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DIVERSITY OF REDUCTIVE DECHLORINATING BACTERIA AND ARCHAEA IN HERBICIDE/DIOXIN-CONTAMINATED SOILS FROM BIEN HOA AIRBASE USING METAGENOMIC APPROACH

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SUMMARY

Heavy herbicide/dioxin contamination of soil was derived a negative effect on the microbial biodiversity, soil quality, animal and human health in Central and South of Vietnam. This is the first time, the application metagenomic tools investigated soil microbial structural community of undetoxified (C - 21,605 ng TEQ/kg dry soil) and bioremediated (BHR - 13.2 ng TEQ/kg dry soil) which could not only help us to explore the potential risks associated with contaminated soils but also provide insights into possible soil bioremediation technology by stimulating indigenous microbes. Four methanogen genera, *Methanosarcina* (24 - 322 OTUs respectively C – BHR samples), *Methanocella* (13 - 63 OTUs), *Methanosaeta* (7 - 42 OTUs) and *Methanococcus* (6 - 69 OTUs) have been dominantly detected in both two metagenomes. Twenty genera of archaea belonging to the phylum Euryarchaeota were found. They could be clustered within 14 different families and nine archaeal genera including unclassified archaea (17 OTUs – C; 145 OTUs - BHR). In metagenome C and BHR, 12 genera of sulfate reducing bacteria (SRB) with different number (2 - 77; 61 - 904 OTUs) respectively were presented. Four SRB genera are dominated in C metagenome, it is linear also in BHR. The highest number is genus *Desulfovibrio* detected in both examined metagenomes. However, the relationship features of these bacterial groups need deeply investigation for understanding their role of reductive dechlorination, anaerobic degradation in herbicide/dioxin contaminated heavy soil and sediment. These results provide additional evidence to explain why heavy herbicide/dioxin contaminated soil was detoxified successfully at Bien Hoa airbase, Vietnam.

Keywords: *Bien Hoa airbase, Herbicide/dioxin contaminated, Metagenomic, Microbial biodiversity.*

INTRODUCTION

During Operation Ranch Hand (1961-1971), the United States Air Force sprayed about 80 million liters of herbicide contain 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) in the south of Vietnam (Cecil, 1986). The Bien Hoa airbases has been referred to as major dioxin "hotspots" due to high dioxin concentrations remained decades after large volumes of Agent Orange and other defoliants were handled at these sites during Vietnam War. About 3,384 m^3 of contaminated soils were treated in bioremediation cell (in Z1 landfill area) using bioremediation technology from Professor Dang Thi Cam Ha and colleagues - Institute of Biotechnology - Vietnam Academy of Science and Technology. After 40 months, the toxicity of dioxin contaminants was reduced from more than 10,000 ng TEQ/kg to 14.12 ng TEQ/kg. However, current knowledge about the process

is mostly based on cultured microbes, which results in less dioxin degradable determinants to be discovered and because traditional microbiological techniques has only isolate and culture a small proportion (0.1 to 1%) of the microorganisms in soil samples (Jeffries *et al.*, 2018).

The effects of mixture of herbicide/dioxin pollution in this region microbial ecology have not been studied at Bien Hoa airport. Although Nguyen Ba Huu was used single strand conformation polymorphism (SSCP), denaturing gradient gel electrophoresis (DGGE), etc. to investigate the diversity of microbial and functional genes in soil samples but a little of microbial diversity had detected in total microorganism (Nguyen Ba Huu *et al.*, 2007).

At bioremediated pilot scales as well as herbicide/dioxin contaminated region at Da Nang airport, many basic studies on obligate and facultative anaerobic bacterial groups capable of utilizing dioxins; 2,4,5-T; 2,4-D as well as their metabolites and soil extract as the sole source of carbon and energy for metabolism. Nguyen Ba Huu had applied modern molecular biology methods such as DGGE, SSCP, MPN-PCR to study microbial community structure in soil and sediment samples at Da Nang airport, the aerobic, obligate anaerobic bacterial groups and specially *Dehalococcoides* genus were detected (Nguyen Ba Huu *et al.*, 2006, 2007, 2008). Alpha-Proteobacteria, Chlorobi, Chloroflexi, Sulfuricurvum, Fusobacteria, Nitrospiraceae such as *Sulfuricurvum kujiense* YK-1, *Fusobacteria unculture, Nitrospiraceae uncultured, Alphaproteobacterium unculture,* etc. and bacteria have not been determined. Obligate dechlorinated bacteria such as *Dehalococcoides* have only been studied initially and have detected the presence of 6 lines in 10 m^3 bioremediated pilot at Da Nang airport (Nguyen Ba Huu *et al.*, 2006).

