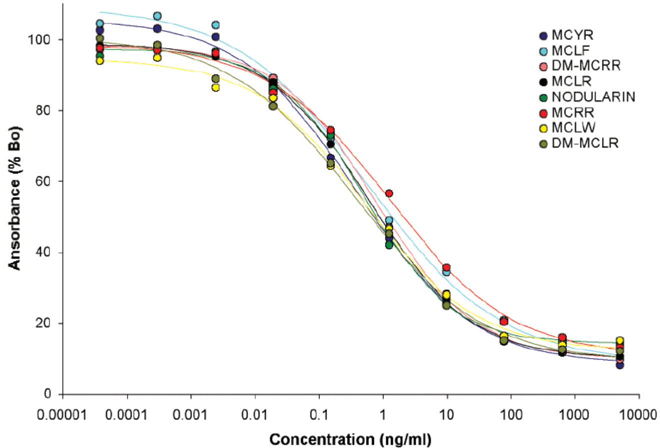


Figure 2.3 ELISA standard curves for cyanobacterial cyclic peptide toxin congeners [31].

Using a series of MC-LR standard solutions, a calibration curve is obtained as absorbance versus log MC concentration. This standard curve in a competitive ELISA exhibits the maximum signal at the lowest concentrations of sample antigen, so that this assay is very sensitive. Figure 2.4 is an example of an ELISA assay standard curve. Its quantification limit is 0.1ug/L (ppb).

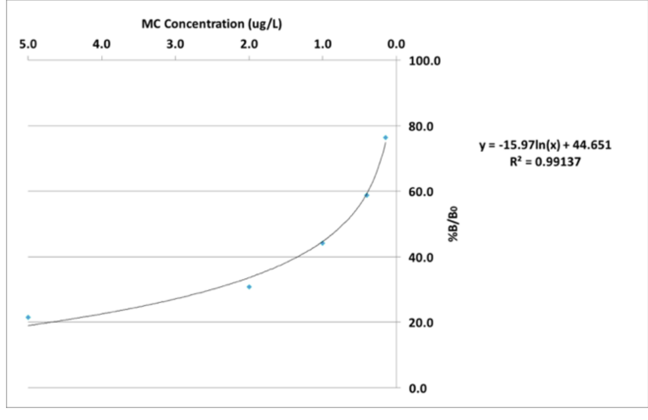


Figure 2.4 Example of the ELISA assay standard curve used in this study

2.7 Treatment of Water Contaminated with Microcystins

It is shown that conventional water treatment, such as coagulation/flocculation, sedimentation and rapid filtration, is not significant to remove MCs from raw water [14]. This is due to the hydrophobic property of MCs as well as their low charge (0, -1 or -2) and relative low molecular weight (909 to 1115), so they are not susceptible to these conventional water treatments [29]. MC is chemically stable and not susceptible to enzymatic hydrolysis by the usual enzymes [28]. MC can degrade slowly at high temperature (40 °C) and at pHs that are either very high (>pH 9) or very low (<pH 1) [32]. The half-life of MCs at pH 1 and 40 °C is 3 weeks; at typical ambient conditions half-life is 10 weeks [32]. MCs break down slowly in full sunlight especially when water-soluble pigments are present [33]. MC can persist for months or even years in dark natural water bodies [34]. Besides, MC cannot be destroyed by boiling [14].

2.8 Biodegradation of Microcystins (MCs)

Biodegradation of MCs is a process which the microorganisms break down MCs through microbial activity. To date, the literature about biodegradation of MCs is dominated by bacterial isolates and not much is related to degrading MCs by archaea or bacterial community. By using endemic bacteria to convert MCs into smaller molecules with much less toxicity, biodegradation is considered the safest and cost effective treatment for mediating cyanobacterial toxicity without generation of carcinogenic byproducts and biotic intrusion [13].

It was suggested that in-situ MC degradation is a bi-phasic degradation process. The bi-phase begins with a rapid phase and followed by a slower phase [35]. To date, the only elucidated biodegradation pathway of microcystin-LR is by isolates of *Sphingomonas sp.* strain (Figure 2.8). It was proposed that MC degradation is a step-by-step enzymatic process [36]. The gene clusters involved in the process are *mlrA, mlrB, mlrC*, and *mlrD* [37]. The most important step is hydrolysis of the Adda-arginine bond to open the cyclic structure by MlrA (microcystinase). The linearized product is reported 160 fold less reactive towards protein phosphatase than the cyclic microcystin. Subsequently, MlrB (putative serine peptidase) cleave the linear product to yield a tetrapeptide intermediate (NH_2-Adda-Glu-Mdha-Ala-OH) and MlrC (putative metallopeptidase) further

break down the intermediate to small peptide and amino acid. MlrD (putative oligopeptide transporter) is the enzyme that transport MC into the cell.

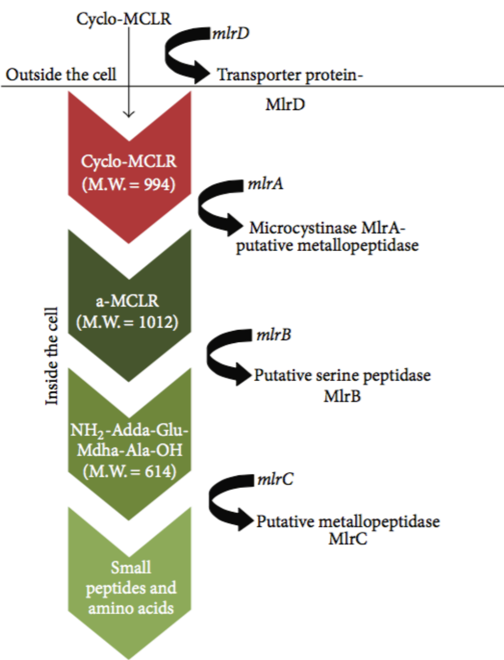


Figure 2.5 The degradative pathway of microcystin-LR and the formation of intermediate products by *Sphingomonas sp.* strain ACM-3962 [37, 38] (MW: molecular weight)

The capability of degrading MC does not generally exist among *Sphingomonas sp.* strain but only *Sphingomonas sp.* strain with gene cluster *mlr*. The *mlr* gene is also found existing in other genus with MC degrading ability. However, some previous studies found that some MC degrading bacteria does not express *mlr* gene [8]. Besides, previous study suggested Adda-arginine bond mentioned above may not be a critical site of cleavage because replacing arginine (MC-LR) by alanine (MC-LA) did not show any effect on biodegradation [8]. These suggest the presence of other degradation pathway.

The bacteria genera able to degrade MC mainly belong to Proteobacteria, including *Sphingomonas*, *Paucibacter*, *Sphingopyxis*, *Burkholderia*, and *Methylobacillus* [8, 9]. They can degrade MC in time scales from hours to days. Some probiotic bacteria also have been reported about their capability on degrading MC incompletely. To date, little is known about the microbial communities associated with MC degradation [39].

2.8.1 Factors affecting biodegradation

Efficiency of biodegradation depends on several environmental factors, including pH, temperature, nutrient conditions, and whether the bacteria have been pre-exposed to MC [40, 41]. The half-life of MC biodegradation ranges from a few hours to 18 days [9], depending on the initial MC concentration, bacterial abundance as well as diversity of the endemic microbial population at the water source.

MC is highly soluble in water, once added, they are ready for cell uptake and degradation. Microorganism in nature may need to acclimatize to it as a food source. This potential lag phase should be taken into account for implication of biodegradation. In-situ applied biodegradation of MC-LR in 1994 for a toxic bloom after algicide treatment occurred after a lag phase of several days [35]. However, lag phase is not observed for microcystin degradation by indigenous bacteria after acclimatization with MC-LR [42].

2.9 Roche/454 pyrosequencing

Roche/454 pyrosequencing is a massively parallel microfluidic sequencing platform developed by 454 Life Sciences Corporation (Branford, Connedticut) in late 2004. It is one of the next generation sequencing (NGS) methods, which can sequence RNA and DNA faster, easier and cheaper than traditional dideoxynucleotide termination DNA sequencing technology invented by Fred Sanger et al in 1977. It also enables analysis of the entire microbial community within a sample. There are three steps in Roche 454 platform: DNA library preparation, amplification by emulsion-based PCR and pyrosequencing based on sequencing-by-synthesis.

DNA Library preparation

This step begins with random fragmentation of genomic double-stranded DNA into small fragments with majority in 300-800bp by nebulization using high nitrogen gas pressure [43]. The fragments are subsequently blunted in order to have both ends nick-ligated with double stranded 454 platform-specific adaptors A and B [44, 45]. Adaptor A provides the first four nucleotides (TCGA) adjacent to primer sequence. This 4-base key sequence is used for the downstream analysis software calibration of light emission by incorporation of a

single nucleotide in pyrosequencing step [45]. Adaptor B is biotin-labelled that enables avidin-biotin purification by streptavidin-coated beads shown in Figure 2.6 [43, 44, 46]. Finally, the non-biotinylated fragments are single stranded by alkali treatment and used as single stranded template DNA (sstDNA) library [43].

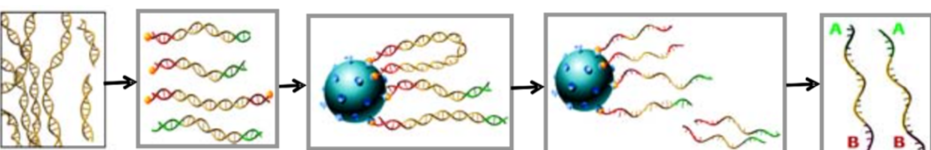


Figure. 2.6 DNA Library preparation

Emulsion PCR (emPCR)

The sstDNA libraries are clonally amplified by emPCR in parallel. Amplification is necessary to create approximately one million of DNA copies for generating required signal intensity for real time detection of sequencing reaction during pyrosequencing. Firstly, each sstDNA library is hybridized to the complementary oligocucleotide sequence immobilized on a micron-sized agarose capture bead [45]. These fragment:bead complex are then individually distributed into a water in oil emulsion with PCR reagents to form microreactors. All beads undergo PCR amplification cycles individually in their microreactor at a time in a single microtube (Figure 2.7). After amplification, the microreactors are broken and the beads are extracted for pyrosequencing.

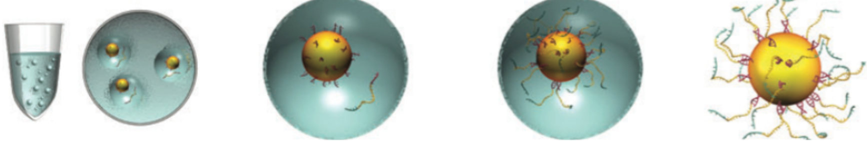


Figure 2.7 Emulsion PCR

Pyrosequencing

Pyrosequencing is based on sequencing-by-synthesis using enzymes: DNA polymerase, sulfurylase, luciferase and substrate: adenosine 5'-phosphosulphate(APS) and luciferin (Figure.2.9). It is carried out using a PicoTitrePlate (PTP) with 1.6 million wells [47]. The beads are deposited into these 44um diameter wells which just one bead fits. Enzyme beads containing sulfurylase and luciferase are loaded into the wells subsequently by centrifugation (Figure.2.8). Sequencing reagents containing buffer and nucleotide are flow across the PTP device in step-wise manner. When a complementary nucleotide is incorporated in the growing DNA strand by polymerase, a pyrophosphate moiety (PPi) will be released. The PPi is converted into ATP by sulfurylase enzyme in the presence of APS. The generated ATP is hydrolysed by luciferase enzyme using luciferin to produce oxyluciferin and luminescent light [48, 49]. The luminescent signal output generated from million sequencing reactions in lockstep are recorded cycle by cycle by a very high sensitivity charge-coupled device (CCD) camera opposite to the PTP.

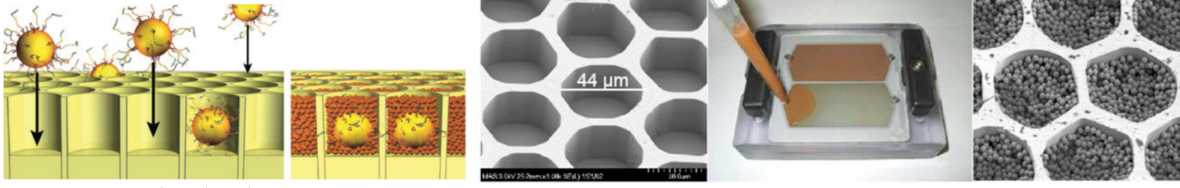


Figure 2.8 Loading beads into PicoTitrePlate

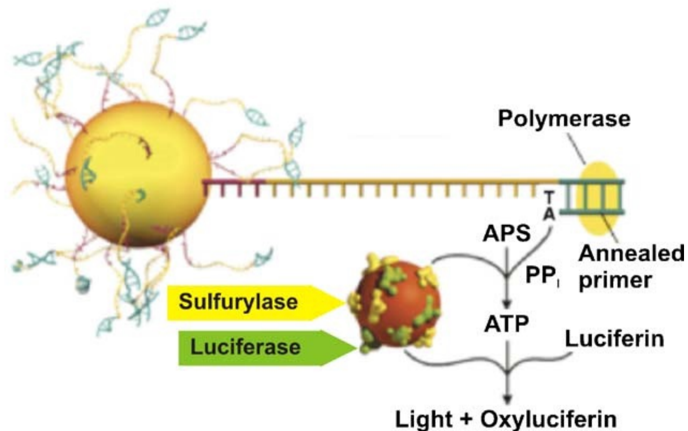


Figure 2.9 Illustration of sequencing-by-synthesis based pyrosequencing

3. MATERIALS AND METHODS

3.1 Sample collection

Two water samples (LSA, LSB) and ten sediment samples (10A, 10B, 11A, 11B, 12A, 12B, 13A, 13B, 14A, 14B) were collected on Feb 24, 2014 from Lake Skinner which is operated by the Metropolitan Water District of Southern California (MWDSC) as drinking water reservoir. As mentioned in Section 1.1, blooms of cyanobacteria and detection of microcystins have been recorded at this lake. Another two water samples (MPA, MPB) were collected on Feb 26, 2014 from a small eutrophic pond at William R Mason Regional Park, Irvine. Duplicate samples were collected at each location using sterile whil-pak bags and transported back to laboratory in UC, Irvine on ice.

3.2 Microcystin and Media

Microcystin stock solutions of MC-LR and MC-LA (1mg/ml in ethanol) with purity of 95% were provided by Cayman Chemical, Miami. The stock solutions were stored at -20°C. Minimum media is the only media used in this study. The composition of minimum media per litre includes 12.8g of Na_2HPO_4-$7H_2O$, 3.0g of KH_2PO_4, 0.5g of NaCl, 1.0g of NH_4Cl, 5.0g of $NaCO_3$, 0.25g of $NaNO_3$, 0.002g of $MgSO_4$, 0.001g of $CaCl_2$. The resulting solution was pH 7.3. Without indication, the media containing 200ug/L MC was prepared by diluting stock of MC in ethanol into fresh minimum media, the estimated concentration of ethanol in the media is 0.395g/L. The media containing 200ug/L MC without ethanol was prepared by reconstituting MC stock after evaporation of ethanol by speed-vac at 10°C into fresh minimum media.

3.3 Enrichment Study and Isolation

5ml of each environmental samples (LSA, LSB, 10A, 10B, 11A, 11B, 12A, 12B, 13A, 13B, 14A, 14B, MPA, MPB) was enriched by 20ml of minimum media containing 200ug/L MC-LR for 7 days at room temperature. At the end of the 7 days, 100ul of samples were inoculated by spread plate technique onto minimum medium agar (1% agar) containing 200ug/L MC-LR as candidate carbon and nitrogen resource for 7 days at room temperature. Single colonies with distinct morphological features and color were transferred from agar plates

to liquid minimum medium containing MC-LR. All isolated bacteria (144 numbers in total) were cultured in microtiter plate and their growth were monitored by measurement of optical density at 600nm (OD600). Control was set up by culturing the isolates in media without MC-LR under same condition. Isolates showing rapid growth in the presence of MC-LR during the study period of 12 days were sub-cultured for subsequent identification and substrate specificity test.

3.4 Identification of candidate MC-degrading bacteria by 16S rRNA sequencing

Discrete colonies of each isolates were grown and purified by 3 times consecutive plate streaking on Luria broth (LB) agar (1% agar). Single colonies from these plates were transferred to LB medium. After visible growth, 1ml of dense culture was transferred into 1.5 ml Eppendorf tubes. After centrifugation, the cell pellets of isolate were submitted for DNA extraction, PCR amplification and sequencing of 16S rRNA gene with bacterial universal primers in both directions at Laragen, Inc. (Los Angeles, CA).

Based on the 16S rRNA sequenceing data, each isolate was assigned the taxonomy of the closest sequences searched from the GenBank data by using the Basic Local Alignment Search Tool (BLAST) of the National Center for Biotechnology Information (NCBI).

3.5 Substrate specificity test

Substrate specificity was investigated by a comparison of bacterial growth in media with the presence of different substrate, either MC-LR or MC-LA. 50ul of inoculum of each isolates screened from enrichment study was sub-cultured into 200ul fresh media with 200ug/L MC-LR or MC-LA. OD value at 600nm was used as index to viable bacterial growth. Isolates showing different level of substrate specificity for biodegradation study were nominated for biodegradation study.

3.6 Culture of isolates and consortia

The consortia mentioned in Section 3.3 were sub-cultured biweekly by having 5ml inoculum of each consortium transferred into 20ml of minimum media containing 200ug/L MC-LR in cell culture flask under ambient room temperature (24 ± 1°C) Candidate isolates were sub-cultured by putting 0.5ml inoculum to 3ml of minimum media containing MC in cell culture tubes under same ambient condition. A set of consortia culture for biodegradation study of MC-LA was obtained by subjecting the original MC-LR enriched culture to fresh media containing 200ug/L MC-LA. Subsamples of each isolate and consortium culture were stored in glycerol 50/50 for future cultivation.

3.7 Biodegradation Study

A series of batch experiments under ambient temperature in dark was undertaken to study the biodegradation. 12 isolates were chosen for the study based on their substrate specificity, morphology, rapid growth in the presence of microcystin. 8 consortia (10B, 11B, 12B, 13A, 14A, LSA, LSB, MP) were chosen for the study based on the distinct samples location, samples types and the bacterial community diversity indicated by identified isolates. Since no MC-LR degradation was observed in the experiment with isolates, no subsequent biodegradation study of isolates was conducted afterwards. There were one and six sets of batch studies done for isolates and consortia respectively and listed as the followings:

Table 3.1 Summary of experimental parameters

	Experiment Code	**Biodegradation Substrate**	**Pre-incubation Substrate**	**Initial OD**
Isolate	MCLR-WE-WE (I)	MC-LR with ethanol	MC-LR with ethanol	0.002
Consortia	MCLR-WE-WE	MC-LR with ethanol	MC-LR with ethanol	0.002
	MCLR-WE-NE	MC-LR with ethanol	MC-LR without ethanol	0.002
	MCLR-NE-NE	MC-LR without ethanol	MC-LR without ethanol	0.0002
	MCLA-WE-WE	MC-LA with ethanol	MC-LA with ethanol	0.002
	MCLA-WE-NE	MC-LA with ethanol	MC-LA without ethanol	0.002
	MCLA-NE-NE	MC-LA without ethanol	MC-LA without ethanol	0.0002

3.7.1 Experiment MCLR-WE-WE (I), MCLR-WE-WE and MCLA-WE-WE

This was the first series of experiment done for both selected isolates and consortia. Isolates/consortia were pre-incubated under ambient temperature (24 ± 1°C) in media containing 200ug/L MC which was prepared by diluting MC stock in ethanol into fresh media. The inoculum of isolates/consortia was then sit on the shaker at 120rmp overnight to use up carbon residual. Right before the experiment, the inoculum was centrifuged and suspended into fresh media containing 200ug/L MC by vortex. The cell density was measured by optical density at absorbency 600nm. In biodegradation, the initial total MC concentration in media was 200ug/L, it was prepared same as the media for pre-incubation. The washed cultures of different consortia were transferred into the media containing 200ug/L MC at same optical density of 0.002. All cultures were maintained in glass erlenmeyer flasks and the flasks were shaken at 120 rmp under ambient room temperature in dark for 7 days. Volume of 1ml of each sample were analyzed daily for the residual MC concentration by ELISA assay described in Section 3.8 and optical density by spectrophotometer at 600nm. Same experiment was conducted without bacteria as the control.

