

Characterization of bacteria community associated with soil arsenic and sulphate contamination based on 16S rRNA gene sequences

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Abstract: In order to investigate molecular diversity of bacteria community from both a normal arable soil (Nor-1) and an arsenic and sulphate polluted soil (Sul-1). Environmental total DNA was directly extracted from two soil samples. The 16S rRNA genes were amplified from the total DNA and construction a clone library. Positive clones were randomly selected from the library and identified by amplified ribosomal DNA restriction analysis (ARDRA) and sequencing, then constructed phylogenetic tree. 23 unique clone sequences from Nor-1 soil were classified into 5 bacterial phylum including Acidobacteria (12.3%, 8/65), Actinobacteria (3.1%, 2/65), Firmicutes (21.5%, 14/65), Nitrospira (3.1%, 2/65) and Proteobacteria (60%, 39/65), while 19 unique clone sequences from Sul-1 soil were classified into 2 bacterial phylum including Firmicutes (29.5%, 13/44), and Proteobacteria (70.5%, 31/44). The result suggested that the high concentration of arsenic and sulphate influenced the bacterial population of Sul-1 soil leading to construction of obviously specific bacteria community. Interestingly, a lot of *Acinetobacter* related sequences have been detected in Sul-1 soil bacteria community including clone Sul11/15, Sul12/7 and Sul12/11. Because *Acinetobacter* strains are often ubiquitous, exhibit metabolic versatility, those related strains may be good targets for exploiting novel arsenic detoxification bacteria.

Keywords: Arsenic and sulphate polluted soil, 16S rRNA gene, ARDRA, Bacteria diversity

基于 16S rRNA 基因序列分析受砷和硫酸盐污染的土壤细菌多样性

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摘要: 为了了解普通耕地土壤(Nor-1)和受砷及硫酸盐污染土壤(Sul-1)中的细菌组成和多样性差异,对2个不同土壤样品直接提取总DNA,通过PCR扩增16S rRNA基因并建立文库,对文库克隆进行核糖体DNA扩增片段酶切分析(ARDRA)和测序,构建系统进化树。从Nor-1土壤样品中测序获得23个16S rRNA基因序列,分析序列系统发育关系表明,共包含Acidobacteria (12.3%, 8/65)、Actinobacteria (3.1%, 2/65)、Firmicutes (21.5%, 14/65)、Nitrospira (3.1%, 2/65)和Proteobacteria (60%, 39/65)等5个不同细菌门。而从Sul-1土壤样品中测序获得19个16S rRNA基因序列,分析序列系统发育关系表明,共包含Firmicutes (29.5%, 13/44)和Proteobacteria (70.5%, 31/44)等2个不同细菌门。结果表明,受高浓度的砷和硫酸盐的影响,Sul-1土壤中细菌群落结构相较于普通耕地土壤(Nor-1)发生了明显的改变,多样性明显下降,但有大量具有较强的污染物降解能力的不动杆菌(*Acinetobacter*)相关序列在Sul-1土壤细菌群落中被发现。

关键词: 砷及硫酸盐污染土壤, 16S rRNA 基因, ARDRA, 细菌多样性

1 Introduction

Fast development of chemical industry in developing country, such as China, has generated large volumes of a variety of ion-rich residues. Inadequate management and abandonment of these residues have resulted in environmental impacts due to contaminants dispersal into soil, air and water^[1]. Sulfuric acid has many applications, and is a central substance in the chemical industry. As an important basic raw material, sulfuric acid is extensively used in chemical, light industry, textile, metallurgical, petrochemical and pharmaceutical sectors. The sulfuric acid production in China uses pyrite as major raw material for years. However, for a long time, China has not made any special pollutant emission standard for the sulfuric acid industry. As a highly polluting industry, the water-based pollutants like arsenic, fluoride and heavy metal ions emitted in the production of sulfuric acid can cause significant harm to human health in China^[2].

Because of widely using pyrite as major raw material in china sulfuric acid industry, a lot of drainage containing high concentration of arsenic and sulphate has been dispersal into soil. Arsenic is considered to be a semimetal with metallic and nonmetallic properties. Arsenic is toxic to not only bacteria, but also other domains of life. In the environment, the biogeochemical cycle of arsenic strongly depends on micro-

bial transformation, which affects the mobility and the distribution of arsenic species in the environment^[3]. Arsenic contaminations in soil and groundwater have been reported in many parts of the world^[4-6]. In China, chronic drinking of arsenic-contaminated groundwater has caused endemic arsenicosis, which has become a major threat to public health^[7]. Hence, remediation of arsenic-contaminated soil and water is one of the major challenges in environmental science and public health. Microorganisms play a crucial role in arsenic geochemical cycling through microbial transformation processes, including reduction, oxidation, and methylation^[8-9]. Low-cost, efficient, and environmentally friendly remediation technologies to remove arsenic from contaminated soil and water are urgently needed. Thus, firstly it is important to elucidate the microbial diverse populations and functional genes associated with arsenic mobility and transport in arsenic polluted soil. However, to fully understand the ecology of such complex arsenic-contaminated soils, it is necessary to analyze different microbial populations simultaneously.