Nguyen Thi Tam Thu and co-authors, 2013 have discovered all 3 groups of dechlorinated bacteria including obligate dehalorespiration (*Dehalococcoides, Dehalogenimonas*), facultative dehalorespiration (*Desulfovibrio, Desulfitobacterium, Desulfuromonas*) and co-metabolic reductive (*Pseudomonas, Shewanella, Clostridium*). Three groups of aerobic bacteria (*Pseudomonas, Azotobacter*), facultative anaerobic (*Vibrio, Klebsiella, Shewanella*) and obligate anaerobic (*Bacteroides, Geobacter, Clostridium*) bacteria, etc. were identified in sample. They are degraded and transformed mixture of herbicide/dioxin and their metabolites are presented in highly toxicity enrichment sample after one year. In addition, the research has identified a diversity of sulfate reducing bacteria in the obligate cultivation at laboratorial scale using DGGE method. The diversity of *Dehalococcoides* and other bacteria plays an important role in reductive dechlorination of chlorinated dioxins under anaerobic conditions in samples soils.

Recently, microorganisms and genes from environmental samples via culture independent have been identify based on previous researchs. The metagenomics which combines sequencing technology and bioinfomatic tools has been applied extensively to understand microbial taxonomy and gene function of microbial communities in various environments. In reality, only 0.001 - 0.1% of microorganism was isolated and cultured with more than 99% of microorganism has not been studied in detail (Riesenfeld *et al.*, 2004). Thus, the wealth of information obtained from the deep metagenomic sequencing makes it possible to capture the genomic information of low-abundance populations and to reveal the multiple activities in soil. Nowadays, the next generation sequencers (NGS) has known Sanger sequencing, Illumina dye sequencing and Ion semiconductor sequencing in combination with bioinformatic technique helping metagenome studies become simpler.

Metadata of microbial communities from different environment including wastewater treatment, soil and air were obtained (Wang *et al.*, 2013). Application of modern bioinformatic

tools to annotate data metagenomics of taxonomy and genes (functional annotations) was needed. There are many different algorithms developed to analyses metadata: MG-RAST (Meyer *et al.,* 2008), EBI-Metagenomics (Hunter *et al.,* 2017), MEGAN, MOCAT, QIIME, MetaHIT and MyTaxa. It is a useful tool to understand unique characteristics of bacteria and genes associated with heavy dioxin contaminated soil and to aid in improvement of methods that could detoxify soil ecosystem damage resulted from heavy herbicide/dioxin.

Bioremediation is the use of microbes (for augmentation) or stimulation of native microbial community for the beneficial removal of contaminants of soil, sediment, and wastewater from industry. The field trail of bioremediation research has traditionally focused heavily on processes from the domain bacteria, however, archaea are often involved as well as the key functions for contaminant removal in "extreme" environments, archaeal processes which are of particular interest for bioremediation. Reductive dehalogenation removes halides from organic compounds, it forms lower halogenated or nonhalogenated products and this process plays one of the main key in anaerobic bioremediation (Krzmarzick *et al.,* 2018). In the degradation of organics in hypersaline environments involved members of both bacteria and archaea that are known in such environments and these are often referred to halobacteria and haloarchaea respectively.

The haloarchaea cluster into a single class (the class Halo-bacteria) within the phylum Euryarchaeota. They are typically cultured at neutral pH and temperatures of 30-45˚C, and they require high salinities of 1.8–5.0 M NaCl (Ding *et al.,* 2017). These isolates were identified as members of *Haloferax* sp. (isolates C-24 and C-27), *Halobacterium piscisalsi* (st. C-37), *Halobacterium salinarum* (st. C-51), *Halorubrum ezzemoulense* (st. C-41 and C-46), *Halorubrum* sp. (st. C-43) and two strains (C-50 and C-52) reported with less than 93% 16S rRNA gene identity to any isolated strains and no publications were associated with this genome (Roh, Song, 2018; Erdoğmuş *et al.,* 2013). A few strains of thermophilic and acidophilic archaea have been found capable of pollutant degradation phenol at 80˚C and pH 3.2 through meta-ring cleavage also (Comte *et al.,* 2013). In nonextreme environments, bacteria are better known to perform hydrocarbon degradation. However, archaea particularly the methanogens, are key bacterial components of the degradation process. Contaminated soil with petroleum and undergoing remediating process was found number of *Methanosarcinales* strains significantly increased by denaturing gradient gel electrophoresis (DGGE) method (Kasai *et al.,* 2005). Another study revealed that archaea were scarce (<1% of the population) in an aquifer above a coaltar DNAPL with only a low abundance of methanogens (Scherr *et al.,* 2016). The role of archaea in oil marine degradation is unclear as well as difficult to amplify DNA by PCR method after cultivation with oil because archaeal populations significantly decrease. Some of archaea bacteria can be found in heavy metal remediation, acid mine drainage and reductive dehalogenation.