3.7.2 Experiment MCLR-WE-NE and MCLA-WE-NE

The procedure was the same as experiments MCLR/LA-WE-WE except that MC stock used as substrate in biodegradation study was ethanol-eliminated by evaporation in speed-vac. It is expected that ethanol consuming bacteria were carried over but not the ethanol which would be used up by the bacteria incubating on the shaker overnight before the biodegradation study.

3.7.3 Experiment MCLR-NE-NE and MCLA-NE-NE

The procedure was similar to experiments MCLR/LA-WE-WE except that MC stock used as substrate in both pre-incubation and biodegradation study was ethanol-eliminated by evaporation in speed-vac. Without the presence of ethanol, the OD value of the inoculum was much lower than those with ethanol (0.395g/L), such that the initial OD value for biodegradation was adjusted to 0.0002.

3.8 Detection of microcystin

MC concentration in samples was measured in duplicate using ELISA assay (ABRAXIS Microcystins/Nodularins (ADDA) ELISA Kit, PN 520011, Microtiter Plate, 96T) following the manufacturer's instruction. Firstly, 1ml of each samples were centrifuged and their supernatant were diluted 40 times for the detection range of ELISA kit. Milli-Q was used for the dilution in order to minimize effects on ELISA by the components in media, such as Ca^{2+}, Mg^{2+} [50]. Milli-Q water (18 MΩ) used in all experiments was obtained through a Milli-Q (Millipore, Bedford, MA) water purification system. Briefly, an aliquot of sample/control was added to a well on the plate to compete for the binding with anti-microcystin antibodies with the antigen immobilized on the well. After incubation at ambient temperature for 90 minutes, the plate was washed three times to remove unbound reagents and then microcystin-enzyme conjugate was added. After incubation for another 30 minutes, the plate was washed again. Finally, substrate was added for color development. After 20 minutes, acid was added to terminate the reaction. The plate was read in the plate reader at absorbency of 450nm. The mean absorbency (B_i) of each standards and samples (i) was divided by the mean absorbency of the zero standard (B_0) to give $\%B_i/B_0$. A standard curve was constructed by plotting $\%B_i/B_0$ for the standard versus their MC concentration (Figure 2.4). The MC concentration of the samples in ppb was then interpolated from the standard curve.

3.9 Kinetic Modelling

Quiroga and Sales Kinetic Model [51] was used to describe the non-linear relationship between MCs concentration and time, and to estimate the biodegradation rates and half-lives of MCs in the consortia culture with MC-degradation capability. The theory of this model is that biodegradation rate is slow at the beginning and increase until reaching the maximum when the microorganisms proliferate. Finally, the biodegradation rate decreases and becomes zero. This model was developed by considering biodegradation of substrate (S) as an autocatalytic process because the reactant (X), microorganism in this study, reproduce during the process:

$$S + X \xrightarrow{k} \sigma X + \cdots ; \ \sigma > 1$$

$$-\frac{dS}{dt} = kSX$$

where k is the rate constant of the process, σ is a stoichiometric constant

The bacterial growth is related to the substrate utilization by cell yield coefficient Y:

$$X = Y(S_0 - S) + X_0; \ Y = \sigma - 1$$

where S_0 is initial concentration of substrate, X_0 is initial concentration of microorganism

The biodegradation rate is:

$$-\frac{dS}{dt} = kS[X_0 + Y(S_0 - S)]$$

$$= kY\left(\frac{X_0}{Y}S + S_0S - S^2\right)$$

$$= -kYS^2 + S(kX_0 + kYS_0)$$

Considering a proportion of substrate is non-biodegradable:

$$S_T = S_B + S_{NB} \ \ and \ \ S_{T0} = S_{B0} + S_{NB}$$

where S_T = total substrate concentration; S_B = biodegradable substrate concentration;
S_{NB} = non-biodegradable substrate concentration; S_{T0} = initial total substrate concentration;
S_{B0} = initial biodegradable substrate concentration

Thus,

$$-\frac{dS}{dt} = -kY(S_T - S_{NB})^2 + (S_T - S_{NB})(kX_0 + kY(S_{T0} - S_{NB})$$

$$-\frac{dS}{dt} = -kY[S_T{}^2 - \left(\frac{X_0}{Y} + (S_{T0} - S_{NB}) + 2S_{NB}\right)S_T + \left(\frac{X_0}{Y}S_{NB} + (S_{T0} - S_{NB})S_{NB} + S_{NB}{}^2\right)]$$

$$-\frac{dS}{dt} = -kYS_T{}^2 + kY\left(\frac{X_0}{Y} + (S_{T0} - S_{NB}) + 2S_{NB}\right)S_T - kY\left(\frac{X_0}{Y}S_{NB} + (S_{T0} - S_{NB})S_{NB} + S_{NB}{}^2\right)]$$

By simplification, the biodegradation rate equation was given by a second degree polynomial of remaining substrate concentration in the medium:

$$-\frac{dS}{dt} = K_2S^2 + K_1S + K_0$$

where K_0, K_1, K_2 are kinetic constants

$K_2 = -kY$

$K_1 = kY\left(\frac{X_0}{Y} + (S_{T0} - S_{NB}) + 2S_{NB}\right)$

$K_0 = -kY\left(\frac{X_0}{Y}S_{NB} + (S_{T0} - S_{NB})S_{NB} + S_{NB}{}^2\right)$

The second degree polynomial was factored:

$$K_2S^2 + K_1S + K_0 = (S + \frac{K_1 - \sqrt{(K_1^2 - 4K_2K_0)}}{2K_2})(S + \frac{K_1 + \sqrt{(K_1^2 - 4K_2K_0)}}{2K_2})$$

$$K_2S^2 + K_1S + K_0 = (S - q)(S - h)$$

where $p = \sqrt{(K_1^2 - 4K_2K_0)}$

$$q = \frac{-K_1 + p}{2K_2}$$

$$h = \frac{-K_1 - p}{2K_2}$$

The biodegradation rate equation was then solved by separating variables and integration to obtain the relationship between the concentration of substrate and time:

$$\int_0^t dt = \int_{S_0}^{S} \frac{-dS}{K_2 S^2 + K_1 S + K_0}$$

$$\int_0^t dt = \int_{S_0}^{S} \frac{-dS}{(S-q)(S-h)}$$

$$t = -\frac{1}{q-h}[\ln(S-q) - \ln(S-h)]_{S_0}^{S}$$

$$pt = \ln[\frac{(S-h)(S_0-q)}{(S-q)(S_0-h)}]$$

$$e^{pt} = \frac{h(S_0-q) - S(S_0-q)}{-S(S_0-h) + q(S_0-h)}$$

$$S = \frac{h(S_0-q) - q(S_0-h)e^{pt}}{(S_0-q) - (S_0-h)e^{pt}}$$

The physical meaning of p, q and h were defined from the equations above. In this model, biodegradation rates are the minimum at the beginning and at the end. dS/dt=0 when S=h or S=q since h and q are the solution of the quadratic equation. This implies that h and q are either maximum concentration of substrate available for utilization by the microorganism or the concentration of non-biodegradable substrate.

$$h = \frac{-K_1 - \sqrt{(K_1^2 - 4K_2K_0)}}{2K_2}$$

$$h = \frac{\left(\frac{X_0}{Y} + S_{B0} + 2S_{NB}\right) + \sqrt{\left(\frac{X_0}{Y} + S_{B0} + 2S_{NB}\right)^2 - 4\left(\frac{X_0}{Y}S_{NB} + S_{B0}S_{NB} + S_{NB}{}^2\right)}}{2}$$

$$h = \frac{\left(\frac{X_0}{Y} + S_{B0} + 2S_{NB}\right) + (\frac{X_0}{Y} + S_{B0})}{2}$$

$$h = \frac{X_0}{Y} + (S_{T0} - S_{NB}) + S_{NB}$$

$$h = \frac{X_0}{Y} + S_{T0}$$

Similarly,

$$q = \frac{\left(\frac{X_0}{Y} + S_{B0} + 2S_{NB}\right) - \left(\frac{X_0}{Y} + S_{B0}\right)}{2}$$

$$q = S_{NB}$$

Thus, h represents the maximum concentration of substrate available for utilization by the microorganism. q represents the concentration of non-biodegradable substrate.

$$p = \sqrt{(K_1^2 - 4K_2K_0)}$$

$$p = \mathrm{kY}\sqrt{\left(\frac{X_0}{Y} + S_{B0} + 2S_{NB}\right)^2 - 4\left(\frac{X_0}{Y}S_{NB} + S_{B0}S_{NB} + {S_{NB}}^2\right)}$$

$$p = \mathrm{kY}\left(\frac{X_0}{Y} + S_{B0}\right)$$

Since $X_0 << YS_{B0}$

$$p \cong \mathrm{kY}S_{B0} = \frac{1}{X_0}\left(-Y\frac{dS}{dt}\right) = \frac{1}{X_0}\frac{dX}{dt}$$

Thus, p represents the maximum specific growth rate of the microorganism.

The experimental data and values predicted by the model was compared. The correlation coefficient was determined by the below equation [52]:

$$R^2 = 1 - \frac{\sum(\mathrm{y} - \mathrm{y_{model}})^2}{\sum(\mathrm{y} - \bar{\mathrm{y}})^2}$$

3.10 Roche/454 pyrosequencing

To access the bacterial abundance and diversity of candidate consortia, 32 number of samples (Table 3.2) were subjected to Roche 454 genome sequencer FLX+ (454 GS-FLX+) amplicon pyrosequencing. Both the DNA extraction and amplicon sequencing was performed by the Research and Testing Laboratory (Lubbock, TX). The 16S rRNA universal eubacterial primers 939F (5'-TTGACGGGGGCCCGCACAAG-3') and 1492R (5'-TACCTTGTTACGACTT-3') were used to amplify approximately 550 bp of the variable regions V6 to V8 (Figure 3.1).

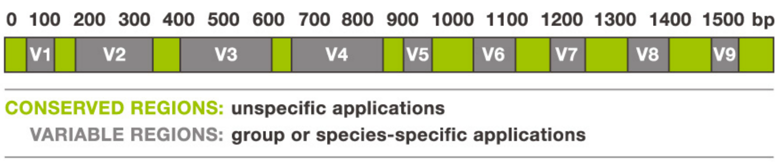


Figure: 3.1 Variable Regions of 16S rRNA
(Source: http://www.alimetrics.net/en/index.php/dna-sequence-analysis)

Table 3.2 Label of Consortia

Source	Consortia	Substrate			
		MC-LR		MC-LA	
		With ethanol	Without ethanol	With ethanol	Without ethanol
Water	10B	10B.LR.WE	10B.LR.NE	10B.LA.WE	10B.LA.NE
	11B	11B.LR.WE	11B.LR.NE	11B.LA.WE	11B.LA.NE
	12B	12B.LR.WE	12B.LR.NE	12B.LA.WE	12B.LA.NE
	13A	13A.LR.WE	13A.LR.NE	13A.LA.WE	13A.LA.NE
	14A	14A.LR.WE	14A.LR.NE	14A.LA.WE	14A.LA.NE
Sediment	LSA	LSA.LR.WE	LSA.LR.NE	LSA.LA.WE	LSA.LA.NE
	LSB	LSB.LR.WE	LSB.LR.NE	LSB.LA.WE	LSB.LA.NE
	MP	MP.LR.WE	MP.LR.NE	MP.LA.WE	MP.LA.NE

3.11 Bacterial 16S rRNA pyrosequening data Analysis

All 16S rRNA pyrosequencing reads were analyzed using QIIME, version 1.9.1 [53]. Firstly, the multiplexed reads were assigned to 32 number of samples based on their specific barcode sequence. Secondly, the key sequences, barcodes and primers were removed and the reads were quality filtered. Most of the default quality requirements were used, which included sequence length of 200-1000 bp, maximum number of ambiguous bases of 6 and a maximum homopolymer length of 6 bp. Besides, the criteria of maximum number of mismatches in primer was increased from 0 to 2 and minimum mean Phred quality scores of 20 was used instead of 25, in order to recover more reads. Filtered reads were then clustered into operational

taxonomic units (OTUs) at 97% sequence similarity using uclust. Taxonomy was searched from Greengenes (GG) reference database (http://greengenes.microbio.me/greengenes_release/gg_13_5/gg_13_8_otus.tar.gz) and assigned to representative OTU using Ribosomal Database Project (RDP) classifier. Prior to the phylogenetically-aware measures, samples were rarefied to same sequencing depth of 824, which is the smallest sequencing depth of all samples. The taxonomic diversity within samples (alpha diversity) was estimated by comparing the Shannon index. The bacterial diversity between samples (beta diversity) was calculated using the unweighted UniFrac distance metric. To visualize the variation of bata diversity indicated by distance matrix, hierarchical cluster analysis of the MC enriched consortia was conducted using Unweighted Pair Group Method with Arithmetic mean (UPGMA).

4. RESULTS

The chapter is divided into three sections. Section 4.1 describes the results for preliminary experiments aimed for screening potential MC degraders while Section 4.2 describes the results for biodegradation study for selected isolates. Section 4.3 describes the results for biodegradation study for consortia.

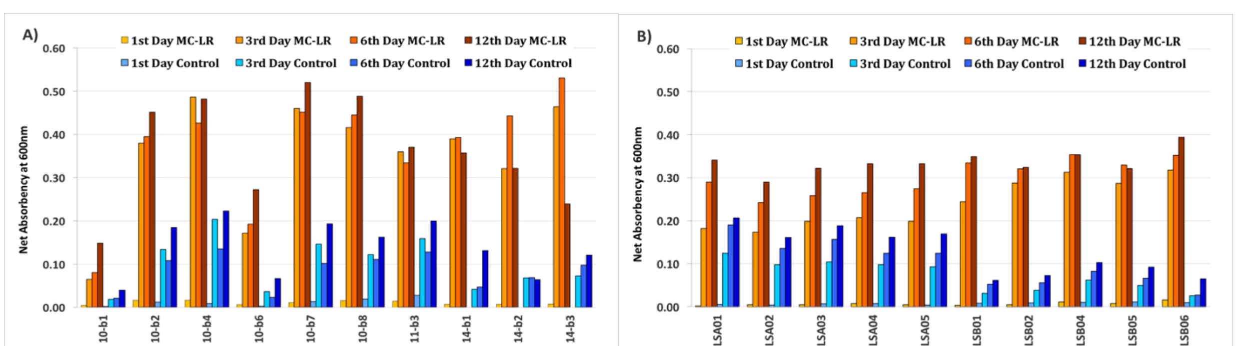
4.1 Screening of isolates for biodegradtion study

Before biodegradation study, candidate isolates were chosen based on their rapid growth in the presence of microcystin, substrate specificity, morphology, and genus. Ultimately, 12 isolates were nominated for the biodegradation study (Table 4.1 and 4.2)

4.1.1 Bacterial Growth in the presence of MC-LR

During the enrichment study, the bacterial growth of 104 isolates in the media with presence of MC-LR was monitored by OD600. 95 out of 104 isolates demonstrated rapid growth in the presence of MC-LR but not in the control (Figure 4.1) They were considered as potential MC degraders.

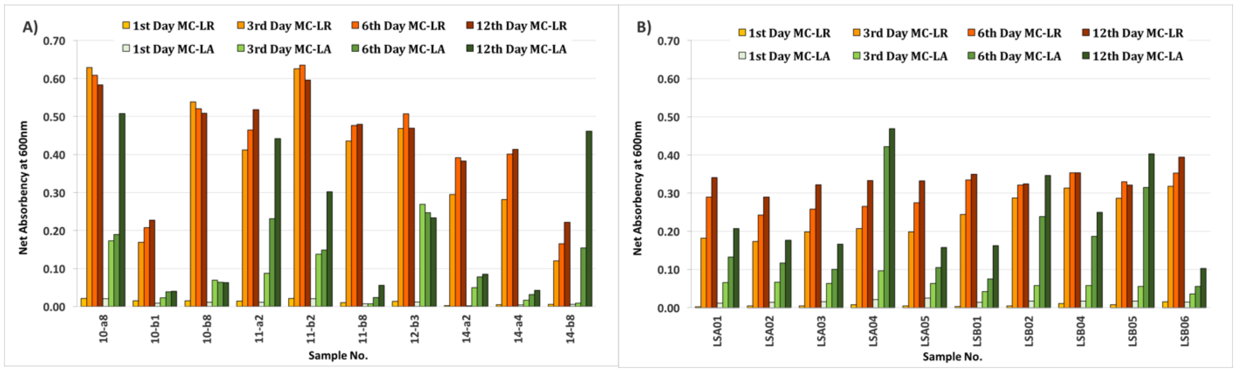
Figure 4.1 Rapid bacterial growth demonstrated by isolates from sediment samples (A) and water samples (B).



4.1.2 Substrate Specificity

Substrate specificity is considered a property determining bacterial biodegradation performance. Besides, there could be more than one MC variant present in cyanoHABs, MC independent biodegrader would be applicable for MC remediation via biofilter. However, universal biodegrader may not be able to degrade MC as efficient as substrate specific degrader. For all these reasons, screening for subsequent biodegradation study was aimed to include the isolates with different level of MC-LR/MC-LA specificity (Figure 4.2).

Figure 4.2 Different level of MC variant specificity demonstrated by isolates from sediment samples (A) and water samples (B)



4.1.3 Morphology of isolates

Diverse morphological features and color of colonies were observed among the isolates. The morphologies of colony after purification by 3 times consecutive plate streaking on LB agar were recorded. The morphological feature of the bacteria nominated for biodegradation study are summarized in Table 4.1.

Table 4.1 Major morphological features of all 12 candidate bacteria

Isolate	Molecular Identification	Colony Color	Macroscopic image	Gram Staining
LSA02	*Pseudomonas brassicacearum*	Light peach		-
P2-D1	*Pseudomonas guineae*	light peach		-
10a6	*Pseudomonas putida*	White		-
11b2	*Acinetobacter kyonggiensis*	White		-
10b1	*Rhodococcus ruber*	White		+
10b7	*Stenotrophomonas rhizophila*	White		-

10b8	*Cupriavidus campinensis*	Clearish white		-
11b8	*Pseudomonas corrugata*	Light peach		-
12b3	*Shinella zoogloeoides*	White		-
12b8	*Brevundimonas bullata*	Light peach		-
14a2	*Rhodococcus triatomae*	White		+
14a4	*Leifsonia shinshuensis*	Yellow		+

4.1.4 Identification of isolates

High genus diversity of the potential degraders was discovered among the isolates. Analysis of bacterial 16S rRNA gene sequences revealed that 95 isolates in enrichment study belong to 11 genera (Figure 4.3): *Pseudomonas sp.; Rhizobium sp.; Rhodococcus sp.; Arthrobacter sp.; Stenotrophomonas sp.; Acinetobacter sp.; Cupriavidus sp.; Shinella sp.; Brevundimonas sp.; Leifsonia sp.*; and *Thermoleophilum sp.*

They are from seven orders and five classes within two phyla: Proteobacteria and Actinobacteria. The majority of the isolates were identified as members of two orders Pseudomonadales and Actinomycetales from the class Gammaproteobacteria and Actinobacteria respectively. A single isolate was identified from the Betaproteobacteria. Another single isolate was identified from the Thermoleophilia.