Since culturable bacteria may only represent 0.1%–1.0% of the total microbial community^[10-11], culture-independent molecular methods, such as profiling soil DNA, are increasingly and widely used in environmental microbiology^[12]. Extraction of DNA and RNA directly from bacteria in soil circumvents

the requirement to grow microorganisms in laboratory culture, avoiding problems associated with the differential growth rates of the estimated 1% that can be grown routinely^[13]. Analysis of 16S rRNA gene indicates the bacteria population in any particular set of conditions and the large, constantly increasing electronic database of gene sequences for the small sub-unit of rRNA gene provides identification of many soil bacteria with varying degrees of certainty to the genus, species or sub-species level^[14].

There are few studies analyzing the bacterial community in arsenic polluted soils or sediments, either by culture-dependent^[15] or culture-independent methods^[16], and to our knowledge, no culture-independent studies of the bacteria community in both arsenic and sulphate polluted soil from the outlet of sulfuric acid chemical factory have been reported. The aim of this study was to evaluate the structural changes occurring in the bacterial populations of an arsenic and sulphate polluted soil by screening environmentally derived gene libraries by Amplified ribosomal DNA restriction analysis (ARDRA) and sequencing.

2 Materials and methods

2.1 Soil samples collection

Two soil samples were collected from Guangdong province of P. R. China. The soil sample named Sul-1 was collected from the outlet of Foshan chemical factory which mainly produce sulfuric acid; the other soil sample named Nor-1 was collected from normal arable soil which has a distance of 200 meter from soil sample Sul-1 collection site. Samples for physicochemical properties analysis were maintained at 4 °C. Samples for DNA extraction were collected into a dry ice-ethanol bath for flash-freezing, and then stored at -70 °C until processing.

2.2 Construction of 16S rRNA gene libraries

The total community DNA of two soil samples

was extracted using previous reported method^[17]. Briefly, the soil sample was grinded in a mortar under liquid nitrogen. The grinded soil was mixed with the extraction buffer (100 mmol/L Tris-HCl; 100 mmol/L EDTA; 100 mmol/L Sodium phosphate; 1.5 mol/L NaCl; 1% [W/V] CTAB. pH 8.0) containing 1 g/L protease K, followed by three freezing-thawing cycles and then incubation at 65 °C for 2 hours. After chloroform-isopentanol extraction, the total DNA was precipitated with cold isopropyl, washed with 70% ice-cold alcohol, and purified by Sephadex G-200.

Primers 27F and 1492R^[18] were used to amplify the 16S rRNA gene fragments with template DNA originating from the two soil samples. The PCR mixture consisted of 1×PCR buffer, 200 mmol/L dNTP, 0.25 mmol/L each of the forward and reverse primers, 0.5 U *Taq* polymerase (TaKaRa, Dalian, China) and 1 μL of DNA in a final volume of 25 μL. Polymerase chain reaction consisted of a denaturing step at 94 °C for 3 min before 30 cycles as follows: 94 °C for 45 s, 55 °C for 45 s and 72 °C for 1.5 min, and a final extension step for 10 min at 72 °C. 5 μL aliquots of the reaction mixtures were analyzed by 1% agarose gel electrophoresis to confirm the presence of products. The amplified 16S rRNA gene segments were purified by QIAquick Gel Extraction Kit (Qiagen, USA) and ligated with pGEM-T easy Vector (Promega, USA) following the protocols recommended by the manufacturer, then electroporated into *E. coli* JM109 by standard method^[19]. Approx. 1 000 white transformants were random collected and stored each one soil 16S rRNA gene library.

2.3 Amplified ribosomal DNA restriction analysis and sequencing

The clones of two soil 16S rRNA gene libraries were extracted using Tiangen Plasmid Extraction Kit (Tiangen, China). one μg aliquots of plasmid was digested separately with the restriction endonuclease *Hinf* I and *Csp6* I for 3 h at 37 °C with buffer supplied by the manufacturer (Promega, USA). Restriction

fragments were analyzed by 1% agarose gel electrophoresis. The gel was digitalized using a scanner and the images were contrasted using the NIH Image Program 1.59 (National Institutes of Health, Bethesda, MD, USA). Each unique pattern clones were selected to further sequencing. Sequencing was performed in Shanghai Sangon Sequencing Centre.

2.4 Phylogenetic analysis

For phylogenetic analysis, the 16S rRNA gene sequences were first screened using the Naive Bayesian Classifier of Ribosomal Database Project II to identify taxonomy^[20]. The 16S rRNA gene sequences of all related available in GenBank database were extracted after using BLAST to determine their closest neighbor. After a complete alignment in clustalX programs (version 1.83)^[21], the same parts of the 16S rRNA gene sequences were selected for phylogenetic analyses using default parameters in the MEGA software package v.3.1^[22]. Phylogenetic distances were calculated by the neighbour joining (NJ) method using the Kimura 2-parameter model in MEGA. The phylogenetic relationships were also confirmed by the Likelihood and parsimony programs in the same package. The bootstrapping supports for the trees were calculated from a sample of 1 000 replicates.