These studies need to strengthen our understanding role of archaea in bioremediation. Discover of various substrates subject to dechlorination by methanogens were reported in 80-90th dedicates provided for further deeply research on interested, mystery field. Metagenomic approach was effectively implemented to evaluate microbial community structure and gene, putative protein function in heavy herbicide/dioxin contaminated soil and obtained results are provided an useful ecological methodology for investigating potential of degradation and toxicant bacterial degraders in this kind of the soils. This paper aims to study microbial diversity of reductive dechlorinated and archaea bacteria in heavy herbicide/dioxin contaminated soil and bioremediated soil using metagenomic tool.

MATERIALS AND METHODS

Site description

The site was located in Bien Hoa airbase (10°58′37″N 106°49′06″E), Dong Nai province, Vietnam. It has significant polluted by mixture of herbicide/dioxins contaminated more than 50 decades.

Sampling

In July 2014, heavy herbicide/dioxin contaminated soil (named C sample) was collected in West-South region of Bien Hoa airport and other bioremediated soil (named BHR sample) from bioremediated region (active landfill), one the part of traditional landfill project. At the same time, approximately 20 kg soil of C sample and BHR were collected for evaluated physicochemical characteristics, total toxicity and DNA isolation. Soil samples used for metagenomic analysis were frozen -20ºC for 1 day before DNA extraction.

Soil physicochemical and total toxicity characteristics

The soil physicochemical characteristics in this studied were analyzed according to the process specified in the Vietnamese standard set (TCVN 6648: 2000; TCVN 8567: 2010; TCVN 8567: 2010; TCVN 8567: 2010; TCVN 8567: 2010; TCVN 5979: 2007; TCVN 8941: 2011; TCVN 6498: 1999; TCVN 8467: 2010) at the Central Analytical Department (CAL), under the Soils and Fertilizers Research Institute (SFRI), Vietnam.

Total soil toxicity (17 congeners of PCDDs and PCDFs) were analyzed by HGC/HMS at the Department of Dioxin and Toxicology Analysis, Center for Environmental Monitoring, Vietnam Environment Administration (Certificate of eligibility for environmental monitoring services VIMCERTS 027).

DNA extraction and Illumina HiSeq 2500 sequencing

Direct DNA extraction from C samples was carried out according to the method of Bourrain (1999) with some modifications. The samples were dissolved in phosphate buffer, treated by ultrasonication and low speed centrifugation. Cells were disrupted by lysozyme, proteinase K and SDS at 70ºC in 5 min. The protein was removed by chloroform/isoamyl alcohol extraction. The DNA was precipitated in isopropanol, dissolved in deionized water and store at 0°C (Bourrain, 1999).

Total DNA of BHR sample were isolated and purified using the PowerSoil® DNA Isolation Kit (MO BIO Laboratories Inc., CA, USA) according to the manufacturer's instructions.

The final DNA concentration and purification were determined using a NanoDrop 2000 UV-vis spectrophotometer and DNA quality was checked by Qubit Fluorometer and 1% agarose gel electrophoresis.

Quality analysis of FASTQ sequence reads

The DNA was measured using the Qubit dsDNA BR Assay Kit (Life Technologies, NY, USA). Metagenomic sequencing was conducted on an Illumina Hiseq 2500 platform by Baseclear Labservices (Baseclear, Leiden, The Netherlands). FASTQ sequence files were generated using the Illumina Casava pipeline version 1.8.3. Quality assessment was based on the Illumina Chastity filtering, the FASTQC v0.10.0 and quality scores per sample were provided as Enclosure.

De novo assembly

Low quality was removed by the Trim sequences and the analysis has been performed using the *De novo* assembly option of the CLC Genomics Workbench version 8.0. Misassemblies and nucleotide disagreement between the Illumina data and the contig sequences are corrected with Pilon v1.8 (Quince *et al.*, 2014).

Prokaryotic genome annotation with Prokka

Genome annotation was performed on the assembled contig or scaffold sequences using the Base Clear annotation pipeline based on the

Prokka Prokaryotic Genome Annotation System v1.6.

Data analysis

The sequencing data were processed using the MG-RAST system (Meyer *et al.,* 2008). The abundance data were identified using the Reference Sequence (RefSeq) database (Pruitt *et al.,* 2005) with the parameters $1e^{-05}$ as the maximum e-value, a minimum identity of 60%, and a minimum alignment length of 15 as the cutoff. Through the workbench tool from MG-RAST server, we generated subsets of the reads annotated in a functional subsystem for taxonomic identification.