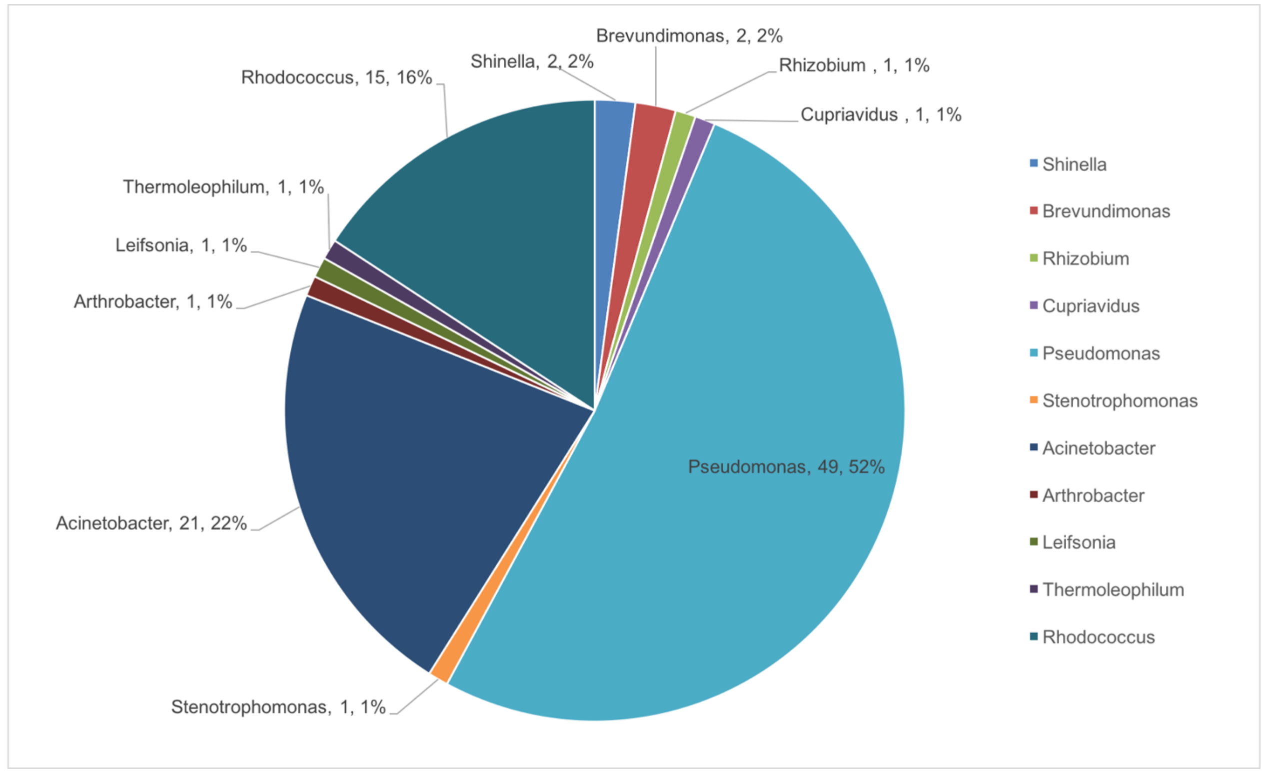


Figure 4.3 Proportions of taxonomy in genus level represented by isolated bacterial strain

(Genus, number of the isolated strains, percentage of different genus)

Table 4.2 Bacterial strains nominated for biodegradation study

Code	Source	Species	Order	Class	Phylum
LSA02	Water	*Pseudomonas brassicacearum*	Pseudomonadales	Gammaproteobacteria	Proteobacteria
P2-D1	Water	*Pseudomonas guineae*	Pseudomonadales	Gammaproteobacteria	Proteobacteria
10a6	Soil	*Pseudomonas putida*	Pseudomonadales	Gammaproteobacteria	Proteobacteria
11b2	Soil	*Acinetobacter kyonggiensis*	Pseudomonadales	Gammaproteobacteria	Proteobacteria
10b1	Soil	*Rhodococcus ruber*	Actinomycetales	Actinobacteria	Actinobacteria
10b7	Soil	*Stenotrophomonas rhizophila*	Xanthomonadales	Gammaproteobacteria	Proteobacteria
10b8	Soil	*Cupriavidus campinensis*	Burkholderiales	Betaproteobacteria	Proteobacteria
11b8	Soil	*Pseudomonas corrugata*	Pseudomonadales	Gammaproteobacteria	Proteobacteria
12b3	Soil	*Shinella zoogloeoides*	Rhizobiales	Alphaproteobacteria	Proteobacteria
12b8	Soil	*Brevundimonas bullata*	Caulobacterales	Alphaproteobacteria	Proteobacteria
14a2	Soil	*Rhodococcus triatomae*	Actinomycetales	Actinobacteria	Actinobacteria
14a4	Soil	*Leifsonia shinshuensis*	Actinomycetales	Actinobacteria	Actinobacteria

4.3 Biodegradation study

To examine MC biodegradation capability, one set and six sets of batch experiment were done for 12 isolates (Table 4.2) and 8 consortia (10B, 11B, 12B, 13A, 14A, LSA, LSB, MP) respectively as described in Section 3. As illustrated in Figure 4.4, none of the isolates was found to degrade MC-LR over a period of 7 days.

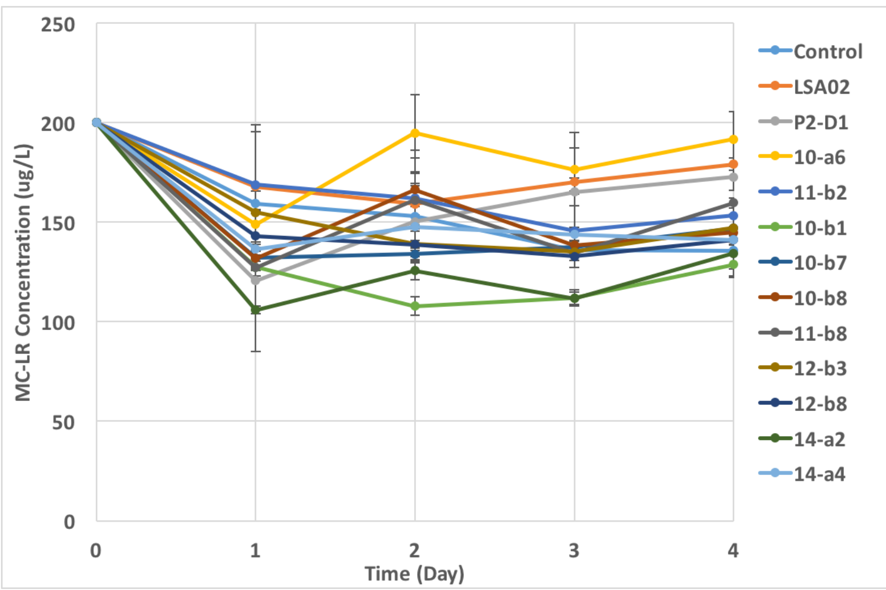


Figure 4.4 Experiment of Isolates - Variation of MC-LR concentration versus incubation time

5 consortia (10B, 11B, 12B, 14A, LSB) demonstrated their ability to degrade MC-LR and MC-LA readily. Most of them were able to degrade MC-LR or MC-LA within 2-3 days (Figure 4.5 and 4.6). The experiment data of MC-degrading consortia was fitted with the Quiroga and Sales Kinetic Model described in Section 3 to calculate the half-time of the biodegradation (Table 4.3).

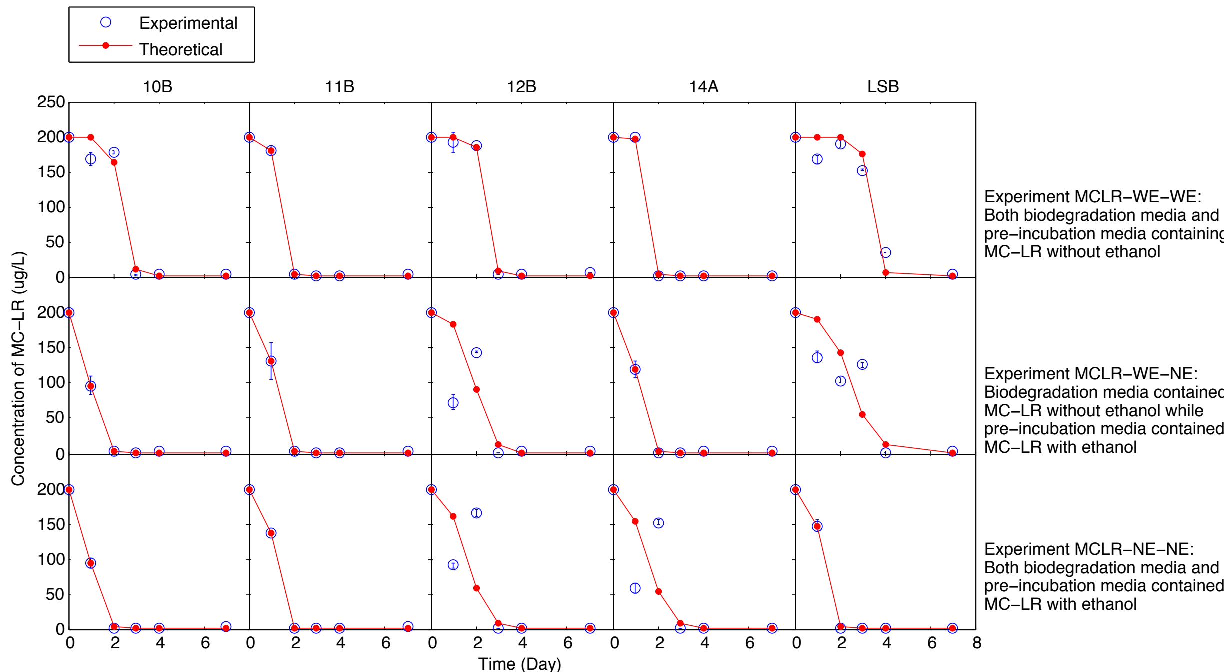


Figure 4.5 Experiment of Consortia - Variation of MC-LR concentration versus incubation time

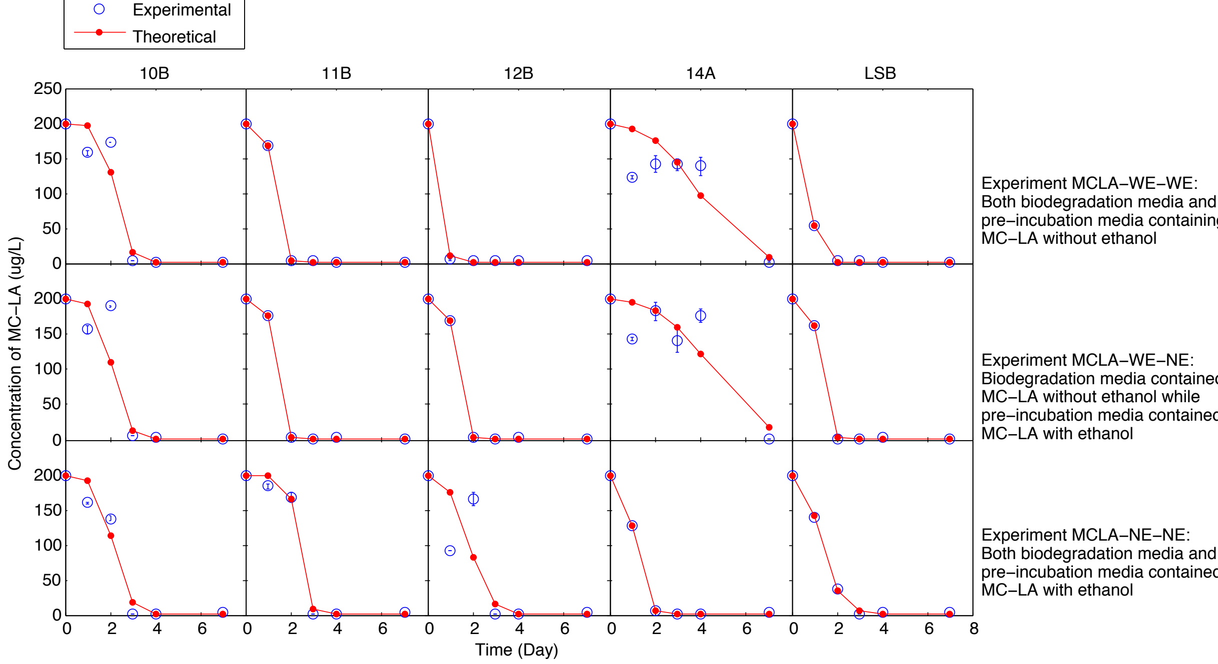


Figure 4.6 Experiment of consortia - Variation of MC-LA concentration versus incubation time

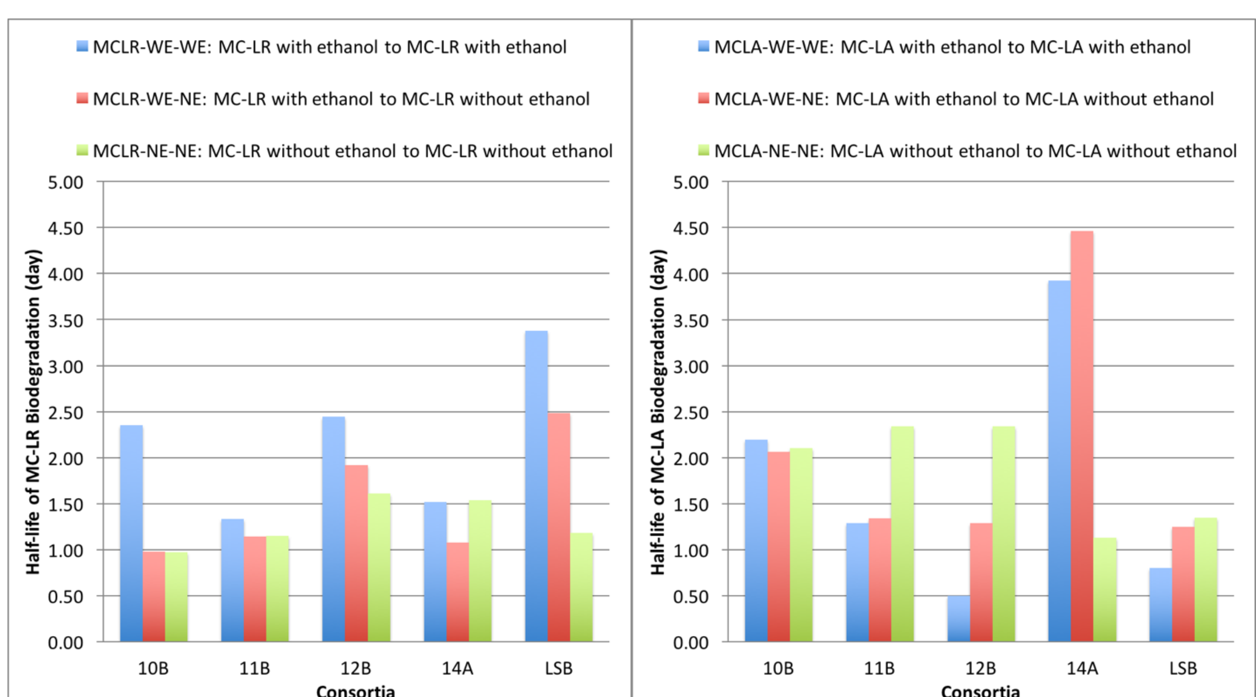
Table 4.3 Values of kinetic parameters obtained by fitting the experimental data to Quiroga and Sales Kinetic Model

		h	q	p	r^2	$t_{1/2}$
Experiment	Consortia	ug/L	ug/L	day^{-1}		day
MCLR-WE-WE	10B	200.01	3.09E-08	4.299	0.962	2.351
	11B	200.04	3.12E-08	6.331	0.999	1.338
	12B	200.00	3.21E-08	5.654	0.997	2.444
	14A	200.00	1.77E-07	7.928	0.999	1.520
	LSB	200.00	2.73E-11	5.296	0.891	3.376
MCLR-WE-NE	10B	203.62	1.01E-06	4.127	0.999	0.980
	11B	201.25	3.10E-08	4.454	0.999	1.142
	12B	202.01	1.74E-10	2.410	0.412	1.917
	14A	201.22	1.39E-07	4.741	0.999	1.079
	LSB	202.22	0.00E+00	1.820	0.546	2.484
MCLR-NE-NE	10B	203.33	1.74E-07	4.240	0.999	0.974
	11B	200.42	3.14E-08	5.373	0.999	1.148
	12B	206.31	1.02E-03	2.180	0.430	1.613
	14A	208.84	1.78E-07	2.080	0.295	1.540
	LSB	200.29	7.84E-01	5.521	0.999	1.185
MCLA-WE-WE	10B	200.23	3.07E-08	3.083	0.893	2.196
	11B	200.15	3.08E-08	5.584	0.999	1.292
	12B	212.05	3.21E-08	5.846	0.997	0.500
	14A	204.62	3.09E-08	0.972	0.250	3.924
	LSB	203.00	2.68E-11	5.241	0.999	0.807
MCLA-WE-NE	10B	200.47	1.31E-03	2.930	0.779	2.068
	11B	200.09	3.14E-08	5.711	0.999	1.341
	12B	200.10	4.07E-08	5.857	0.999	1.292
	14A	203.41	3.06E-08	0.920	0.534	4.464
	LSB	200.14	7.36E-01	5.830	0.999	1.252
MCLA-NE-NE	10B	200.76	1.65E-07	2.650	0.942	2.107
	11B	200.00	3.52E-08	4.727	0.991	2.340
	12B	203.97	3.63E-08	2.146	0.509	2.340
	14A	201.73	1.76E-07	4.207	0.999	1.133
	LSB	209.62	1.24E+00	2.327	0.999	1.346

Remark: h is maximum MC quantity for biomass formation; p is specific maximum growth rate of microorganism; q is non-biodegradable MC concentration; r^2 is correlation coefficient; $t_{1/2}$ is the half-life of MC biodegradation

In the biodegradation study, 4 out of 5 consortia from sediment samples (10B,11B,12B and 14A) and 1 out of 3 consortia from water samples (LSB) demonstrated capability of degrading both MC-LR and MC-LA. As illustrated in Figure 4.7, the half-lives of MC degradation found in this study ranges from 12 hours to 4.5 days. Consortia 10B and 11B showed relatively low substrate specificity. Consortia 12B and LSB is more MC-LA favorable while consortia 14A favors MC-LR.

Figure 4.7 MC-LR (Left) and MC-LA (Right) biodegradation performance of different consortia



4.4 Microbial Community Analysis

Samples were subjected to 16S ribosomal RNA gene pyrosequencing (454 Life Sciences). A total of 269,092 16S rRNA sequencing reads were obtained for 32 consortia samples subjected to 454 pyrosequencing. The average read length is 540bp. 16S data processing and diversity estimates were performed using QIIME, version 1.9.1 [53] The sequencing reads have been clustered into 30,846 OTUs based on 97% similarity. After quality control, 102,586 reads (38.1% of all reads) remained (Table 4.4) and the number of reads per sample ranged from 824 to 9136 (mean=3205).