RESULTS AND DISCUSSION

This is the first time, microbial diversity of heavy contaminated herbicide/dioxin soils was investigated by the use of metagenomic tool. The aim of this study was to determine diversity of two metagenomes of herbicide/dioxin contaminated and bioremediated soils in Bien Hoa airbase.

Data analysis of metagenomes C and BHR samples

The basic parameters of soil samples contaminated with herbicide/dioxin were determined including: pH, temperature, humidity, humus, nitrogen, total carbon, arsenic (Table 1). Contaminated and bioremediated soils have the same characteristics of very low humidity, humus and total nitrogen. The difference is shown in the pH value, heavily polluted soil has an alkalic pH (8.19) while the treated soil has pH close to neutral (6.67) which is more suitable for many types of microorganisms to grow well. The amount of arsenic was found below requirement for agricultural production soil by national standard regulation.

Table 1. Basic properties of the soil samples.

Parameters		Analysis method	Unit	Samples	
				BHR	**C**
Moisture		TCVN 6648:2000	(%)	0.73	0.52
Mechanical composition	Raw sand	TCVN 8567:2010	(%)	0.16	0.04
	Fine sand	TCVN 8567:2010	(%)	21.52	53.80
	Limon	TCVN 8567:2010	(%)	37.48	26.12
	Lightning	TCVN 8567:2010	(%)	9.26	15.14
pH_{KCl}		TCVN 5979:2007		6.67	8.19
Total organic matter (OM)		TCVN 8941:2011	(%)	0.78	2.12
Total nitrogen		TCVN 6498:1999	(% N)	0.16	0.04
Arsenic		TCVN 8467:2010	(mg/kg)	3.23	11.69
Total Dioxin/Furan, PCDD/F – TEQ (WHO2005)		EPA 8280B	(ngTEQ/kg)	**13.2**	**21,605**

The total toxicity of herbicide/dioxin was also analyzed because it has a great impact on the microbiota and it is also the special feature of samples in this study in order to inform with previous studies. Obtained data showed that total toxicity of untreated soil sample in Bien Hoa (C sample) was very high (21,605 ng TEQ/kg dry soil) while bioremediated soil was only 13.2 ng TEQ/kg dry soil which meets to the standard requirement for often used agricultural production soil (under 40 ng TEQ/kg dry soils – decided number 13/2012/TT-BTNMT - the regulations of Ministry of Agriculture and Rural Development Vietnam).

Results obtained from quality statistics after sequencing, *de novo* assembly and annotation were shown in Table 2. The number of reads and sample yield was huge with high scores of Phred to estimate the quality of the consensus sequences. Numbers of reads in sample C (64,339,446) was higher than those of sample BHR (61,600,474).

After assembly, different results were observed in two examined samples and are shown in Table 3. The result of sequence analysis on the database of Refseq showed that the archaea had a low rate in the sample with only 0.13 % (106 representative hit in total 80,157 hits of C sample) and 1,24 % (3,333 representative hit in total 268,770 hits of BHR sample) in the total number of analytical procedures .

Abundance of archaea diversity with methanogen and halophilic groups will be discribed in detail of below chapter, but their exactly role in herbicide/dioxin contaminated soil requires futher studies. Archaea are also commonly reported as a part of microbial communities dechlorinating chloroethenes. The ability of methanogens to dechlorinate suggests that these archaea may contribute to dechlorination activities even in systems dominated by organohalide respirers. The roles and antagonism of archaea in reductive dechlorination systems are likely complex, it needs more efforts for investigation of each archaea bacterial physiological activity in the structural community (Krzmarzick *et al.,* 2018).

Table 2. Quality statistics after CASAVA and FastQC analysis.

Samples	Number of reads	Sample yield (in MB)	Average quality scores (Phred)
C	64,339,446	15,635	37.83
BHR	61,600,474	15,522	33.98

Table 3. De novo assembly statistics.

Contents	C	BHR
Number of sequences	12,133	119,555
Sum (bp)	61,478,000	215,533,770
GC contents (%)	65.10	58.72
Maximum contig size	1,070,898	261,394
Minimum contig size	700	700
Average contig size	5,067	1,802
N25	104,014	5,218
N50	28,891	2,114
N75	4,081	1,142
Number of gaps	8	0
Size of gaps	74	0

Diversity of methanogens in metagenomes of C and BHR samples

In the metagenomes of the two samples, 20 genera of archaea belonging to the phylum Euryarchaeota were found, which can be further clustered into 14 different families, including unclassified archaea (17 OTUs - C and 145 OTUs - BHR). The number of genera recovered in the

metagenome of sample C was not as high as that in the BHR bioremediated soil (Figure 1). From a recent review, Krzmarzick *et al.,* (2018) showed that there was evidence of the degradability of crude oil, PAH and metabolic products and these genotypes. They play a very important role in bioremediation of these compounds.