Table 4.4 Summary of sequences used for the analysis

Consortia	Number of reads qualified for analysis	Number of reads retrieved	Percentage (%)
10B.LR.WE	10,757	8,781	81.6
11B.LR.WE	3,284	2,108	64.2
12B.LR.WE	3,707	3,283	88.6
13A.LR.WE	7,942	1,155	14.5
14A.LR.WE	4,836	4,308	89.1
LSA.LR.WE	5,104	4,455	87.3
LSB.LR.WE	7,529	5,717	75.9
MP.LR.WE	5,441	4,820	88.6
10B.LA.WE	5,112	4,500	88.0
11B.LA.WE	14,511	2,394	16.5
12B.LA.WE	7,973	6,735	84.5
13A.LA.WE	12,031	2,082	17.3
14A.LA.WE	11,694	9,136	78.1
LSA.LA.WE	3,827	3,157	82.5
LSB.LA.WE	5,219	4,620	88.5
MP.LA.WE	3,980	3,392	85.2
10B.LR.NE	10,448	2,224	21.3
11B.LR.NE	3,136	2,056	65.6
12B.LR.NE	14,261	2,821	19.8
13A.LR.NE	15,870	2,380	15.0
14A.LR.NE	11,534	1,994	17.3
LSA.LR.NE	13,766	2,497	18.1
LSB.LR.NE	10,183	1,571	15.4
MP.LR.NE	3,016	1,932	64.1
10B.LA.NE	11,163	1,716	15.4
11B.LA.NE	8,948	1,270	14.2
12B.LA.NE	7,449	824	11.1
13A.LA.NE	5,200	1,050	20.2
14A.LA.NE	5,988	3,531	59.0
LSA.LA.NE	14,754	2,816	19.1
LSB.LA.NE	13,438	2,232	16.6
MP.LA.NE	6,991	1,029	14.7
Total	269,092	102,586	38.1

32 bacterial class were revealed, including Acidobacteria, Solibacteres, Acidimicrobiia, Actinobacteria, Thermoleophilia, Fimbriimonadia, Bacteroidia, Cytophagia, Flavobacteriia, Sphingobacteriia, Saprospirae, Chlamydiia, Chlorobia, Anaerolineae, Thermomicrobia, Chloroplast, Oscillatoriophycideae, Deferribacteres, Fibrobacteria, Bacilli, Clostridia, Gemmatimonadetes, Planctomycetia, Alphaproteobacteria (23.6%), Betaproteobacteria (30.0%), Deltaproteobacteria, Epsilonproteobacteria, Gammaproteobacteria (41.0%), Mollicutes, Opitutae, Methylacidiphilae and Pedosphaerae (Figure 4.8 and Appendix A-1, A-2). Proteobacteria is the dominant phylum.

Alphaproteobacteria, Betaproteobacteria and Gammaproteobacteria are three main class of bacteria among all consortia samples and they all belong to Proteobacteria. There are shifts among these dominant classes, but no significant shift from these classes to other classes for substrate changed or ethanol eliminated.

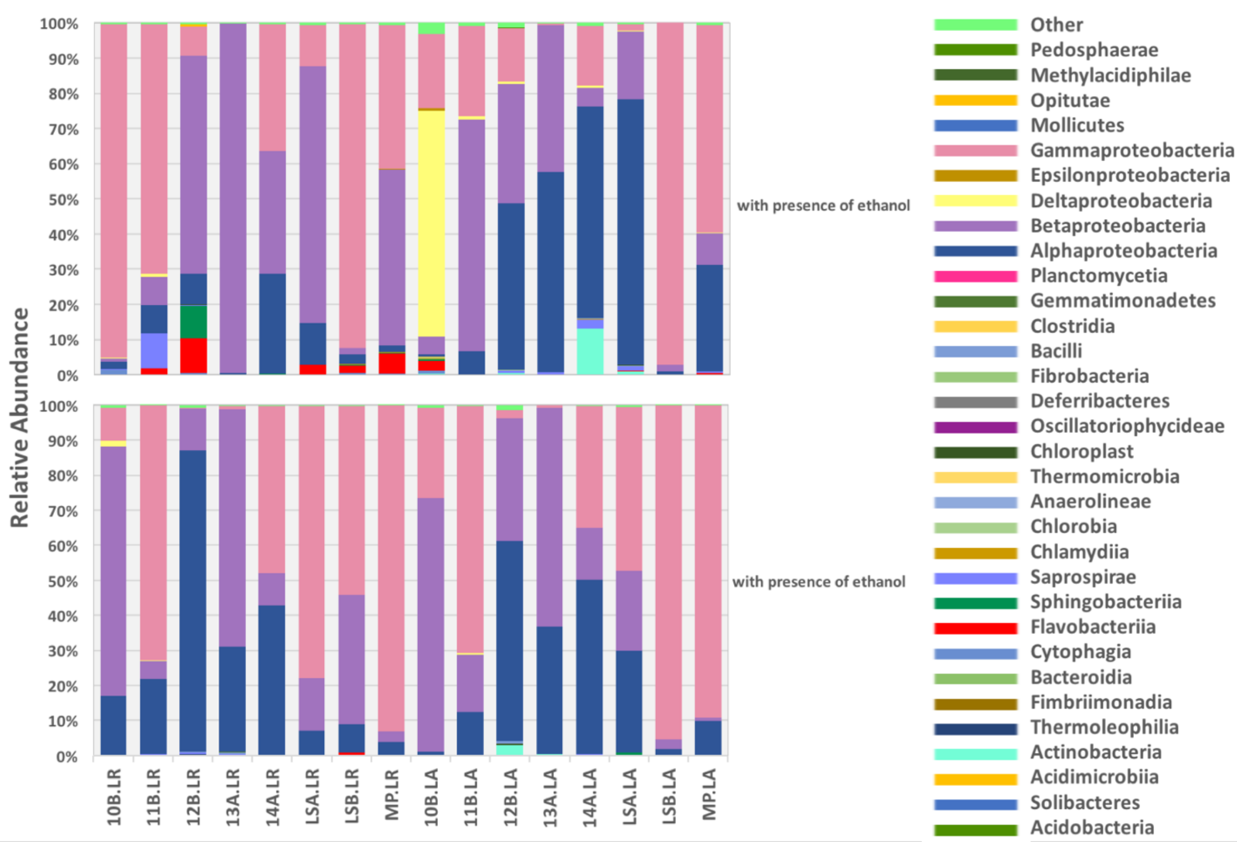


Figure 4.8 Class-level phylogenetic analysis of consortia samples that were subjected to 454 pyrosequencing (consortia samples enriched in MC-LR and MC-LA with ethanol (Top) and without ethanol (Bottom))

With the presence of ethanol, the diversity of bacterial structure in class level changed significantly by culturing the MC-LR enriched consortia in MC-LA. The comparison is shown in Table 4.5, the abundance of betaproteobacteria and gammaproteobacteria of all samples decreased from 41.2% and 44.3% to 22.8% and 29.7% respectively, while the abundance of alphaproteobacteria and deltaproteobacteria of all samples increased from 8.1% and 0.1% to 34.8% and 8.3% respectively. Besides, there are 13 of 32 bacterial classes,

including Acidobacteria, Solibacteres, Acidimicrobiia, Thermoleophilia, Chlorobia, Anaerolineae, Thermomicrobia, Deferribacteres, Fibrobacteria, Clostridia, Planctomycetia, Mollicutes and Pedosphaerae, were detected in MC-LA enriched samples but not in their original culture, MC-LR enriched samples.

On the contrast, there is no significance difference of the relative abundance of bacteria in class level observed between consortia enriched in MC-LR and MC-LA without presence of ethanol (Table 4.6)

Table 4.5 Microbial community compositions at class level of consortia in the presence of ethanol

Class	Abundance in MC-LR enriched samples (%)	Abundance in MC-LA enriched samples (%)	Difference (%)
Acidobacteria	-	0.006	0.006
Solibacteres	-	0.005	0.005
Acidimicrobiia	-	0.015	0.015
Actinobacteria	0.016	1.829	1.812
Thermoleophilia	-	0.001	0.001
Fimbriimonadia	0.022	-	-0.022
Bacteroidia	0.008	-	-0.008
Cytophagia	0.314	0.119	-0.195
Flavobacteriia	2.843	0.444	-2.399
Sphingobacteriia	1.220	0.054	-1.166
Saprospirae	1.260	0.714	-0.546
Chlamydiia	0.028	0.058	0.031
Chlorobia	-	0.004	0.004
Anaerolineae	-	0.037	0.037
Thermomicrobia	-	0.004	0.004
Chloroplast	0.027	0.031	0.005
Oscillatoriophycideae	0.004	-	-0.004
Deferribacteres	-	0.006	0.006
Fibrobacteria	-	0.006	0.006
Bacilli	0.012	0.055	0.043
Clostridia	-	0.025	0.025
Gemmatimonadetes	0.004	0.006	0.003
Planctomycetia	-	0.008	0.008
Alphaproteobacteria	8.059	34.760	26.701
Betaproteobacteria	41.217	22.762	-18.455
Deltaproteobacteria	0.132	8.327	8.195
Epsilonproteobacteria	0.004	0.091	0.087
Gammaproteobacteria	44.346	29.679	-14.667
Mollicutes	-	0.003	0.003
Opitutae	0.077	0.006	-0.071
Methylacidiphilae	0.003	-	-0.003
Pedosphaerae	-	0.038	0.038
Other	0.405	0.909	0.503

Table 4.6 Microbial community compositions at class level of consortia in the absence of ethanol

Class	Abundance in MC-LR enriched samples (%)	Abundance in MC-LA enriched samples (%)	Difference (%)
Acidobacteria	-	-	-
Solibacteres	-	-	-
Acidimicrobiia	-	-	-
Actinobacteria	0.082	0.424	0.342
Thermoleophilia	0.009	0.000	-0.009
Fimbriimonadia	-	-	-
Bacteroidia	-	-	-
Cytophagia	-	-	-
Flavobacteriia	0.121	0.022	-0.099
Sphingobacteriia	-	0.080	0.080
Saprospirae	0.139	0.050	-0.089
Chlamydiia	-	-	-
Chlorobia	-	-	-
Anaerolineae	-	-	-
Thermomicrobia	-	-	-
Chloroplast	0.004	0.061	0.056
Oscillatoriophycideae	-	-	-
Deferribacteres	-	-	-
Fibrobacteria	-	-	-
Bacilli	0.089	0.079	-0.009
Clostridia	-	-	-
Gemmatimonadetes	0.016	0.000	-0.016
Planctomycetia	-	-	-
Alphaproteobacteria	27.008	24.706	-2.302
Betaproteobacteria	27.492	28.442	0.950
Deltaproteobacteria	0.192	0.059	-0.133
Epsilonproteobacteria	-	-	-
Gammaproteobacteria	44.471	45.673	1.201
Mollicutes	-	-	-
Opitutae	-	-	-
Methylacidiphilae	-	-	-
Pedosphaerae	-	-	-
Other	0.378	0.405	0.027

In the plot of Shannon index (Figure 4.9), none of the samples has its curve reached a plateau, but all of them started leveling off. This suggests that the sequencing depths for the analysis were adequate to represent the whole community structure. The Shannon index shows that the consortia without ethanol generally have higher order of diversity than those with the presence of ethanol. The Shannon index also indicates that, for the consortia enriched in MC-LR with ethanol, 11B has the highest diversity, following in descending order by LSB, 14A, 13A, MP, LSA, 10B and 12B. For the consortia enriched in MC-LA with ethanol, the highest Shannon index also is 11B, following in descending order by 13A, 14A, LSA, MP, 12B, 10B and LSB. Being enriched in MCs without ethanol, all consortia from water samples (LSA, LSB, MP)

have higher Shannon index than those from sediment samples (10B, 11B, 12B, 13A, 14A). Shannon index ranging from high to low, LSA, MP, LSB, 12B, 14A, 11B, 13A and 10B is for the consortia enriched in MC-LR without ethanol while LSB, LSA, MP, 14A, 11B, 12B, 10B, 13A is for those enriched in MC-LA without ethanol (Figure 4.9 and Table 4.7).

Different Shannon indexes were found after culturing the consortia previously enriched in MC-LR into MC-LA (Figure 4.10 and Table 4.7). For consortia with ethanol, 10B, 11B and LSB have higher Shannon index after being cultured in MC-LA while 12B, 13A, 14A, LSA and MP have lower. For consortia without ethanol, all MC-degrading consortia have increase in Shannon index while all MC non-degrading consortia have decrease in Shannon index after being cultured in MC-LA.

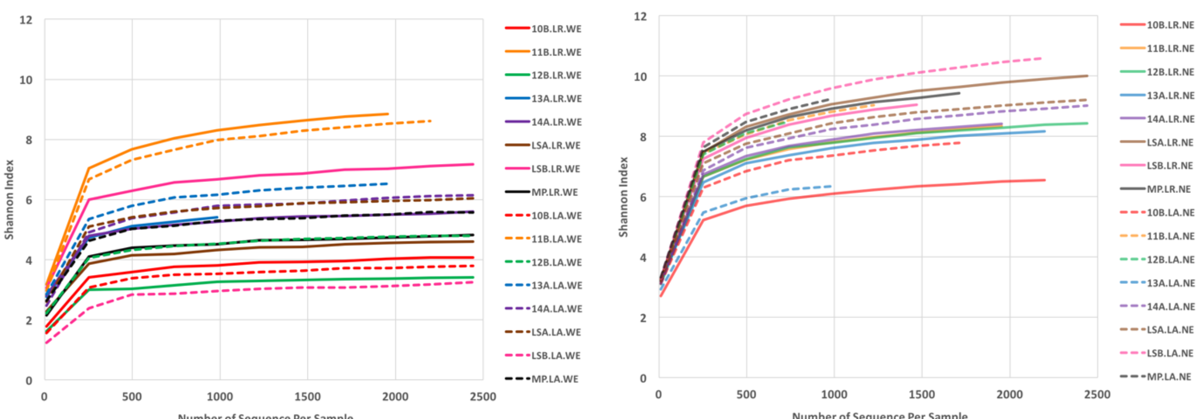


Figure 4.9: Shannon's diversity curves of 16S rDNA sequences of the samples enriched in MCs with ethanol (Left) and without ethanol (Right)

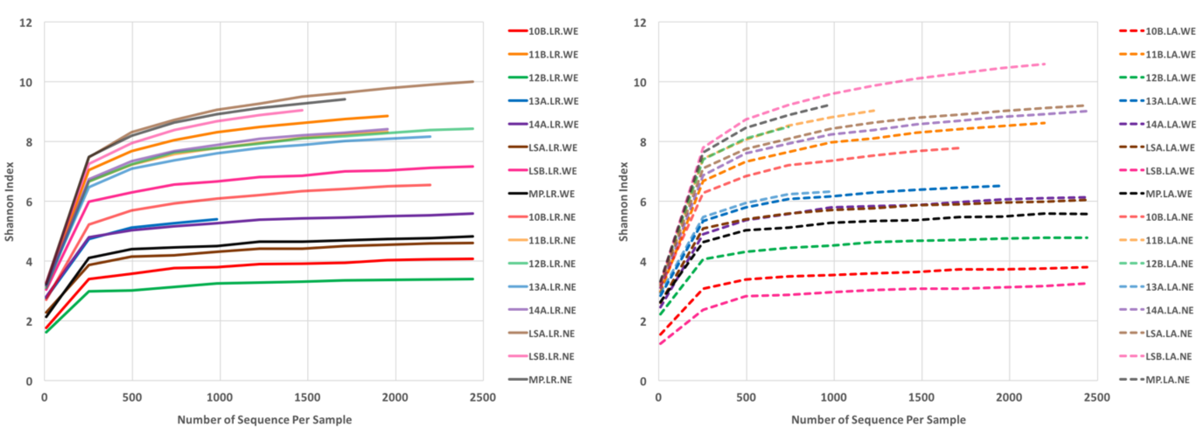


Figure 4.10 Shannon's diversity curves of 16S rDNA sequences of the samples enriched in MC-LR (Left) and MC-LA (Right)

Table 4.7 Table of Shannon index of microbial communities in the samples with different substrate

Consortia	MC-LR		MC-LA		Difference			
	(a)With ethanol	(b)Without ethanol	(c)With ethanol	(d)Without ethanol	Impact of ethanol		Impact of MC-LA	
					(b)-(a)	(d)-(c)	(c)-(a)	(d)-(b)
10B	4.25	6.57	3.89	7.79	2.32	3.90	-0.36	1.22
11B	8.87	8.37	8.65	9.05	-0.50	0.39	-0.22	0.67
12B	3.47	8.47	4.90	8.53	5.00	3.63	1.43	0.06
13A	5.50	8.20	6.53	6.41	2.70	-0.12	1.03	-1.79
14A	5.71	8.43	6.31	9.12	2.72	2.81	0.60	0.69
LSA	4.72	10.02	6.08	9.27	5.30	3.20	1.36	-0.74
LSB	7.29	9.08	3.40	10.60	1.80	7.20	-3.88	1.52
MP	4.92	9.45	5.71	9.26	4.53	3.55	0.78	-0.20

(Red: MC degrading consortia; Black: MC non-degrading consortia)

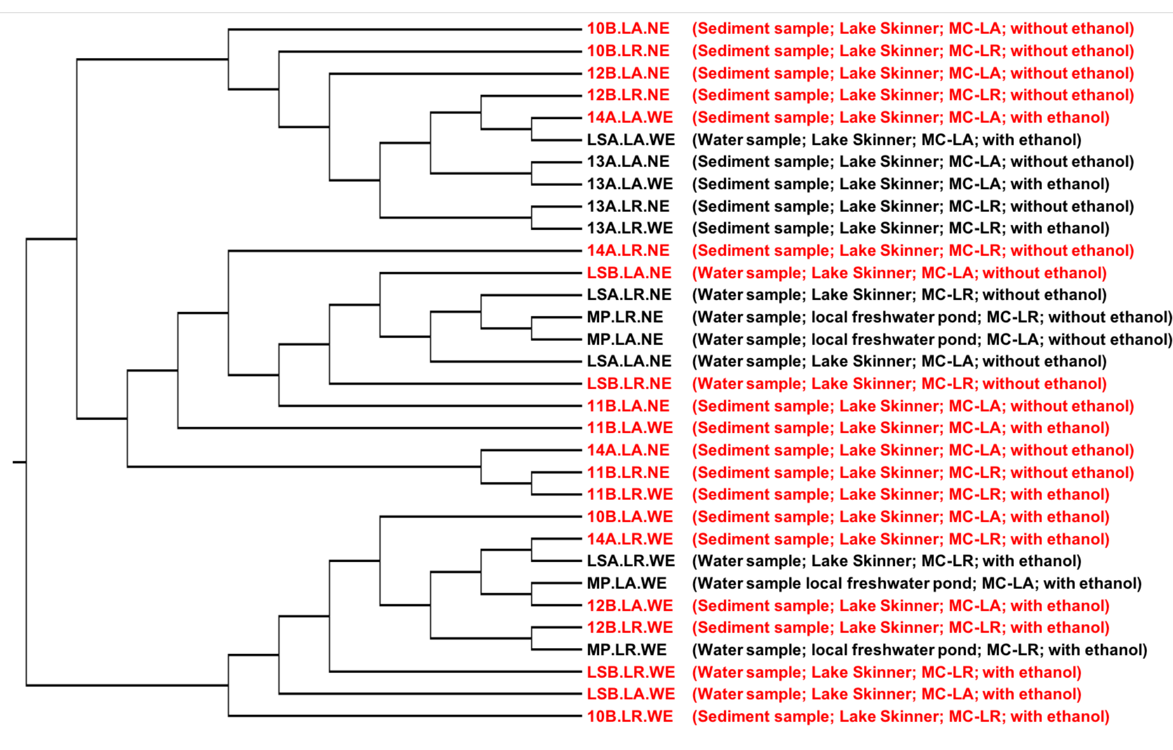


Figure 4.11 Dendrogram of UPGMA cluster analysis of all bacterial communities

(Red: MC degrading consortia; Black: MC non-degrading consortia)

Cluster analysis of bacterial communities based on UniFrac distances is illustrated in Figure 4.11. The closer the samples are clustered, the more similar their overall phylogenetic relationship is. The consortia were grouped into two major clusters. First cluster consists of some consortia with ethanol and all consortia without ethanol. The second cluster is composed of only consortia with ethanol. The consortia have not been clustered solely based on neither destination, MC-variant nor MC degrading capability.

For consortia enriched in MCs with the presence of ethanol, most of the consortia clustered together with their own communities associated with different MC variants, except 14A and LSA which are also characterized by the highest increase in Shannon index after being enriched in MC-LA (Figure 4.12). For consortia enriched in MCs with absence of ethanol, all the consortia clustered together with their own communities being cultured in different MC variants. In this group of consortia, water consortia (LSA, LSB and MP) closely clustered together, as did the MC non-degrading consortia (LSA, MP and 13A) (Figure 4.13).

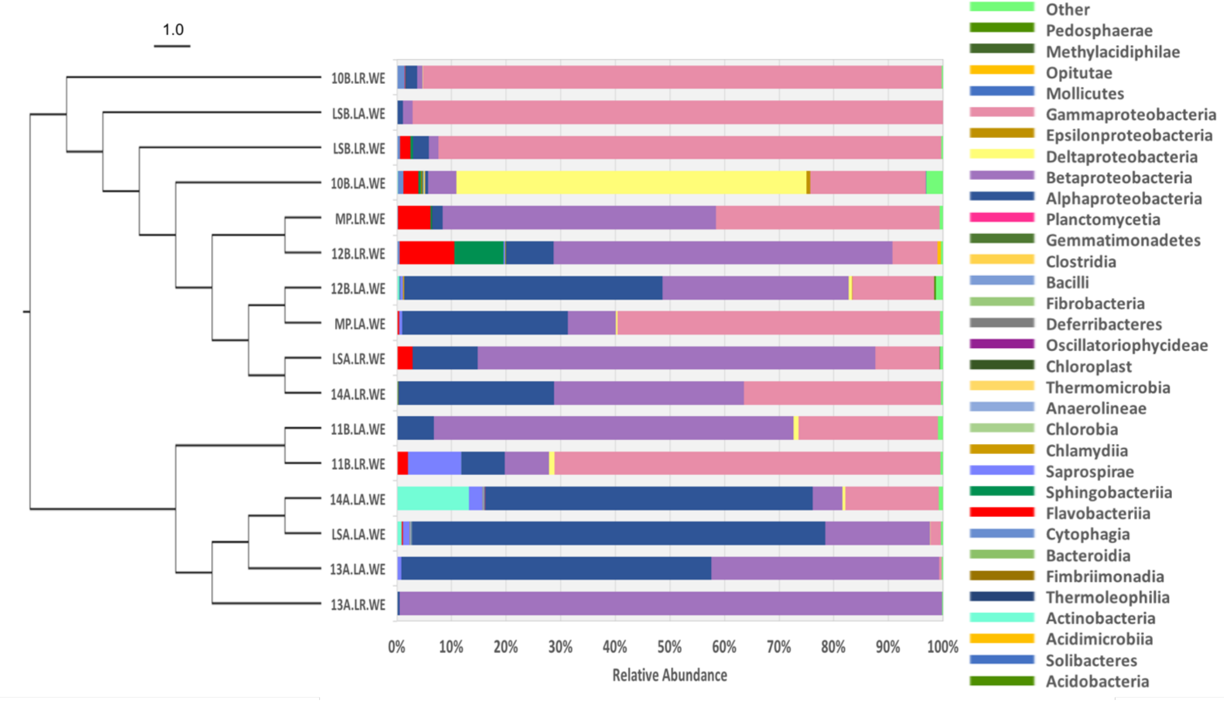


Figure 4.12 Dendrogram (Left) of UPGMA cluster analysis and relative abundance (Right) of bacterial communities of consortia enriched in MCs with presence of ethanol

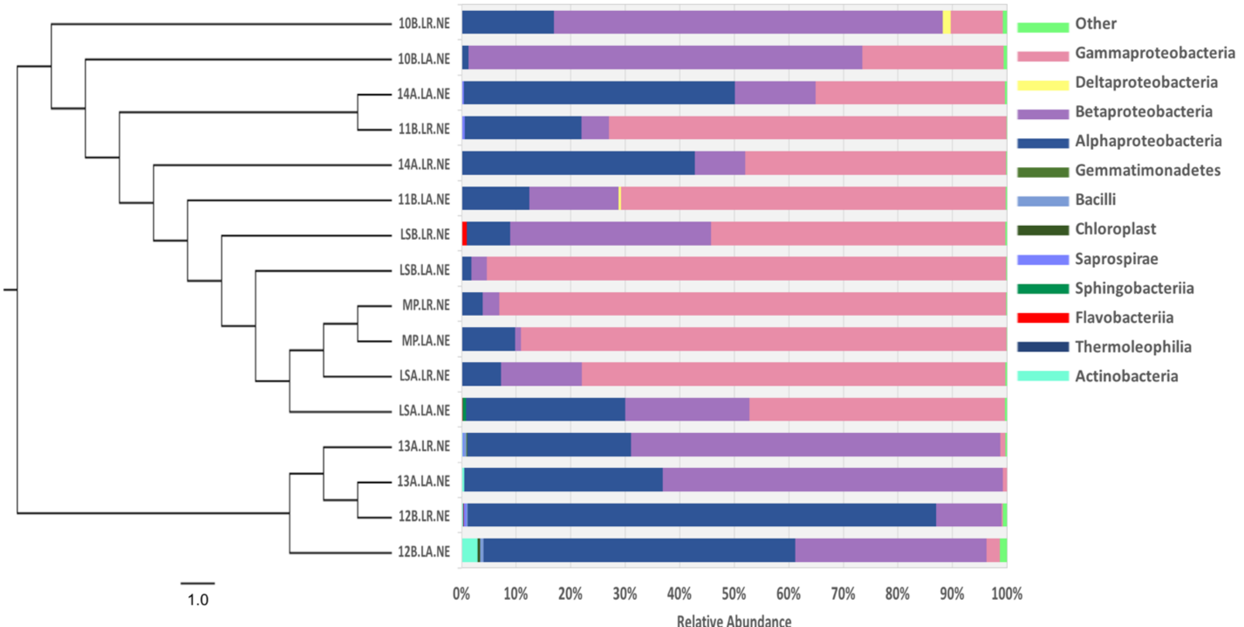


Figure 4.13 Dendrogram (Left) of UPGMA cluster analysis and relative abundance (Right) of bacterial communities of consortia enriched in MCs with absence of ethanol

5. DISCUSSION

5.1 Biodegradation Study of Isolates

High genus diversity was discovered among the strains isolated in the enrichment study. 6 out of 11 genera have not been reported for any capability to degrade microcystin, including *Acinetobacter sp.*; *Cupriavidus sp.*; *Shinella sp.*; *Brevundimonas sp.*; *Leifsonia sp.*; and *Thermoleophilum sp.* [9]. Over the biodegradation study period of 7 days, none of them showed any capability of degrading MC-LR. This can be explained that the MC biodegraders in the consortia samples are not culturable. In fact, there are lots of bacteria who are abundant in nature but unculturable [54]. Unable to isolate any MC degrader may also because they were not identified as an isolate in the first plating of the consortia. Additional study was done to compare the growth of these isolates in the presence of MC-LR with or without ethanol or in the presence of ethanol without MC-LR, the result confirmed that they are ethanol consumer rather than MC degrader.

5.2 Biodegradation Study of Consortia

In the biodegradation study, 4 out of 5 number of consortia from sediment samples and 1 out of 3 number of consortia from water samples demonstrated capability of degrading both MC-LR and MC-LA. The half-lives of MC degradation found in this study ranges from 12 hours to 4.5 days. Previous study found that the half lives of MC biodegradation by isolates and consortia are from a few hours to 18 days and from 12 hours to 14 days respectively [9, 11, 39, 55]. The finding in this study is encouraging for continuous study on MC biodegradation by consortia. It is not only because obtained biodegradation rate is competitive to those by isolates, but also because there is potentially more than one degradation pathway occurring in consortia [8, 53]. Synergistic degradation in consortia potentially allows faster and higher degree biodegradation.

Generally, no obvious lag phase is observed for the biodegradation. This can be explained by two reasons. Firstly, the bacteria of degrading consortia already acclimatized MC as their food source since they were pre-incubated in MC [42]. The second reason is that the enzyme secreted by the bacteria to degrade MC may exist in the inoculum, so that both the enzyme and MC are ready to interact once the experiment begin.

After realizing bacteria in consortia may grow solely on ethanol. Experiment MCLR-WE-NE, MCLR-NE-NE, MCLA-WE-NE and MCLA-NE-NE were conducted to examine the effect induced by the presence of ethanol itself and its consumers on the performance of the MC degrading consortia. For MC-LR, the half-lives of biodegradation among all consortia with absence of ethanol are generally faster than those with presence of ethanol. However, this was not the case in the experiment of MC-LA without ethanol. To explain the faster degradation rate, it is considered that the ethanol consuming bacteria may compete with MC-degrading bacteria for the limited nutrient in media. Elimination of these ethanol-required bacteria by incubating them without ethanol made the nutrient more available for the MC-degrading bacteria to grow and do their work. This is supported by the previous study showing that MC degrader performs better in nutrient rich media [41].

It is possible that ethanol-required bacteria may be also the symbionts who are responsible to perform the downstream degradation of linearized MC or tetrapeptide. ELISA used in this study targets Adda moiety, it cannot distinguish degraded or non-degraded MC [31]. Elimination of the bacteria who do the downstream degradation work will result in slower the evaluated degradation rate. There might exist a balance of promoting MC-degraders and prohibiting linearized MC/tetrapeptide degraders. Such balance would depend on both the bacterial abundance and diversity of the consortia. This explains that no general trend is observed for the change of MC degrading performance among the consortia from the presence to absence of ethanol.

In experiment MCLR-NE-NE and MCLA-NE-NE, it was unable to have the initial OD of consortia as high as the OD used to conduct experiment MCLR-WE-WE, MCLR-WE-NE, MCLA-WE-WE and MCLA-WE-NE under the same condition. 10-fold lower OD was used instead. Ethanol consuming bacteria may be the majority in consortia and contributed significantly for the optical density of the consortia. In fact, low dose of ethanol can stimulate bacterial growth to a higher cell density [56]. However, such large decrease in initial

OD did not necessary result in lower MC degradation rate. Some consortia performed more less the same (10B and 11B in MC-LR, 10B and LSB in MC-LA) or even better (12B and LSB in MC-LR, 14A in MC-LA). This is unfair to compare the degradation performance of MCLR-NE-NE and MCLA-NE-NE directly with other set of experiments provide that the inoculum of the former was incubated in MCs without ethanol. Having these good biodegradation performances without high cell density required will be an incentive factor to implement biodegradation by these consortia. Besides, based on the low substrate specificity and high consistency in performance observed among all sets of experiment, consortia 10B and 11B should be given priority for further study on MC biodegradation by consortia.

Overall, most of the MC degrading consortia found in this study can degrade MCs from 200ug/L down below the detection limit of ELISA kit in 2 to 3 days. However, it is difficult to directly compare the biodegradation performance reported by other authors because there is great variability in the concentration of MC used, the type of MC, the incubation time and the methodologies used to quantify degradation [9]. And there is no standard algorithm for evaluation of MC degrading ability.

Besides, ELISA is mainly used for screening purpose and it is not able to distinguish and quantify specific degraded product [25, 31]. Provided that biodegradation is used for detoxify the MC-contaminated water and degraded MC product maybe non-toxic [28], completely breaking down MC is not necessary. For degrading cyclic MC to linear MC, ELISA may be over-conservative and underestimating the biodegradation rate.

Biodegradation should not be evaluated by a single analytical method. This study gives us the insight on MC biodegradation study by consortia and helps to screen out the consortia with MC-degrading ability. Further study by using instrumental analysis is expected to evaluate their performance, understand cleavage position of different MC variants as well as the degradation pathway by looking at the transformed products.

5.3 Community analysis of MC degrading consortia

Changing the substrate from MC-LR to MC-LA caused significant diversity change in bacterial community among all samples. If this change is solely due to substrate specificity, it should not occur in MC non-degrading consortia community. It is suggested that some bacteria are less tolerant to MC-LA than MC-LR. Although no biocidal effect on laboratory bacteria was found previously [14], a previous study assessing the impact of microcystins on the growth of aquatic bacteria suggests that microcystin can induce reduction of bacterial growth [57]. This explains why there were changes in composition of MC non-degrading bacterial communities.

For MC degrading consortia, the growth of MC-LA degraders or/and their symbionts also contributed to the change in community structure. It is queried that if this shift caused by subjecting to different MC variants is reversible. It may be interesting to evaluate their consistency of MC degrading ability after being enriched in different MC-variant.

Having more bacterial classes revealed, the consortia cultured in MC-LA with ethanol shows higher diversity in class level than their original culture (Table 4.5). This is explained that the bacteria who cannot tolerant or degrade MC-LA may abundantly exist in samples cultured in MC-LR and their abundance make the presence of those bacteria who are detected in sample of MC-LA negligible. As discussed above, ethanol consuming bacteria dominantly exist in these samples and ethanol potentially enhance the growth of MC non-degrading bacteria. Abundant presence of these bacteria severely make the other bacteria difficult to be detected. This suggests the need of phylogenetic analysis of consortia sample cultured in MC without ethanol in order to reveal the actual MC degraders.

The consortia enriched in MCs without ethanol have lesser number of classes revealed (Table 4.6) but higher diversity in genus level reflected by Shannon index than those enriched in MCs with ethanol (Table 4.7). This suggests that those bacterial classes revealed in consortia with ethanol used ethanol as their energy source and

they are able to grow along with MC. Some bacteria may rapidly proliferate on ethanol. The rapid proliferation of a small number of bacteria resulted in lower Shannon index. This observation is consistent to the previous study showing that a few bacteria in MC degrading bacterial community can easily degrade lots of organic compounds along with microcystin [11].

For consortia enriched in MCs without ethanol, cluster analysis shows that MC non-degrading consortia from water samples (LSA, MP) shares similar bacterial community structures and MC non-degrading consortia from sediment sample (13A) is also isolated form other MC degrading consortia in the dendrogram (Figure 4.13). However, for consortia enriched in MCs with ethanol, it seems that there is similarity shared in both MC degrading and MC non-degrading consortia, such that there is no clear clustering to distinguish them. This suggests that presence of ethanol concealed the difference of community structure between MC degrading consortia and MC non-degrading consortia by having the ethanol consuming bacteria grow dominantly.

MC degrading strains may belong to the genus that exist in MC non-degrading consortia. The dominant bacterial classes revealed in community analysis are Alphaproteobacteria, Betaproteobacteria and Gammaproteobacteria which are also the three main class of bacteria in the microorganism community associated with MC releasing genus, *Microcystis aeruginosa* [58]. The isolates found capable of degrading MC in previous studies also mainly belong to these three classes [9]. Not only MC degrading consortia have these classes in majority but also the MC non-degrading consortia (Figure 4.8). The overlapping of community structure in genus level is clearly observed (Appendix B). Previous studies looked at the bacterial structure of microbial community associated with MC producer or MC degradation [58, 59], sorted out the dominant bacterial class or even genus and considered them the potential player in MC degradation. But no one compares the composition between those MC degrading community and MC non-degrading community and realizes that they may share the similar community structure and the MC degrading strains belong to the genus that commonly exist.

Based on the reason mentioned above and the fact that MC non-degrading bacteria can grow on the carbon released by the dead bacteria, it is hypothesized that MC degrader is not necessary the dominant group in the MC degrading consortia and the major bacteria may just accompany the MC degrader and have no ability to degrade MC. This shows the complexity of characterizing the MC degrading consortia.

MC biodegradation capability depends on bacterial diversity of the consortia. However, no correlation between MC degrading capability and taxonomic diversity is found in this study. This suggests that diversity itself is not a good indicator for MC biodegradation ability. The only selective response of bacterial communities to MC is that a decrease in diversity was evidenced by Shannon index for each of the MC non-degrading consortia after amendment of MC variant.

The community structure as well as MC degradation capability of the consortia used in this study are different, even through most of them (10B,11B,12B,13A,14A, LSA and LSB) are from the same river. These differences indicate that biogeological parameters significantly influence the community formation.

The clustering analysis demonstrated that the community structure of 10B is relatively distinct from other consortia. Some unique genera are found in 10B. It is the only consortium has benthic cyanobacteria *Phormidium sp.* whose some isolates have been confirmed as strong microcystin producers [2, 3]. 10B is the only consortia has *Aeromonas sp.*, which is capable of remineralization of many organic materials and was found abundantly exist in bacterial community associated with microcystin producer, Microcystis sp. [60]. A recent study shows that *Aeromonas sp* is able to use microcystin as its major energy source [61]. These component of community structure may contribute to the MC degradation and relate to the relative consistent biodegradation performance under different experiments. However, their potential role in the process cannot be accessed based on the results obtained in this study.

Overall, looking at the community structure at only one stage in biodegradation study is not sufficient to reveal the selective response of the MC degrading community to the MC biodegradation or the role of bacteria playing in the process. Previous study showed that the community structure changed during the

biodegradation process [11]. This supports the need to monitor the bacterial diversity along the biodegradation.

6. CONCLUSION

This study investigated the biodegradation of MC by bacterial isolates and consortia which are originated from lake with toxic cyanobacterial bloom record. And the bacterial communities of the consortia were characterized. The main conclusions are listed as the followings.

1. None of the strains isolated can degrade MC-LR. The isolates are fast growing bacteria and they are able to use ethanol as their carbon source.
2. 5 of 8 consortia were found capable to degrade both MC-LR and MC-LA efficiently in the presence or absence of ethanol. The biodegradations had half lives from 12 hours to 4.5 days without obvious lag phase. 2 of them demonstrated relatively consistent performance on degradation of MC-LR and MC-LA.
3. Presence of ethanol was found significantly shaped the community structure and affected the MC degradation ability of different consortia differently. Amendment of MC variants also affected the community.
4. No clear correlation between bacterial diversity and MC degradation capability was found. And the dominant taxonomies found in the MC degrading consortia are significantly overlapped with MC non-degrading consortia. Monitoring the change in community structure along the biodegradation study may be a better way to understand the response of consortia to MC degradation.