The methanogen group was also present in the metagenome and it was most abundant in the BHR sample (Fig. 2). This is because during the last stage of the detoxification in active landfill, the bioremediated cells BHR was saturated with water. The total toxicity of the sample has decreased below the permissible level. Some genera were not only low in number, but in sample C they have not been found. Four genera, *Methanosarcina* (24 - 322 OTUs respectively C – BHR samples), *Methanocella* (13 - 63 OTUs), *Methanosaeta* (7 - 42 OTUs) and *Methanococcus* (6 - 69 OTUs) have been detected in this metagenome. Moreover, representatives of the order *Methanomicrobiales, Methanomethylovorans, Methanobacterium* and *Methanothrix* that have been known for halogen eliminating ability of some compounds were not detected. The representatives of this genus often live with the bacteria reducing sulphate (synthrophic). Studies have been published many decades ago and have recently shown that methanogens could respire with organic halogen compounds by using them as intermediate electron acceptors. Consortia of archaea and obligatory anaerobic bacteria *Dehalococcoides* have been shown to reductively dechlorinate pentachlorophenol, perchlorethylene, trichloroethene, chloroform, trichlorofluoromethane and 1,2-dichloroethane. Various strains of *Methanosarcina* were found to dehalogenate pentachlorophenol, perchloroethylene, trichloroethene, chloroform and trichlorofluoromethane and it was important for the dechlorination of vinyl chloride in an enriched. *Methanosarcina,* and *Methanosaeta* were reported as significant components of the well-studied and highly enriched KB-1 organochloride dechlorinating culture and their archaea were also found in metagenomic samples. Some of methanogen group such as *Methanobacterium ivanovii* strain T1N degrade pentachlorophenol, *Methanothermobacter marburgensis* and *Methanothrix soehngenii* (DSM 2139) dechlorinate 1,2-dichloroethane through dihaloelimination to the product ethylene and through hydrogenolysis to chloroethane. *Methanobacterium congolense* founded in the well-studied chloroethene-dechlorinating ANAS culture, etc. but they haven't show in this study.

The ability to dehalogenate in methanogens which are needed for methanogenesis so that archaea are also commonly reported as a part of microbial communities dechlorinating chloroethenes. All most these bacteria are present in both metagenomes with different number is shown the linker between their original.

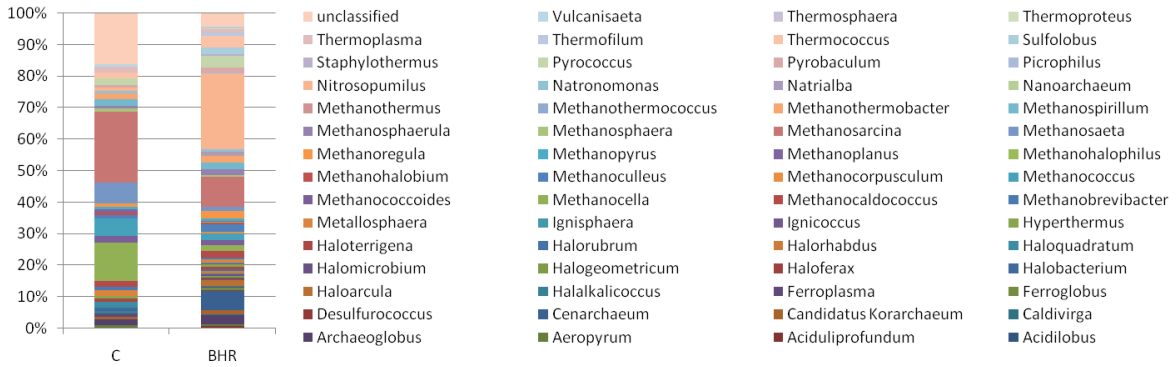

Figure 1. Composition of Archaea metagenomes based on RefSeq database at genus level with the abundance of more than 3,000 was showed. C: Contaminated soil; BHR: Bioremediation soil.