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APPENDIX

Appendix A-1: Relative abundance in percentage of taxonomy in class level among consortia enriched in MC with ethanol

	MC-LR								MC-LA							
	10B	11B	12B	13A	14A	LSA	LSB	MP	10B	11B	12B	13A	14A	LSA	LSB	MP
Acidobacteria	-	-	-	-	-	-	-	-	0.04	-	-	-	-	-	-	-
Solibacteres	-	-	-	-	-	-	-	-	0.02	-	0.01	-	-	-	-	-
Acidimicrobiia	-	-	-	-	-	-	-	-	-	-	0.04	-	0.01	0.06	-	-
Actinobacteria	0.03	-	0.03	-	-	0.04	-	0.02	0.16	-	0.39	0.10	13.17	0.82	-	-
Thermoleophilia	-	-	-	-	-	-	-	-	-	-	-	-	0.01	-	-	-
Fimbriimonadia	0.08	0.09	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bacteroidia	-	-	0.06	-	-	-	-	-	-	-	-	-	-	-	-	-
Cytophagia	1.33	-	0.43	-	-	0.04	0.54	0.17	0.93	-	0.01	-	-	-	-	-
Flavobacteriia	0.08	1.90	9.96	-	0.12	2.78	1.98	5.93	2.80	-	0.04	-	0.02	0.22	0.02	0.44
Sphingobacteriia	-	-	9.08	-	0.07	-	0.38	0.23	0.40	-	0.03	-	-	-	-	-
Saprospirae	0.02	9.72	0.24	0.09	-	-	-	-	0.07	0.13	0.61	0.72	2.46	1.20	0.02	0.50
Chlamydiia	0.01	-	0.09	-	0.05	-	0.05	0.02	0.11	-	0.16	-	0.10	0.10	-	-
Chlorobia	-	-	-	-	-	-	-	-	-	-	0.03	-	-	-	-	-
Anaerolineae	-	-	-	-	-	-	-	-	-	-	-	-	0.04	0.25	-	-
Thermomicrobia	-	-	-	-	-	-	-	-	-	-	-	-	0.03	-	-	-
Chloroplast	0.08	-	0.03	-	-	-	-	0.10	0.16	-	-	-	0.03	0.06	-	-
Oscillatoriophycideae	0.03	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Deferribacteres	-	-	-	-	-	-	-	-	0.04	-	-	-	-	-	-	-
Fibrobacteria	-	-	-	-	-	-	-	-	0.04	-	-	-	-	-	-	-
Bacilli	-	0.09	-	-	-	-	-	-	0.18	-	0.01	-	0.15	0.10	-	-
Clostridia	-	-	-	-	-	-	-	-	0.20	-	-	-	-	-	-	-
Gemmatimonadetes	-	-	0.03	-	-	-	-	-	-	-	-	-	0.02	-	-	0.03
Planctomycetia	-	-	-	-	-	-	-	-	-	-	-	-	0.07	-	-	-
Alphaproteobacteria	2.03	7.97	8.74	0.43	28.53	11.94	2.90	1.93	0.56	6.60	47.29	56.72	59.98	75.58	1.04	30.31
Betaproteobacteria	1.02	8.06	62.05	99.31	34.75	72.77	1.75	50.02	5.16	65.87	34.05	41.74	5.47	19.20	1.80	8.81
Deltaproteobacteria	0.03	1.00	-	-	-	0.02	-	-	64.13	0.92	0.65	-	0.55	0.10	-	0.27
Epsilonproteobacteria	0.01	-	-	-	-	-	-	0.02	0.71	-	0.01	-	-	-	-	-
Gammaproteobacteria	94.96	70.68	8.25	-	36.10	11.78	92.04	40.95	21.16	25.56	15.04	0.48	17.09	1.93	97.12	59.05
Mollicutes	-	-	-	-	-	-	-	-	0.02	-	-	-	-	-	-	-
Opitutae	-	-	0.58	-	-	-	0.03	-	0.04	-	-	-	-	-	-	-
Methylacidiphilae	-	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-
Pedosphaerae	-	-	-	-	-	-	-	-	0.02	-	0.28	-	-	-	-	-
Other	0.27	0.47	0.43	0.17	0.39	0.58	0.31	0.60	3.04	0.92	1.31	0.24	0.79	0.38	-	0.59

Appendix A-2: Relative abundance in percentage of taxonomy in class level among consortia enriched in MC without ethanol

	MC-LR								MC-LA							
	10B	11B	12B	13A	14A	LSA	LSB	MP	10B	11B	12B	13A	14A	LSA	LSB	MP
Actinobacteria	-	0.10	0.28	0.21	-	-	0.06	-	-	-	2.91	0.48	-	-	-	-
Thermoleophilia	-	-	0.07	-	-	-	-	-	-	-	-	-	-	-	-	-
Flavobacteriia	-	-	0.04	-	-	0.04	0.89	-	-	-	-	-	-	0.18	-	-
Sphingobacteriia	-	-	-	-	-	-	-	-	-	-	-	-	-	0.64	-	-
Saprospirae	-	0.44	0.46	0.21	-	-	-	-	-	-	-	-	0.40	-	-	-
Chloroplast	-	-	0.04	-	-	-	-	-	-	-	0.49	-	-	-	-	-
Bacilli	-	-	0.25	0.46	-	-	-	-	-	-	0.61	-	0.03	-	-	-
Gemmatimonadetes	-	-	-	0.13	-	-	-	-	-	-	-	-	-	-	-	-
Alphaproteobacteria	16.95	21.40	85.89	30.08	42.73	7.17	7.96	3.88	1.22	12.44	57.16	36.38	49.65	29.19	1.79	9.82
Betaproteobacteria	71.27	5.06	11.98	67.69	9.28	14.82	36.79	3.05	72.26	16.30	35.07	62.38	14.87	22.76	2.82	1.07
Deltaproteobacteria	1.48	0.05	-	-	-	-	-	-	-	0.47	-	-	-	-	-	-
Gammaproteobacteria	9.49	72.86	0.25	0.88	47.79	77.61	53.98	92.91	25.87	70.55	2.43	0.76	34.69	46.80	95.25	89.02
Other	0.81	0.10	0.74	0.34	0.20	0.36	0.32	0.16	0.64	0.24	1.33	-	0.37	0.43	0.13	0.10

Appendix B: Relative abundance in percentage of taxonomy in genus level among consortia

#OTU ID	10B.LR.WE	11B.LR.WE	12B.LR.WE	13A.LR.WE	14A.LR.WE	LSA.LR.WE	LSB.LR.WE	MP.LR.WE	10B.LA.WE	11B.LA.WE	12B.LA.WE	13A.LA.WE	14A.LA.WE	LSA.LA.WE	LSB.LA.WE	MP.LA.WE	10B.LR.NE	11B.LR.NE	12B.LR.NE	13A.LR.NE	14A.LR.NE	LSA.LR.NE	LSB.LR.NE	MP.LR.NE	10B.LA.NE	11B.LA.NE	12B.LA.NE	13A.LA.NE	14A.LA.NE	LSA.LA.NE	LSB.LA.NE	MP.LA.NE
Unclassified;Other;Other;Other;Other;Other	-	-	-	-	-	-	-	-	-	-	0.01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;Other;Other;Other;Other;Other	0.08	-	0.12	0.17	-	-	-	0.02	0.58	0.17	0.12	-	0.22	0.19	-	0.12	0.13	-	0.07	0.13	0.05	0.08	-	-	0.23	0.16	-	-	-	0.14	0.09	-
k__Bacteria;p__Acidobacteria;c__Acidobacteria-6;o__;f__;g__	-	-	-	-	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Acidobacteria;c__Acidobacteria-6;o__iii1-15;f__;g__	-	-	-	-	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Acidobacteria;c__Solibacteres;o__Solibacterales;f__Solibacteraceae;g__	-	-	-	-	-	-	-	-	0.02	-	0.01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Actinobacteria;Other;Other;Other;Other	-	-	-	-	-	-	-	-	-	-	-	-	0.01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Actinobacteria;c__Acidimicrobiia;o__Acidimicrobiales;f__;g__	-	-	-	-	-	-	-	-	-	-	0.04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Actinobacteria;c__Acidimicrobiia;o__Acidimicrobiales;f__Microthrixaceae;g__	-	-	-	-	-	-	-	-	-	-	-	-	0.01	0.06	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;Other;Other	-	-	-	-	-	-	-	-	0.02	-	-	-	0.79	0.03	-	-	-	-	0.04	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__ACK-M1;g__	-	-	-	-	-	-	-	-	0.04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__ACK-M1;g__Planktophila	-	-	-	-	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Gordoniaceae;Other	-	-	-	-	-	-	-	-	-	-	-	-	0.09	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Gordoniaceae;g__Millisia	-	-	-	-	-	-	-	-	-	-	-	-	0.04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Microbacteriaceae;Other	-	-	-	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Microbacteriaceae;g__	-	-	-	-	-	0.02	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Microbacteriaceae;g__Candidatus Rhodoluna	-	-	-	-	-	-	-	-	0.04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Microbacteriaceae;g__Clavibacter	0.01	-	-	-	-	0.02	-	-	-	-	0.01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Microbacteriaceae;g__Cryocola	-	-	-	-	-	-	-	-	-	-	0.28	-	-	-	-	-	-	0.10	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Microbacteriaceae;g__Labedella	-	-	-	-	-	-	-	-	-	-	0.04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Microbacteriaceae;g__Leifsonia	0.01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Microbacteriaceae;g__Yonghaparkia	0.01	-	-	-	-	-	-	-	-	-	0.03	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Mycobacteriaceae;g__Mycobacterium	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.04	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Nocardiaceae;Other	-	-	-	-	-	-	-	-	-	-	-	-	0.19	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Nocardiaceae;g__Rhodococcus	-	-	0.03	-	-	-	-	-	-	-	-	0.10	12.05	0.79	-	-	-	-	0.21	0.21	-	-	-	-	-	-	2.31	0.48	-	-	-	-
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Nocardiaceae;g__Segniliparus	-	-	-	-	-	-	-	-	-	-	-	-	0.01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Nocardioidaceae;g__Pimelobacter	-	-	-	-	-	-	-	-	-	-	0.01	-	-	-	-	-	-	-	-	-	-	-	0.06	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Propionibacteriaceae;g__Propionibacterium	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.61	-	-	-	-	-
k__Bacteria;p__Actinobacteria;c__Thermoleophilia;o__Gaiellales;Other;Other	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.04	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Actinobacteria;c__Thermoleophilia;o__Gaiellales;f__;g__	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.04	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Actinobacteria;c__Thermoleophilia;o__Solirubrobacterales;f__;g__	-	-	-	-	-	-	-	-	-	-	-	-	0.01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Armatimonadetes;c__[Fimbriimonadia];o__;f__;g__	-	0.09	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Armatimonadetes;c__[Fimbriimonadia];o__[Fimbriimonadales];f__;g__	0.08	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Bacteroidetes;Other;Other;Other;Other	0.02	-	0.21	-	-	-	-	-	0.04	-	-	-	0.01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__SB-1;g__	-	-	0.06	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Bacteroidetes;c__Cytophagia;o__Cytophagales;Other;Other	0.05	-	0.09	-	-	-	0.02	-	0.04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Bacteroidetes;c__Cytophagia;o__Cytophagales;f__Cyclobacteriaceae;Other	-	-	-	-	-	0.02	-	-	0.04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Bacteroidetes;c__Cytophagia;o__Cytophagales;f__Cytophagaceae;g__	0.02	-	-	-	-	0.02	-	0.06	0.84	-	0.01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Bacteroidetes;c__Cytophagia;o__Cytophagales;f__Cytophagaceae;g__Dyadobacter	1.24	-	0.21	-	-	-	0.52	0.10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Bacteroidetes;c__Cytophagia;o__Cytophagales;f__Cytophagaceae;g__Hymenobacter	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Bacteroidetes;c__Cytophagia;o__Cytophagales;f__Flammeovirgaceae;Other	-	-	0.03	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Bacteroidetes;c__Cytophagia;o__Cytophagales;f__Flammeovirgaceae;g__Marinoscillum	-	-	0.09	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Bacteroidetes;c__Flavobacteriia;o__Flavobacteriales;f__;g__	-	-	-	-	-	-	-	-	-	-	0.01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Bacteroidetes;c__Flavobacteriia;o__Flavobacteriales;f__Cryomorphaceae;g__Fluviicola	-	-	0.37	-	-	-	-	-	0.38	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Bacteroidetes;c__Flavobacteriia;o__Flavobacteriales;f__Flavobacteriaceae;Other	-	0.14	0.18	-	0.05	0.20	0.12	0.56	0.40	-	-	-	-	0.03	-	0.03	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Bacteroidetes;c__Flavobacteriia;o__Flavobacteriales;f__Flavobacteriaceae;g__Aequorivita	-	1.76	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Bacteroidetes;c__Flavobacteriia;o__Flavobacteriales;f__Flavobacteriaceae;g__Bizionia	-	-	-	-	-	-	-	-	-	-	-	-	0.01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Bacteroidetes;c__Flavobacteriia;o__Flavobacteriales;f__Flavobacteriaceae;g__Flavobacterium	0.06	-	9.05	-	0.05	2.40	1.84	5.27	1.80	-	0.01	-	0.01	0.19	0.02	0.41	-	-	-	-	-	0.04	0.89	-	-	-	-	-	-	0.14	-	-
k__Bacteria;p__Bacteroidetes;c__Flavobacteriia;o__Flavobacteriales;f__Flavobacteriaceae;g__Gillisia	0.02	-	-	-	0.02	0.18	0.02	0.10	0.18	-	0.01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Bacteroidetes;c__Flavobacteriia;o__Flavobacteriales;f__Flavobacteriaceae;g__Snuella	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.04	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Bacteroidetes;c__Flavobacteriia;o__Flavobacteriales;f__Flavobacteriaceae;g__Ulvibacter	-	-	0.37	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.04	-	-
k__Bacteria;p__Bacteroidetes;c__Flavobacteriia;o__Flavobacteriales;f__[Weeksellaceae];g__Chryseobacterium	-	-	-	-	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Bacteroidetes;c__Flavobacteriia;o__Flavobacteriales;f__[Weeksellaceae];g__Cloacibacterium	-	-	-	-	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Bacteroidetes;c__Sphingobacteriia;o__Sphingobacteriales;Other;Other	-	-	0.06	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Bacteroidetes;c__Sphingobacteriia;o__Sphingobacteriales;f__;g__	-	-	9.02	-	-	-	-	-	0.11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Bacteroidetes;c__Sphingobacteriia;o__Sphingobacteriales;f__Sphingobacteriaceae;Other	-	-	-	-	-	-	0.33	0.15	0.04	-	0.01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.18	-	-
k__Bacteria;p__Bacteroidetes;c__Sphingobacteriia;o__Sphingobacteriales;f__Sphingobacteriaceae;g__	-	-	-	-	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Bacteroidetes;c__Sphingobacteriia;o__Sphingobacteriales;f__Sphingobacteriaceae;g__Mucilaginibacter	-	-	-	-	-	-	-	-	0.09	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Bacteroidetes;c__Sphingobacteriia;o__Sphingobacteriales;f__Sphingobacteriaceae;g__Pedobacter	-	-	-	-	0.05	-	0.05	0.08	0.09	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.43	-	-
k__Bacteria;p__Bacteroidetes;c__Sphingobacteriia;o__Sphingobacteriales;f__Sphingobacteriaceae;g__Solitalea	-	-	-	-	0.02	-	-	-	0.04	-	0.01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.04	-	-
k__Bacteria;p__Bacteroidetes;c__[Saprospirae];o__[Saprospirales];f__Chitinophagaceae;Other	-	0.43	-	-	-	-	-	-	-	0.04	0.04	-	0.37	-	-	-	-	0.05	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Bacteroidetes;c__[Saprospirae];o__[Saprospirales];f__Chitinophagaceae;g__	-	7.69	-	-	-	-	-	-	0.02	-	0.12	0.72	1.67	0.98	-	0.41	-	0.34	0.14	0.21	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Bacteroidetes;c__[Saprospirae];o__[Saprospirales];f__Chitinophagaceae;g__Chitinophaga	-	1.19	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Bacteroidetes;c__[Saprospirae];o__[Saprospirales];f__Chitinophagaceae;g__Ferruginibacter	-	0.33	-	0.09	-	-	-	-	-	-	-	-	0.36	-	-	0.09	-	0.05	0.32	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Bacteroidetes;c__[Saprospirae];o__[Saprospirales];f__Chitinophagaceae;g__Flavihumibacter	-	-	0.24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Bacteroidetes;c__[Saprospirae];o__[Saprospirales];f__Chitinophagaceae;g__Lacibacter	-	-	-	-	-	-	-	-	-	-	-	-	0.01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Bacteroidetes;c__[Saprospirae];o__[Saprospirales];f__Chitinophagaceae;g__Parasegitibacter	-	0.09	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Bacteroidetes;c__[Saprospirae];o__[Saprospirales];f__Chitinophagaceae;g__Sediminibacterium	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Bacteroidetes;c__[Saprospirae];o__[Saprospirales];f__Chitinophagaceae;g__Terrimonas	-	-	-	-	-	-	-	-	0.04	0.08	0.45	-	0.04	0.22	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	0.40	-	-	-
k__Bacteria;p__Chlamydiae;c__Chlamydiia;o__Chlamydiales;Other;Other	-	-	0.03	-	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Chlamydiae;c__Chlamydiia;o__Chlamydiales;f__;g__	0.01	-	-	-	0.05	-	0.05	-	0.07	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Chlamydiae;c__Chlamydiia;o__Chlamydiales;f__Parachlamydiaceae;Other	-	-	-	-	-	-	-	-	-	-	-	-	0.01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Chlamydiae;c__Chlamydiia;o__Chlamydiales;f__Parachlamydiaceae;g__Candidatus Protochlamydia	-	-	-	-	-	-	-	-	-	-	0.16	-	0.09	0.10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Chlamydiae;c__Chlamydiia;o__Chlamydiales;f__Rhabdochlamydiaceae;g__Candidatus Rhabdochlamydia	-	-	0.03	-	-	-	-	0.02	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Chlamydiae;c__Chlamydiia;o__Chlamydiales;f__Simkaniaceae;Other	-	-	0.03	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Chlorobi;Other;Other;Other;Other	-	-	-	-	-	-	-	-	0.02	-	0.09	-	-	-	-	-	0.05	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Chlorobi;c__Chlorobia;o__Chlorobiales;f__Chlorobiaceae;g__Chlorobium	-	-	-	-	-	-	-	-	-	-	0.03	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Chlorobi;c__OPB56;o__;f__;g__	-	-	-	-	-	-	-	-	0.60	-	0.73	-	-	-	-	-	0.40	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Chloroflexi;c__Anaerolineae;o__SBR1031;f__A4b;g__	-	-	-	-	-	-	-	-	-	-	-	-	0.04	0.25	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Chloroflexi;c__Thermomicrobia;o__AKYG1722;f__;g__	-	-	-	-	-	-	-	-	-	-	-	-	0.03	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Cyanobacteria;Other;Other;Other;Other	-	-	-	-	-	-	-	-	-	-	-	-	0.03	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Cyanobacteria;c__Chloroplast;Other;Other;Other	-	-	-	-	-	-	-	-	-	-	-	-	0.01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Cyanobacteria;c__Chloroplast;o__Euglenozoa;f__;g__	0.07	-	0.03	-	-	-	-	-	0.11	-	-	-	0.02	0.06	-	-	-	-	0.04	-	-	-	-	-	-	-	0.49	-	-	-	-	-
k__Bacteria;p__Cyanobacteria;c__Chloroplast;o__Stramenopiles;f__;g__	0.01	-	-	-	-	-	-	0.10	0.04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Cyanobacteria;c__Oscillatoriophycideae;o__Oscillatoriales;f__Phormidiaceae;Other	0.01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Cyanobacteria;c__Oscillatoriophycideae;o__Oscillatoriales;f__Phormidiaceae;g__Phormidium	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Deferribacteres;c__Deferribacteres;o__Deferribacterales;f__Deferribacteraceae;g__Flexistipes	-	-	-	-	-	-	-	-	0.04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__FCPU426;c__;o__;f__;g__	-	-	-	-	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Fibrobacteres;c__Fibrobacteria;o__258ds10;f__;g__	-	-	-	-	-	-	-	-	0.04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Firmicutes;Other;Other;Other;Other	-	-	-	-	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;Other;Other	-	-	-	-	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.24	-	0.03	-	-	-
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Paenibacillaceae;Other	-	0.05	-	-	-	-	-	-	-	-	-	-	0.07	-	-	-	-	-	0.04	0.38	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Paenibacillaceae;g__Cohnella	-	-	-	-	-	-	-	-	-	-	-	-	-	0.10	-	-	-	-	0.21	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Paenibacillaceae;g__Paenibacillus	-	0.05	-	-	-	-	-	-	0.13	-	0.01	-	0.09	-	-	-	-	-	-	0.08	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Staphylococcaceae;g__Staphylococcus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.36	-	-	-	-	-
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Streptococcaceae;g__Streptococcus	-	-	-	-	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae;g__Clostridium	-	-	-	-	-	-	-	-	0.11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Firmicutes;c__Clostridia;o__Thermoanaerobacterales;f__;g__	-	-	-	-	-	-	-	-	0.04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Firmicutes;c__Clostridia;o__Thermoanaerobacterales;f__Thermoanaerobacteraceae;g__Thermoanaerobacter	-	-	-	-	-	-	-	-	0.04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__GN02;c__GKS2-174;o__;f__;g__	0.01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Gemmatimonadetes;c__Gemmatimonadetes;o__;f__;g__	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.13	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Gemmatimonadetes;c__Gemmatimonadetes;o__Ellin5290;f__;g__	-	-	-	-	-	-	-	-	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Gemmatimonadetes;c__Gemmatimonadetes;o__Gemmatimonadales;f__Gemmatimonadaceae;g__Gemmatimor	-	-	0.03	-	-	-	-	-	-	-	-	-	-	-	-	0.03	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__OD1;c__ZB2;o__;f__;g__	-	-	-	-	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__OP11;c__OP11-3;o__;f__;g__	-	-	-	-	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__OP11;c__OP11-4;o__;f__;g__	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__OP3;c__koll11;o__GIF10;f__kpj58rc;g__	0.01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Planctomycetes;c__Planctomycetia;o__Gemmatales;f__Isosphaeraceae;g__Singulisphaera	-	-	-	-	-	-	-	-	-	-	-	-	0.01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Planctomycetes;c__Planctomycetia;o__Planctomycetales;f__Planctomycetaceae;g__Planctomyces	-	-	-	-	-	-	-	-	-	-	-	-	0.05	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;Other;Other;Other;Other	0.15	0.47	0.06	-	0.37	0.58	0.26	0.52	1.64	0.75	0.37	0.24	0.51	0.19	-	0.47	0.22	0.10	0.67	0.21	0.15	0.28	0.32	0.16	0.41	0.08	1.34	-	0.37	0.28	0.04	0.10
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;Other;Other;Other	0.03	0.09	0.12	-	0.51	0.09	0.12	0.04	0.07	0.04	0.36	0.48	2.35	1.77	-	0.15	-	0.19	2.98	1.09	0.05	-	-	0.16	-	0.08	2.43	0.19	0.48	0.04	-	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__;f__;g__	-	-	-	-	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Caulobacterales;Other;Other	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.10	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Caulobacterales;f__Caulobacteraceae;Other	-	-	-	-	0.56	0.09	-	-	0.02	-	-	-	0.15	-	-	0.03	-	-	-	-	-	-	-	0.05	-	-	0.36	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Caulobacterales;f__Caulobacteraceae;g__	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.57	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Caulobacterales;f__Caulobacteraceae;g__Brevundimonas	0.07	-	-	-	9.54	1.12	-	-	0.04	-	-	-	0.65	0.10	0.04	0.06	0.09	-	0.04	-	-	0.12	-	-	-	-	0.49	-	-	1.21	-	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Caulobacterales;f__Caulobacteraceae;g__Mycoplana	0.09	-	-	-	0.09	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Caulobacterales;f__Caulobacteraceae;g__Nitrobacteria	-	-	-	-	0.02	-	-	-	-	-	-	-	0.24	0.03	-	-	-	-	-	-	-	-	-	-	-	-	1.94	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Caulobacterales;f__Caulobacteraceae;g__Phenylobacterium	0.02	-	0.03	-	-	-	-	-	-	-	-	-	0.02	0.06	-	-	-	-	-	-	-	-	-	-	-	-	0.12	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Caulobacterales;f__Caulobacteraceae;g__Streptomyces	-	-	-	-	0.23	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;Other;Other	0.10	-	0.27	-	0.53	0.56	0.09	0.04	-	0.46	0.55	1.01	2.66	3.01	0.15	0.32	1.66	0.29	3.12	2.40	0.85	0.36	0.25	0.05	0.35	0.55	7.40	1.14	2.32	1.31	0.04	0.29
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__;g__	0.06	-	-	-	-	-	-	-	-	-	0.03	0.05	0.19	2.22	-	-	-	-	0.04	0.04	0.30	-	-	0.05	-	-	0.24	-	0.09	-	-	0.10
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Aurantimonadaceae;g__	-	-	-	-	0.02	0.09	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Beijerinckiaceae;Other	-	-	-	-	-	-	-	-	-	-	-	-	0.07	0.10	-	-	-	-	-	0.04	0.05	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Beijerinckiaceae;g__Methylovirgula	0.02	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Beijerinckiaceae;g__Salinarimonas	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.04	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Bradyrhizobiaceae;Other	-	-	-	0.09	-	0.11	-	-	-	0.50	0.01	0.14	0.11	-	0.02	0.09	1.84	1.90	4.57	0.29	5.87	0.04	-	-	-	0.94	0.36	0.38	2.24	0.11	-	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Bradyrhizobiaceae;g__	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.05	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Bradyrhizobiaceae;g__Afipia	-	-	-	-	0.02	0.02	-	-	0.02	0.42	0.04	-	0.07	-	0.02	0.06	2.83	3.70	1.06	0.50	8.58	0.04	-	0.05	0.06	1.57	0.24	0.29	4.84	0.07	-	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Bradyrhizobiaceae;g__Bosea	-	0.05	-	0.17	0.12	0.65	-	-	-	0.92	0.10	0.24	0.16	0.25	0.13	0.15	9.13	13.62	27.44	19.71	22.97	3.48	-	0.05	0.17	5.12	1.94	1.81	15.26	3.34	0.09	0.10
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Bradyrhizobiaceae;g__Bradyrhizobium	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.08	-	-	-	-	-	-	-	-	-	-	-	-