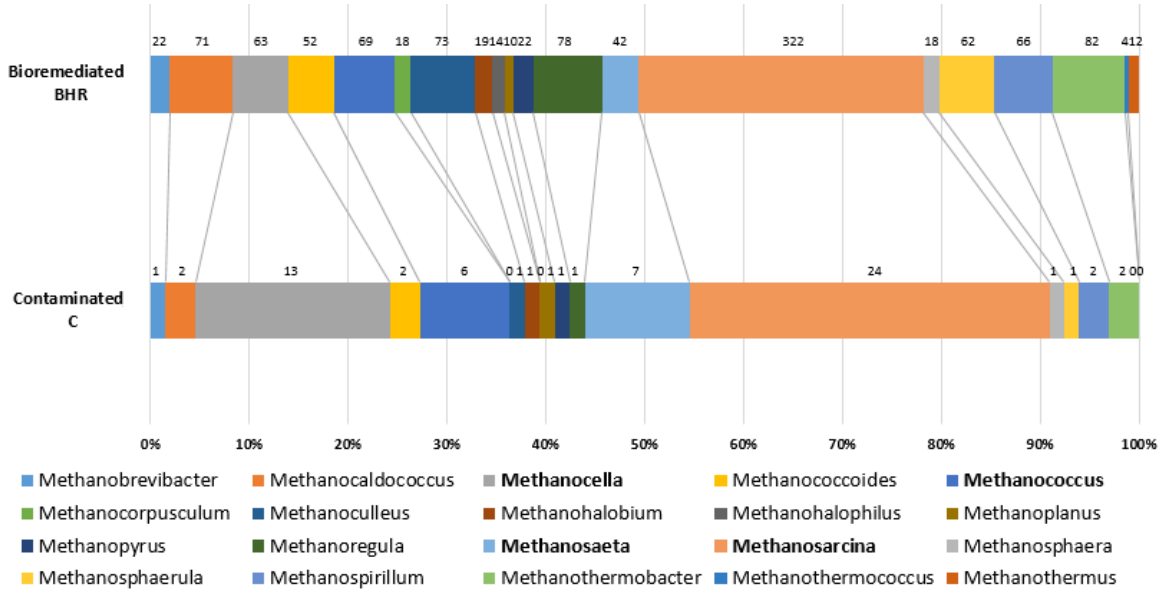

Figure 2. Diversity of methanogenic archaea in the studied samples

Diversity of halophilic archaea in metagenome of the studied samples

Metagenomics study of the two samples revealed that nine archaeal genera belonging to the Halobacteriaceae family were present in the samples but their relative abundance in sample C was very low than BHR sample (Fig. 3).

Archaea belonging to the halophilic group (*Halogeometricum*, *Halomicrobium*) capable of degrading oils or xenobiotic compounds and thus might play important roles in bioremediation of xenobiotic substances such as dioxins, herbicide in soils and sediments (Fig 3). In addition, groups of respiratory dechlorinating bacteria found by DGGE including both obligatory (*Dehalococcoides*, *Dehalogenimonas*) in both bioremediated samples from Bien Hoa and Da Nang by enrichment cultivation (Nguyen Thi Tam Thu *et al.*, 2013)

Archaea can oxidize the aromatic ring in the ortho position through oxidation by catechol 1,2-dioxygenase or 3,4-dioxygenase protocatechuate. Many representatives of these halophytes have been shown to decompose the following aromatic compounds: crude oils, pyrene, anthracene, naphthalene, phenanthrene, pristane, biphenyl toluene, benzene, and benzoate, hylnapthalene, alkanes (C9-C40), benzene, n-hexadecane, n-octadecane, p-hydroxybenzoate, benzoic, p-hydroxybenzoic acid, tetradecane, hexadecane, eicosane, heneicosane, acenaphthene, 9-methyl anthracene, benzoate -hydroxybenzoate, cinnamate, phenylpropionate, 4-hydroxybenzoic acid, heptadecane, C8-C34 n-alkanes, biphenyl benz [a] anthracene, tween, etc. Also strain *Haloterrigena mahii* sp. H13 could decompose 1,2-dichloroethane, naphthalene/anthracene, γ-hexachlorocyclohexane, 1-2-met naphthalene, However, the role of the nine genera halophilic archaea in the bioremediated soil of this study in the detoxification of dioxins, other herbicide and their metabolites remaining to be uncovered in future studies.

In the metagenome of the two samples, the majority of microbes were bacteria (C with 99.19% and BHR with 98.17%), while very few were eukaryotic and archaeal. In this study, we focus on sulfate reducing bacteria and methanogens.

When evaluating the microbial diversity obtained from these metagenomes, it showed

that Proteobacteria was the most abundant phylum in soils heavily contaminated with herbicide/dioxin, followed by Actinobacteria. Bacteroidetes and Firmicutes were found at relatively high abundance samples BHR. Chloroflexi and Acidobacteria contributed more than 2% of the BHR metagenome (Table 4).