k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Bradyrhizobiaceae;g__Nitrobacter	-	-	-	0.17	-	-	-	-	-	-	-	-	0.08	-	0.02	-	-	-	-	-	0.15	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Bradyrhizobiaceae;g__Oligotropha	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.05	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Bradyrhizobiaceae;g__Pseudomonas	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.05	0.04	-	-	-	-	-	-	-	-	-	0.03	-	-	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Brucellaceae;g__Ochrobactrum	0.05	-	-	-	0.14	0.29	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.12	0.06	0.05	-	-	-	-	-	14.45	0.09	6.71
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Hyphomicrobiaceae;Other	0.13	-	-	-	-	0.07	-	-	-	0.25	0.70	2.74	0.01	0.32	-	0.09	0.05	-	0.46	0.13	0.10	-	-	0.10	-	0.16	0.97	-	0.40	-	-	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Hyphomicrobiaceae;g__	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.06	-	-	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Hyphomicrobiaceae;g__Devosia	0.34	-	0.15	-	0.02	-	-	0.48	0.02	0.13	0.30	0.19	1.02	1.77	0.02	0.06	-	-	-	0.21	0.35	-	-	-	-	0.08	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Hyphomicrobiaceae;g__Filomicrobium	-	-	-	-	-	-	-	-	-	0.25	1.47	5.04	-	0.25	-	1.15	-	-	-	-	-	-	-	-	0.06	-	0.36	0.10	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Hyphomicrobiaceae;g__Hyphomicrobium	-	-	-	-	-	1.03	-	-	-	2.34	40.50	37.46	0.01	11.31	-	24.29	-	0.05	0.04	0.04	-	-	-	-	0.06	0.16	2.67	0.86	0.14	-	-	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Hyphomicrobiaceae;g__Pannonibacter	0.34	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.05	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Hyphomicrobiaceae;g__Parvibaculum	0.06	-	0.61	-	-	-	-	-	0.02	-	0.91	-	0.10	0.06	-	-	0.13	0.05	2.20	2.73	0.10	-	-	1.19	-	1.10	8.74	0.38	1.08	-	-	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Hyphomicrobiaceae;g__Rhodoplanes	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.02	-	-	-	0.04	-	0.10	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Methylocystaceae;g__	-	-	-	-	-	-	-	-	-	-	-	-	0.01	-	-	-	-	-	0.04	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Phyllobacteriaceae;Other	0.14	0.05	0.37	-	0.02	-	0.37	0.06	0.02	0.42	0.21	1.44	1.30	0.98	0.09	0.71	-	0.10	-	0.46	0.45	0.16	0.83	-	0.06	0.94	10.32	0.86	10.65	0.04	0.27	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Phyllobacteriaceae;g__	-	-	-	-	0.23	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Phyllobacteriaceae;g__Aliihoeflea	0.01	-	0.06	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Phyllobacteriaceae;g__Aminobacter	0.02	-	0.03	-	-	-	0.84	0.50	0.02	0.04	0.25	0.53	0.09	0.98	0.15	1.09	-	-	-	0.25	0.05	-	0.51	-	-	0.39	4.13	0.48	3.34	-	0.27	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Phyllobacteriaceae;g__Aquamicrobium	-	-	-	-	0.02	-	-	-	-	-	-	-	-	0.03	-	-	-	-	-	-	-	0.04	-	-	-	-	-	-	-	0.07	-	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Phyllobacteriaceae;g__Chelativorans	-	-	0.12	-	-	-	-	0.04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Phyllobacteriaceae;g__Defluviibacter	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.05	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Phyllobacteriaceae;g__Hoeflea	-	-	-	-	-	-	0.02	0.02	-	0.04	0.03	0.05	0.13	0.16	-	0.06	-	-	-	-	-	-	0.13	-	-	0.08	3.40	-	2.35	-	0.04	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Phyllobacteriaceae;g__Mesorhizobium	0.10	-	2.80	-	0.21	0.09	0.05	-	0.02	0.08	0.37	0.53	0.14	0.51	0.15	0.94	-	-	-	0.46	0.15	-	0.57	-	-	0.24	3.52	0.10	2.89	-	0.18	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Phyllobacteriaceae;g__Nitratireductor	-	-	-	-	-	-	-	-	-	-	-	-	0.08	0.03	-	-	-	-	-	-	-	-	-	-	-	-	0.12	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Phyllobacteriaceae;g__Phyllobacterium	-	-	-	-	-	-	-	-	-	-	0.03	0.05	-	-	-	-	-	-	-	0.04	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Phyllobacteriaceae;g__Pseudaminobacter	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.03	-	-	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Phyllobacteriaceae;g__Thermovum	-	-	-	-	-	-	-	-	-	-	-	-	0.01	0.03	-	-	-	-	0.04	-	-	-	-	-	-	-	0.24	0.10	0.11	-	-	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Rhizobiaceae;Other	-	-	0.03	-	0.51	0.22	-	-	0.02	0.13	0.03	0.82	0.33	0.16	0.04	0.03	-	-	0.14	0.04	0.70	0.48	-	0.05	0.06	0.08	1.21	1.52	0.06	1.56	0.18	0.29
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Rhizobiaceae;g__	-	-	-	-	-	0.09	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.04	-	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Rhizobiaceae;g__Agrobacterium	-	-	-	-	0.05	-	0.04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Rhizobiaceae;g__Ensifer	0.01	-	-	-	-	-	-	-	-	-	0.03	0.91	0.01	0.19	0.02	0.03	-	-	-	0.13	-	-	-	-	-	-	0.49	13.24	0.06	0.07	-	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Rhizobiaceae;g__Kaistia	-	-	-	-	-	-	-	-	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	0.12	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Rhizobiaceae;g__Rhizobium	-	-	0.34	-	5.32	2.13	-	-	-	-	0.10	1.06	0.02	0.19	0.04	0.15	-	-	-	-	-	0.88	-	-	-	-	-	0.29	-	1.95	0.13	0.29
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Rhizobiaceae;g__Shinella	0.02	-	0.06	-	0.21	0.09	-	-	-	0.04	0.04	1.34	1.98	2.41	-	-	-	-	0.50	0.04	1.35	1.36	-	0.16	0.17	0.39	2.06	9.14	0.17	4.30	-	1.07
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Rhizobiaceae;g__Sinorhizobium	-	-	-	-	-	-	-	-	-	-	-	-	0.01	0.03	-	-	-	-	0.04	-	-	-	-	-	-	-	-	1.05	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Xanthobacteraceae;Other	-	-	-	-	-	0.27	-	-	-	0.04	-	-	0.01	0.98	-	-	-	-	-	-	-	-	-	-	-	-	0.12	-	-	0.04	-	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Xanthobacteraceae;g__	-	-	-	-	-	3.95	-	-	-	-	-	-	-	20.53	-	-	-	-	-	-	-	-	-	-	-	-	0.24	-	-	0.36	-	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Xanthobacteraceae;g__Ancylobacter	-	-	0.06	-	-	0.45	-	-	-	-	-	-	-	0.03	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.14	-	0.49
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Xanthobacteraceae;g__Angulomicrobium	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.12	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Xanthobacteraceae;g__Azorhizobium	-	-	-	-	-	-	-	-	-	-	-	-	-	0.03	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Xanthobacteraceae;g__Starkeya	-	-	-	-	-	-	-	-	-	-	-	-	-	13.15	-	-	-	-	-	-	-	-	-	-	-	-	0.12	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Xanthobacteraceae;g__Xanthobacter	-	-	-	-	0.28	0.07	-	-	-	-	0.01	-	0.03	0.38	-	0.03	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodobacterales;f__Hyphomonadaceae;g__Hyphomonas	0.01	-	-	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.57	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodobacterales;f__Rhodobacteraceae;Other	-	-	0.03	-	-	0.02	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.03	-	-	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodobacterales;f__Rhodobacteraceae;g__Octadecabacter	-	-	-	-	-	-	-	-	-	-	0.19	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodobacterales;f__Rhodobacteraceae;g__Planktotalea	-	-	-	-	-	-	-	-	-	-	0.06	-	0.01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodobacterales;f__Rhodobacteraceae;g__Pseudorhodobacter	0.01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodobacterales;f__Rhodobacteraceae;g__Rhodobacter	-	-	-	-	-	0.04	-	-	0.07	-	-	-	-	-	-	-	-	-	-	-	-	-	0.70	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodobacterales;f__Rhodobacteraceae;g__Sulfitobacter	-	-	-	-	-	-	-	-	-	-	0.03	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodobacterales;f__Rhodobacteraceae;g__slope	-	-	-	-	-	-	-	-	-	-	0.06	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodospirillales;Other;Other	-	0.28	0.06	-	-	-	-	-	-	-	-	-	1.07	0.06	-	-	-	0.05	0.99	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodospirillales;f__Acetobacteraceae;g__Roseomonas	-	-	-	-	0.07	0.07	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.08	-	-	-	-	-	-	-	0.07	-	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodospirillales;f__Rhodospirillaceae;Other	-	0.43	0.06	-	-	-	-	-	0.02	-	0.01	-	2.11	0.38	-	-	0.22	-	4.61	-	-	-	-	-	-	-	0.12	-	-	0.04	-	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodospirillales;f__Rhodospirillaceae;g__	0.01	-	-	-	-	-	-	-	-	-	0.12	-	-	-	-	-	0.31	-	0.07	0.59	0.05	-	-	-	0.06	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodospirillales;f__Rhodospirillaceae;g__Azospirillum	-	7.02	-	-	-	-	-	-	0.09	-	0.33	-	44.53	12.04	-	-	-	1.17	36.83	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodospirillales;f__Rhodospirillaceae;g__Enhydrobacter	0.16	-	0.03	-	-	-	0.09	0.04	-	-	0.15	-	0.09	0.25	-	-	0.13	-	0.07	0.08	-	-	0.25	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodospirillales;f__Rhodospirillaceae;g__Phaeospirillum	0.10	-	3.11	-	-	-	0.18	0.31	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.59	1.14	-	-	-	-	-	-	0.22	0.49
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodospirillales;f__Rhodospirillaceae;g__Reyranella	0.06	-	-	-	-	-	-	-	-	-	0.03	0.05	0.07	-	-	-	0.09	-	0.43	0.34	0.05	-	0.06	-	-	-	0.12	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rickettsiales;Other;Other	-	-	-	-	-	-	0.02	-	-	-	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rickettsiales;f__;g__	-	-	-	-	-	-	1.08	0.10	0.02	-	-	-	-	-	-	-	-	0.05	-	-	-	-	0.13	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rickettsiales;f__Pelagibacteraceae;g__	-	-	-	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rickettsiales;f__Rickettsiaceae;g__	-	-	0.27	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;Other;Other	-	-	-	-	0.79	0.04	-	-	-	-	-	-	-	0.03	-	0.06	0.09	-	-	-	-	-	0.06	-	-	0.08	0.24	0.10	0.14	-	-	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__;g__	-	-	-	-	-	-	-	-	0.04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Erythrobacteraceae;Other	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Erythrobacteraceae;g__Erythromicrobium	-	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Erythrobacteraceae;g__Porphyrobacter	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae;Other	0.02	-	-	-	0.53	0.04	-	0.04	-	0.13	0.07	0.19	0.01	0.10	0.02	0.38	0.09	-	0.07	-	0.15	-	0.06	-	0.06	0.08	0.61	0.10	0.34	-	0.04	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae;g__	-	0.05	-	-	7.92	0.13	-	-	-	-	-	-	-	-	0.04	-	-	-	-	-	-	-	-	-	-	0.08	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae;g__Sphingobium	-	-	-	-	0.49	0.04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.12	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae;g__Sphingomonas	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.03	-	-	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae;g__Sphingopyxis	0.03	-	0.12	-	-	-	-	0.19	-	0.33	0.15	1.73	0.03	0.67	-	0.24	0.22	0.15	0.07	0.34	-	-	2.04	-	0.12	0.16	1.46	3.71	2.07	-	0.22	-
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae;g__Sphingosinicella	-	-	-	-	-	-	-	0.02	-	0.04	-	0.67	-	-	0.02	0.15	0.05	0.05	-	-	0.20	-	0.13	-	-	0.16	-	0.57	0.45	-	-	-
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;Other;Other;Other	-	0.05	0.58	3.29	0.02	0.07	-	1.78	0.24	0.67	0.30	0.67	0.04	0.22	0.13	0.06	0.45	-	0.35	1.81	-	0.12	0.06	-	3.73	0.08	0.85	0.10	0.09	0.25	0.09	-
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;Other;Other	0.01	0.09	1.37	1.13	-	-	0.04	1.12	0.13	0.58	0.48	1.68	0.21	0.19	-	0.03	0.94	0.15	0.14	1.76	-	-	0.06	-	2.86	-	0.36	0.19	-	-	0.04	-
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__;g__	-	-	-	-	-	-	-	0.04	0.02	-	-	-	-	-	-	-	-	-	-	0.21	-	-	-	-	0.29	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Alcaligenaceae;Other	-	0.14	-	1.47	-	-	-	0.02	0.07	0.96	0.16	1.54	0.11	0.03	0.13	-	4.99	0.73	2.84	7.35	-	-	-	-	2.86	0.71	3.88	2.86	0.06	-	-	-
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Alcaligenaceae;g__	-	1.42	-	-	-	-	-	-	0.11	0.58	0.27	0.10	0.16	0.57	0.09	-	6.70	2.77	2.38	-	-	-	-	-	0.99	0.47	2.79	1.14	0.31	0.04	-	-
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Alcaligenaceae;g__Achromobacter	-	-	-	0.09	-	-	-	-	-	-	-	0.24	-	-	0.02	-	0.09	-	-	-	-	-	-	-	0.47	-	-	2.57	0.23	-	-	-
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Alcaligenaceae;g__Bordetella	-	-	-	-	-	-	-	-	-	0.08	-	0.10	-	-	0.02	-	-	-	-	0.08	-	-	-	-	0.41	-	0.12	1.52	0.03	-	-	-
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Alcaligenaceae;g__Candidimonas	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.27	-	0.25	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Alcaligenaceae;g__Castellaniella	-	-	-	1.13	-	-	-	-	-	0.67	0.13	9.32	0.05	-	-	-	-	-	0.11	5.13	-	-	-	-	3.15	5.04	5.10	13.52	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Alcaligenaceae;g__Denitrobacter	-	-	-	1.73	-	-	-	-	-	0.92	0.49	9.70	0.01	0.10	-	-	-	-	0.35	6.55	-	0.04	-	-	4.14	4.49	4.61	31.05	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Alcaligenaceae;g__Kerstersia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.04	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Alcaligenaceae;g__Paralcaligenes	-	-	-	-	-	-	-	-	-	-	-	-	0.01	0.03	-	-	3.24	-	2.27	-	-	-	-	-	0.06	-	0.85	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Alcaligenaceae;g__Pusillimonas	-	0.62	-	-	-	-	-	-	0.02	0.54	-	-	-	0.03	-	-	0.09	0.97	-	0.04	-	-	-	-	-	0.79	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Burkholderiaceae;Other	-	-	-	-	-	-	-	-	-	-	0.01	-	-	-	-	-	0.27	-	-	0.13	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Burkholderiaceae;g__	-	-	-	-	-	-	-	-	-	-	0.03	-	-	-	-	-	0.05	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;Other	0.31	0.19	0.24	0.09	2.07	3.68	0.23	2.51	0.22	16.79	1.28	2.79	1.35	1.05	0.30	1.83	-	0.19	1.17	1.51	3.26	0.68	12.79	1.66	0.64	0.79	6.07	0.95	11.13	0.75	0.40	0.10
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__	-	-	-	-	-	0.02	0.02	-	0.02	0.25	0.03	0.05	-	-	-	-	-	-	-	0.17	0.05	-	0.06	-	0.06	-	0.12	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Acidovorax	0.03	0.05	0.09	-	32.54	68.78	0.77	2.51	0.22	2.51	0.55	-	0.01	-	0.26	0.59	0.72	0.05	-	0.04	0.05	13.98	2.16	0.16	2.10	-	-	-	0.25	21.66	1.70	0.87
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Alicycliphilus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.04	-	-	-	-	-	0.05	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Aquamonas	0.03	-	-	-	-	0.02	-	0.02	0.02	-	0.09	0.05	0.01	-	-	0.03	0.05	-	-	-	0.40	-	-	0.21	0.06	-	-	-	0.17	-	-	-
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Brachymonas	0.10	-	0.24	-	-	-	-	-	-	0.04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Caenimonas	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.06	-	-	-	-	-	-	0.06	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Curvibacter	-	-	-	-	-	0.02	-	0.02	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Diaphorobacter	-	4.51	-	-	-	-	-	-	-	0.08	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.39	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Giesbergeria	-	-	-	-	-	-	-	0.08	-	0.13	0.01	-	-	-	-	0.15	-	-	-	-	0.15	-	0.06	0.83	0.06	-	-	-	1.10	-	0.04	-
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Hydrogenophaga	0.41	-	0.73	-	-	-	-	0.73	0.02	34.92	2.87	-	-	-	-	2.27	-	-	-	0.04	1.45	-	-	0.21	0.35	3.23	0.12	-	0.99	-	0.13	-
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Leptothrix	-	-	-	-	-	-	-	-	-	-	-	-	0.01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Limnobacter	-	-	-	-	-	-	-	0.06	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.04	-
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Limnohabitans	-	-	-	-	0.07	0.02	-	0.12	0.62	-	0.01	-	-	-	-	-	-	-	-	-	-	-	0.38	-	-	-	-	-	-	0.04	-	-
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Methylibium	-	-	-	-	-	-	-	0.02	0.02	-	-	-	-	-	-	0.38	-	-	-	-	0.10	-	0.83	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Pelomonas	-	-	-	-	-	-	-	0.04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Polaromonas	-	-	-	-	-	0.02	0.56	4.54	-	-	-	-	-	-	-	0.03	-	-	-	-	-	-	12.48	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Pseudacidovorax	-	-	-	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__RS62	-	-	-	-	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Ramlibacter	-	-	-	-	-	-	-	-	-	-	0.67	-	0.77	11.85	-	1.36	-	-	0.50	-	-	-	-	-	-	-	3.28	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Rhodoferax	0.01	-	-	-	-	-	-	-	-	0.17	-	-	-	-	-	-	-	-	-	-	-	-	0.06	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Simplicispira	-	0.09	-	0.09	-	-	-	0.08	0.07	4.89	5.12	15.42	2.50	4.40	0.69	1.95	0.05	-	1.45	4.16	3.56	-	-	-	0.41	0.24	6.31	4.95	0.20	-	0.04	-
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Variovorax	-	-	-	-	0.05	0.07	-	0.02	0.13	0.33	0.03	-	-	-	-	0.03	-	-	-	-	-	-	4.65	-	-	-	-	-	0.03	-	-	0.10
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Xylophilus	-	-	-	-	-	-	-	0.02	0.02	0.25	0.01	-	0.15	-	-	-	-	-	-	-	-	-	2.93	-	-	-	-	-	0.25	-	-	-
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__mine	-	-	-	-	-	-	-	-	-	-	-	-	-	0.03	-	-	-	-	-	-	-	-	-	-	-	-	0.12	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Oxalobacteraceae;Other	0.01	-	1.13	3.55	-	-	-	1.18	0.69	-	1.43	-	0.01	0.03	-	-	7.28	-	-	2.18	-	-	-	-	3.50	-	-	0.19	-	0.04	-	-
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Oxalobacteraceae;g__	-	-	-	-	-	-	-	-	0.13	-	16.60	0.05	-	-	-	-	32.28	-	0.07	1.81	-	-	-	-	-	-	-	2.67	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Oxalobacteraceae;g__Cupriavidus	-	-	-	86.75	-	-	-	-	1.71	0.21	1.84	-	-	-	-	-	8.36	-	-	34.20	-	-	-	-	46.10	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Oxalobacteraceae;g__Herbaspirillum	-	-	-	-	-	-	-	-	-	-	0.27	-	0.01	-	-	-	0.18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Oxalobacteraceae;g__Herminiimonas	-	-	0.30	-	-	-	-	0.42	-	-	-	-	-	-	-	-	0.05	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Oxalobacteraceae;g__Janthinobacterium	-	-	-	-	-	0.07	-	-	0.04	-	-	-	-	0.06	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Oxalobacteraceae;g__Massilia	-	-	-	-	-	-	-	0.02	0.04	-	1.34	0.05	-	-	-	-	5.22	-	0.04	0.29	-	-	-	-	-	-	-	0.48	0.03	-	-	-
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Oxalobacteraceae;g__Polynucleobacter	0.03	-	-	-	-	-	-	0.04	0.27	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Methylophilales;f__Methylophilaceae;g__	0.02	-	-	-	-	-	0.02	0.02	0.07	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Methylophilales;f__Methylophilaceae;g__Methylotenera	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Neisseriales;f__Neisseriaceae;g__Deefgea	0.01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Procabacteriales;f__Procabacteriaceae;g__	-	-	-	-	-	-	-	-	0.16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Procabacteriales;f__Procabacteriaceae;g__Leeia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.06	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Rhodocyclales;f__Rhodocyclaceae;Other	-	-	0.27	-	-	-	0.02	1.22	-	-	0.04	-	-	-	-	-	-	0.10	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Rhodocyclales;f__Rhodocyclaceae;g__	-	-	-	-	-	-	-	0.17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Rhodocyclales;f__Rhodocyclaceae;g__C39	-	-	-	-	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Rhodocyclales;f__Rhodocyclaceae;g__KD1-23	-	-	-	-	-	-	-	-	-	0.29	0.01	-	0.05	0.06	0.11	0.06	-	-	0.04	0.21	0.20	-	-	-	-	-	0.49	0.19	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Rhodocyclales;f__Rhodocyclaceae;g__Methyloversatilis	-	-	57.08	-	-	-	-	33.13	-	-	-	-	-	0.54	-	-	-	-	-	-	-	-	0.06	-	-	0.08	-	-	-	-	0.31	-
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Rhodocyclales;f__Rhodocyclaceae;g__Rhodocyclus	-	0.90	-	-	-	-	0.11	0.02	-	-	-	-	-	-	-	-	-	0.10	-	-	-	-	0.13	-	-	-	-	-	-	-	-	-