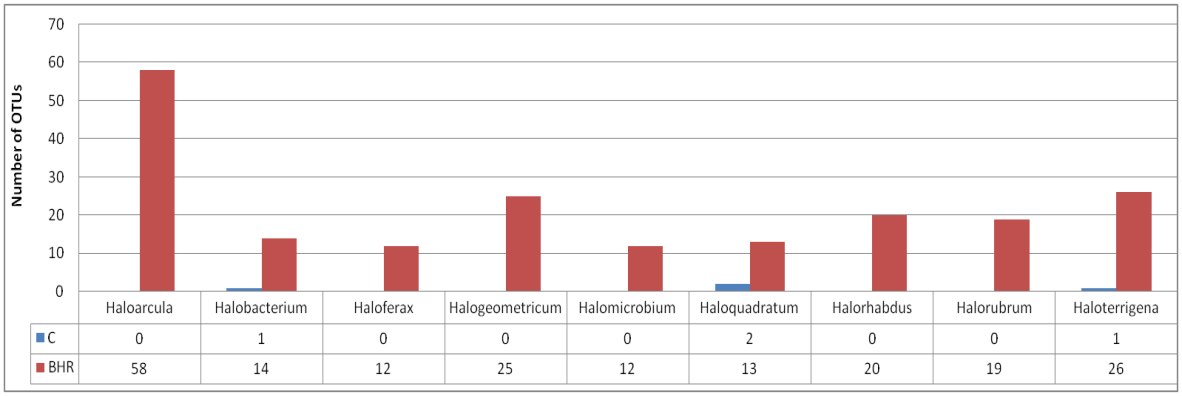

Figure 3. Diversity of halophilic archaea in the metagenome of the studied samples.

Table 4. Diversity of sulfate reducing microorganisms in metagenomes of samples C and BHR.

Phylum	Class	Order	Family	Genus	C (OTUs)	BHR (OTUs)
Firmicutes	Clostridia	Clostridiales	Peptococcaceae	*Desulfitobacterium*	15	**312**
Proteobacteria	Deltaproteobacteria	Desulfobacterales	Desulfobacteraceae	*Desulfobacterium*	7	151
Proteobacteria	Deltaproteobacteria	Desulfobacterales	Desulfobacteraceae	*Desulfococcus*	8	**232**
Proteobacteria	Deltaproteobacteria	Desulfovibrionales	Desulfohalobiaceae	*Desulfohalobium*	4	93
Proteobacteria	Deltaproteobacteria	Desulfovibrionales	Desulfomicrobiaceae	*Desulfomicrobium*	7	157
Proteobacteria	Deltaproteobacteria	Desulfovibrionales	Desulfohalobiaceae	*Desulfonatronospira*	2	61
Proteobacteria	Deltaproteobacteria	Desulfobacterales	Desulfobulbaceae	*Desulfotalea*	6	139
Firmicutes	Clostridia	Clostridiales	Peptococcaceae	*Desulfotomaculum*	22	**291**
Proteobacteria	Deltaproteobacteria	Desulfovibrionales	Desulfovibrionaceae	*Desulfovibrio*	77	**904**
Chrysiogentes	Chrysiogentes (class)	Chrysiogenales	Chrysiogenaceae	*Desulfurispirillum*	4	81
Proteobacteria	Deltaproteobacteria	Desulfobacterales	Desulfobulbaceae	*Desulfurivibrio*	2	**151**
Proteobacteria	Deltaproteobacteria	Desulfuromonadales	Desulfuromonadaceae	*Desulfuromonas*	5	157

In this study, we focused on sulfate reducing bacteria since there have been lines of evidence to show that they might be able to degrade herbicide, dioxin compounds, have or without chlorines such as 2,3,7,8-TCDD with representatives of some genera *Sphingomonas* , *Streptomyces, Achromobacter, Burkholderia Ralstonia, Pseudomonas, Rhodococcus; Beijerinckia, Bacillus; Terrabacter; Burkholderia; Klebsiella; Alcaligens, Paenibacillus, Arthrobacter*, etc. Chloroflexi, Firmicutes and Proteobacteria dominated the microbial communities of anaerobic enrichment samples with a total toxicity of more than 41,265 pg TEQ/g soils. Bacteria of Chlorobi, Chloroflexi, Sulfuricurvum, Fusobacteria, Nitrospira were found in the herbicide contaminated sediment sample in the previous studies using DGGE method (Dam Thuy Hang *et al.*, 2010). Nguyen Thi Tam Thu and co-

authors, 2013 also detected those above mentioned genera in three samples set up from Bien Hoa site. In addition, group of the facultative (*Desulfovibrio, Desulfitobacterium, Desulfuromonas*). *Pseudomonas, Shewanella, Clostridium, Bacteroides* and *Geobacter* which were also present in both bioremediated samples from Bien Hoa and Da Nang by enrichment cultivation (Nguyen Thi Tam Thu *et al.*, 2013). In metagenome C, 12 genera of sulfate reducing bacteria with different number (2- 77 OTUs), but in BHR metagenome 12 genera also detected but with rather higher OTUs varied 61- 904. Four genera are dominated in C metagenome, it is linear also in BHR. Difference is only in OTUs number, the highest number is *Desulfovibrio* in both examined metagenomes. This genus were detected in almost anaerobic soil and sediment of different environment. This phenomena was also present in BHR which was maintained at more strictly anaerobic condition showed higher relative abundance of sulfate reducing bacteria.

CONCLUSION

Soils heavy contaminated with herbicide/dioxin need to be treated to reduce the risk for the environment and human health of many generations. Although being highly toxin, the high concentration of herbicide/dioxin is a selective condition to enrich microorganisms that can adapt and use the contaminants for growth. There have been numerous studies on isolating, classifying and testing the ability of microorganisms to degrade herbicide/dioxin conducted at contaminated sites to develop a biological based technology for the treatment of pollution by herbicide/dioxin in particular and by organic compounds in general. On the other hand, culture-independent techniques such as DGGE, SSCP as well as the studies on mechanism of degradation, metabolism, and mineralization of herbicide/dioxin were also conducted. To continue the success of the previous studies, the metagenomic tool was used in this research to provide better understanding of microbial diversity and clarify more which microorganisms play a key role in degradation of contaminants at the Bien Hoa airbase. All most these bacteria are present in both metagenomes with different number is shown the linker between their original. Four genera are dominated in C metagenome, it is linear also in BHR. Difference is only in OTUs number, the highest number is *Desulfovibrio* in both examined metagenomes and this phenomena was also present in our BHR bioremediated sample. Studies such as our current work have generated useful information about the diversity of microbes in contaminated soil with herbicide/dioxin. Further studies are needed to focus on decifer the roles of methanogens, sulfate reducing bacteria, etc. and to establish a conceptual model of the microbial network at the herbicide/dioxin bioremediated sites. The results provide additional evidence to explain why heavy herbicide/dioxin contaminated soil was detoxified successfully at Bien Hoa airbase, Dong Nai province, Vietnam.

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ĐA DẠNG VI KHUẨN VÀ VI KHUẨN CỔ KHỬ LOẠI CHLOR TRONG ĐẤT NHIỄM CHẤT DIỆT CỎ/DIOXIN Ở SÂN BAY BIÊN HÒA SỬ DỤNG CÔNG CỤ METAGENOMIC

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TÓM TẮT

Đất ô nhiễm nặng chất diệt cỏ/dioxin có tác động tiêu cực đến đa dạng vi sinh vật, chất lượng đất, sức khỏe động vật và con người ở miền Trung và miền Nam Việt Nam. Đây là lần đầu tiên công cụ metagenomic được sử dụng trong điều tra đa dạng cấu trúc vi sinh vật trong đất ô nhiễm chưa được khử độc (C - 21.605 ng TEQ/kg đất khô) và đất đã xử lý bằng phương pháp sinh học (BHR – 13,2 ng TEQ/kg đất khô), điều này không chỉ giúp phát hiện những rủi ro tiềm ẩn liên quan đến đất bị ô nhiễm mà còn cung cấp những hiểu biết sâu sắc về công nghệ xử lý sinh học đất ô nhiễm chất diệt cỏ/dioxin bằng cách kích thích hệ vi sinh vật bản địa. Bốn chi methanogen, *Methanosarcina* (24 - 322 OTUs tương ứng mẫu C - BHR), *Methanocella* (13 - 63 OTUs), *Methanosaeta* (7 - 42 OTUs) và *Methanococcus* (6 - 69 OTUs) đã được phát hiện chiếm đa số ở cả hai metagenomes. Hai mươi chi cổ khuẩn thuộc ngành Euryarchaeota đã được tìm thấy. Chúng được phân nhóm thuộc 14 họ khác nhau và 9 chi cổ khuẩn bao gồm cả các chi chưa được phân loại (17 OTUs - C; 145 OTUs - BHR). Trong metagenome C và BHR, 12 chi vi khuẩn khử sulfat (SRB) với số lượng khác nhau (2 - 77; 61 - 904 OTU) đã được trình bày. Bốn chi SRB chiếm ưu thế trong metagenome C cũng xuất tương ứng trong metagenome BHR. Chi *Desulfovibrio* được phát hiện và chiếm số lượng cao nhất trong cả hai metagenome. Tuy nhiên, mối liên hệ của các nhóm vi khuẩn này cần được nghiên cứu sâu để hiểu rõ được vai trò của chúng với quá trình khử clo ở điều kiện phân hủy kỵ khí trong đất và trầm tích nhiễm nặng chất diệt cỏ/dioxin. Những kết quả này cung cấp thêm bằng chứng để giải thích tại sao đất bị nhiễm nặng chất diệt cỏ/dioxin được khử độc thành công tại sân bay Biên Hòa, Việt Nam.

Từ khóa: *Sân bay Bien Hoa, Ô nhiễm chất diệt cỏ/dioxin, Metagenomic, Đa dạng vi sinh vật.*