k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Rhodocyclales;f__Rhodocyclaceae;g__Uliginosibacterium	-	-	-	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Tremblayales;f__;g__	0.01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;Other;Other;Other	-	-	-	-	-	0.02	-	-	0.42	0.04	-	-	0.01	-	-	-	0.05	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Bdellovibrionales;Other;Other	-	-	-	-	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Bdellovibrionales;f__Bacteriovoracaceae;g__Peredibacter	-	-	-	-	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Bdellovibrionales;f__Bdellovibrionaceae;g__Bdellovibrio	0.03	1.00	-	-	-	-	-	-	63.22	0.88	0.28	-	-	-	-	0.27	1.39	0.05	-	-	-	-	-	-	-	0.47	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Desulfovibrionales;f__Desulfohalobiaceae;g__Desulfonauticus	-	-	-	-	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Desulfovibrionales;f__Desulfovibrionaceae;g__Desulfovibrio	-	-	-	-	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Myxococcales;Other;Other	-	-	-	-	-	-	-	-	0.07	-	-	-	0.01	-	-	-	0.05	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Myxococcales;f__;g__	-	-	-	-	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Myxococcales;f__Cystobacteraceae;g__Cystobacter	-	-	-	-	-	-	-	-	-	-	-	-	0.04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Myxococcales;f__Haliangiaceae;g__	-	-	-	-	-	-	-	-	0.02	-	-	-	0.48	0.10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Myxococcales;f__Polyangiaceae;Other	-	-	-	-	-	-	-	-	0.07	-	0.04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Myxococcales;f__Polyangiaceae;g__	-	-	-	-	-	-	-	-	0.20	-	0.18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Myxococcales;f__Polyangiaceae;g__Byssovorax	-	-	-	-	-	-	-	-	-	-	0.13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Syntrophobacterales;f__Syntrophaceae;Other	-	-	-	-	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Syntrophobacterales;f__Syntrophobacteraceae;g__Desulfoglaeba	-	-	-	-	-	-	-	-	-	-	0.01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Epsilonproteobacteria;o__Campylobacterales;f__Campylobacteraceae;g__	-	-	-	-	-	-	-	-	0.67	-	0.01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Epsilonproteobacteria;o__Campylobacterales;f__Campylobacteraceae;g__Arcobacter	0.01	-	-	-	-	-	-	0.02	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Epsilonproteobacteria;o__Campylobacterales;f__Helicobacteraceae;Other	-	-	-	-	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;Other;Other;Other	0.21	1.80	0.15	-	0.35	0.36	2.66	0.60	2.96	3.34	0.70	-	0.50	-	0.48	2.09	0.54	2.43	0.04	-	1.00	6.17	2.74	5.49	2.62	5.12	-	-	1.25	2.91	8.42	4.57
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Aeromonadales;f__Aeromonadaceae;g__Aeromonas	-	-	-	-	-	-	-	-	0.11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Aeromonadales;f__Succinivibrionaceae;g__Anaerobiospirillum	-	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Alteromonadales;f__Alteromonadaceae;g__Gilvimarinus	-	-	-	-	-	-	-	-	0.04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Alteromonadales;f__Shewanellaceae;g__	-	-	-	-	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Alteromonadales;f__[Chromatiaceae];g__	-	-	0.03	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Alteromonadales;f__[Chromatiaceae];g__Rheinheimera	-	-	-	-	-	-	-	-	0.04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacteriales;f__Enterobacteriaceae;Other	-	-	-	-	-	-	-	0.02	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacteriales;f__Enterobacteriaceae;g__Kluyvera	-	-	-	-	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacteriales;f__Enterobacteriaceae;g__Pseudomonas	0.10	0.47	0.58	-	0.39	1.37	9.52	0.75	0.04	8.52	0.15	-	-	-	0.19	5.34	0.05	0.19	-	-	3.81	33.36	14.51	26.19	0.41	11.89	-	0.10	1.36	17.08	51.25	19.24
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Legionellales;Other;Other	-	-	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Legionellales;f__;g__	-	-	-	-	-	-	0.04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Legionellales;f__Coxiellaceae;g__Aquicella	-	-	-	-	-	-	0.07	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Legionellales;f__Coxiellaceae;g__Rickettsiella	-	-	-	-	-	-	0.07	-	0.04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Legionellales;f__Legionellaceae;g__	-	-	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Legionellales;f__Legionellaceae;g__Legionella	0.01	-	-	-	-	-	-	-	-	-	-	-	-	-	0.13	-	-	-	-	-	0.10	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Methylococcales;f__Crenotrichaceae;g__Crenothrix	0.07	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Methylococcales;f__Methylococcaceae;Other	-	-	-	-	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;Other;Other	-	-	-	-	-	-	-	-	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Moraxellaceae;Other	-	-	-	-	-	-	-	-	-	-	-	-	0.94	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Moraxellaceae;g__Acinetobacter	-	-	-	-	-	-	-	-	-	-	-	-	13.04	0.19	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Moraxellaceae;g__Alkanindiges	-	-	-	-	-	-	-	-	-	-	-	-	0.93	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Pseudomonadaceae;Other	0.08	0.90	0.15	-	0.05	0.04	2.59	0.37	0.13	0.21	0.19	-	-	-	1.13	0.38	0.31	1.65	-	-	4.36	-	3.44	-	0.29	1.57	-	-	1.30	0.07	0.72	0.29
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Pseudomonadaceae;g__	0.03	-	-	-	0.02	0.02	0.05	0.02	-	-	0.01	-	-	-	0.04	-	0.05	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Pseudomonadaceae;g__Azomonas	-	-	-	-	-	-	0.04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.05	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Pseudomonadaceae;g__Pseudomonas	0.73	67.46	4.51	-	4.09	7.61	72.82	3.84	16.96	13.12	12.00	-	-	-	93.42	49.44	7.73	68.53	-	0.04	37.81	37.97	29.54	61.08	22.38	51.65	0.24	0.48	30.30	24.79	31.94	47.04
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;Other;Other	-	-	-	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Sinobacteraceae;Other	-	-	0.03	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Sinobacteraceae;g__Steroidobacter	-	-	-	-	-	-	-	-	-	-	-	0.05	-	-	-	-	0.05	0.05	0.11	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Xanthomonadaceae;Other	6.05	-	0.37	-	2.62	0.29	0.98	2.88	0.36	0.13	0.25	0.10	0.33	0.06	0.37	0.27	0.18	-	0.11	0.13	0.10	0.04	0.57	0.05	0.06	0.24	0.61	0.10	0.14	0.32	0.90	1.17
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Xanthomonadaceae;g__	0.06	-	-	-	-	-	2.41	1.29	-	-	-	-	-	-	0.02	0.18	-	-	-	-	-	-	3.12	-	-	-	-	-	-	-	-	0.10
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Xanthomonadaceae;g__Achromobacter	0.01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Xanthomonadaceae;g__Dokdonella	-	0.05	-	-	-	-	-	-	-	0.13	0.07	-	-	-	1.06	1.03	-	-	-	0.46	-	-	-	-	-	-	0.12	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Xanthomonadaceae;g__Luteibacter	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Xanthomonadaceae;g__Luteimonas	-	-	-	-	-	-	-	-	-	-	0.01	-	0.18	0.03	-	-	-	-	-	0.04	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Xanthomonadaceae;g__Lysobacter	0.01	-	-	-	-	-	-	-	0.04	0.08	0.18	0.29	0.88	0.38	-	0.03	0.09	-	-	-	0.05	-	-	-	-	-	0.49	0.10	0.06	-	-	-
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Xanthomonadaceae;g__Pseudoxanthomonas	61.47	-	2.04	-	0.26	0.02	0.72	29.85	0.27	0.04	1.32	-	0.04	0.13	0.09	0.15	0.40	-	-	-	-	-	0.06	-	0.06	-	-	-	0.06	-	1.61	0.10
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Xanthomonadaceae;g__Rhodanobacter	-	-	-	-	-	-	-	-	-	-	-	0.05	-	-	0.02	0.03	-	-	-	0.21	-	-	-	-	-	-	0.12	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Xanthomonadaceae;g__Silanimonas	-	-	-	-	-	-	-	-	-	-	-	-	-	0.03	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Xanthomonadaceae;g__Stenotrophomonas	18.49	-	0.06	-	27.62	2.04	0.02	0.02	-	-	-	-	0.01	-	0.11	0.12	-	-	-	-	-	0.08	-	-	-	0.08	-	-	0.23	1.28	-	16.04
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Xanthomonadaceae;g__Thermomonas	-	-	-	-	-	-	-	-	0.07	-	0.01	-	0.22	1.11	0.04	-	-	-	-	-	0.55	-	-	-	-	-	0.85	-	-	-	-	-
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Xanthomonadaceae;g__Xanthomonas	7.38	-	0.34	-	0.12	-	0.04	1.29	-	-	0.13	-	-	-	-	-	0.09	-	-	-	-	-	-	0.05	0.06	-	-	-	-	0.32	0.40	0.39
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Xanthomonadaceae;g__Xylella	0.25	-	-	-	0.58	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.04	-	0.10
k__Bacteria;p__TM6;c__SBRH58;o__;f__;g__	-	-	-	-	-	-	-	0.06	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__TM6;c__SJA-4;o__;f__;g__	-	-	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__TM6;c__SJA-4;o__S1198;f__;g__	-	-	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__TM7;c__TM7-1;o__;f__;g__	-	-	0.03	-	-	-	0.02	-	0.04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__TM7;c__TM7-3;o__EW055;f__;g__	-	-	-	-	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Tenericutes;c__Mollicutes;o__;f__;g__	-	-	-	-	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Verrucomicrobia;c__Opitutae;o__Opitutales;f__Opitutaceae;Other	-	-	-	-	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Verrucomicrobia;c__Opitutae;o__Opitutales;f__Opitutaceae;g__	-	-	0.58	-	-	-	0.04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Verrucomicrobia;c__Opitutae;o__[Cerasicoccales];f__[Cerasicoccaceae];g__	-	-	-	-	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Verrucomicrobia;c__[Methylacidiphilae];o__Methylacidiphilales;Other;Other	-	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Verrucomicrobia;c__[Pedosphaerae];o__[Pedosphaerales];Other;Other	-	-	-	-	-	-	-	-	-	-	0.01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
k__Bacteria;p__Verrucomicrobia;c__[Pedosphaerae];o__[Pedosphaerales];f__;g__	-	-	-	-	-	-	-	-	0.02	-	0.27	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Remark: The first five OTUs in genus level with highest relative abundance in the associated samples were highlighted from high to low in red, orange, yellow, green and blue