2.5 Nucleotide sequence accession numbers

The nucleotide sequences determined in this study have been deposited in the National Center for Biotechnology Information (NCBI) database under accession No. JF747160–JF747201.

3 Results

3.1 Physicochemical properties of the soil samples

Both Sul-1 soil sample from the outlet of Foshan chemical factory and Nor-1 soil from a normal arable soil were characterized by clay loam texture and a low content of organic matter, Sul-1 soil was characterized

with a moderately acid pH while Nor-1 was characterized with a neutralized pH (Table 1). Sulphate concentration in Sul-1 soil sample is as high as 560 mg/kg, while there is low sulphate concentration of 16 mg/kg in sample Nor-1. This may suggested that the outlet soil of Foshan chemical factory have been polluted because of long time draining with exceeded levels of sulphate imposed by the Chinese Integrated wastewater discharge standard for industrial sites (5 mg/kg). The Sur-1 soil sample also has been polluted by arsenic with higher concentration of 124 mg/kg (Table 1).

Table 1 Physicochemical properties analysis of soil samples from the outlet of Foshan chemical factory (Sul-1) and normal arable soil (Nor-1)

表1 佛山化工厂出水口土壤样品(Sul-1)和普通耕地土壤样品(Nor-1)理化性质

Parameters 参数	Nor-1	Sul-1
Textural classes 结构特征(%)		
Clay 粘粒	45.0	40.0
Sand 砂粒	25.0	30.0
Silt 粉粒	30.0	30.0
pH	6.5	5.5
SO ₄ 硫酸根(ppm)	16.0	560.0
Fe ²⁺ 铁(ppm)	ND	10.0
As 砷(ppm)	ND	124.0
Organic matter 有机质(%)	6.5	3.8

Note: ND: Not determined.

注: ND: 未检测到.

3.2 Bacteria community diversity of normal arable soil sample

A total of 65 clones of Nor-1 soil 16S rRNA gene library were checked by amplified ribosomal DNA restriction analysis and 23 clones with unique double digestion banding patterns (data not shown) were selected for sequencing. After checked by the Naive Bayesian Classifier of Ribosomal Database Project II, The 23 unique clone sequences were classified into 5 bacterial phylum including Acidobacteria (12.3%, 8/65), Actinobacteria (3.1%, 2/65), Firmicutes (21.5%, 14/65), Nitrospira (3.1%, 2/65) and Proteobacteria

(60%, 39/65) (Table 2). In the case of the Acidobacteria, Clone Nor1/2, Nor1/4 and Nor1/6 belong to Acidobacteria. Their closest relatives were from uncultured bacteria with sequence identity of between 95 and 99%. Clone Nor12/2, Nor12/4, Nor12/13 and Nor11/11 were classified into phylum Firmicutes, three genus were identified including *Clostridium*, *Desulfotomaculum* and *Oscillospira* in phylum Firmicutes. In the case of Proteobacteria, the major clones belong to Betaproteobacteria (61.5%, 24/39) and Gammaproteobacteria (28.2%, 11/39). In Betaproteobacteria, three sequences clustered as Comamonadaceae, but other genus, including *Acidovorax*, *Achromobacter* and *Naxibacter* were also represented

in the set, and only one clone was identified into each genus. Clone Nor11/5 was affiliated with *Achromobacter xylosoxidans* strain M66 with 99% sequence identity. This bacterium normally inhabits aquatic sources in the environment and hospital as well as the human gut, but may cause nosocomial and community-acquired infections, but this bacterium is not obviously danger to health human. Clone Nor11/10, Nor11/12 and Nor12/19 were affiliated with Comamonadaceae are the most abundant composition of Nor-1 soil bacteria community, and may have important roles in Nor-1 soil bacteria community. The rest clone Nor1/15 and Nor1/16 were affiliated with uncultured bacteria with sequence identity of between 98

Table 2 Taxonomic affiliation of 16S rRNA gene sequences from a normal arable soil (Nor-1) according to their ARDRA patterns
表 2 根据 ARDRA 分析普通耕地土壤样品(Nor-1)中 16S rRNA 基因序列的分类位置

Clone 克隆子	Phylogenetic assignment 系统进化关系	Closest relative strain/clone and GenBank accession number 最高亲缘关系菌株/克隆及其序列号	Identity 相似度(%)	Number 数量
Nor1/2	Acidobacteria	Uncultured acidobacterium sp. clone 64 (EU223942)	99	2
Nor1/4		Uncultured acidobacteria bacterium clone 01 (AB252947)	95	4
Nor1/6		Uncultured acidobacteria bacterium clone 2h-29 (FJ444657)	99	2
Nor1/5	Actinobacteria	<i>Micrococcus lylae</i> strain d10 (AJ298940)	99	2
Nor12/2	Firmicutes	<i>Clostridium crotonatorovans</i> strain R6B (AY742899)	96	2
Nor12/13		<i>Clostridium metallolevans</i> strain ASI1 (DQ133569)	92	2
Nor12/4		<i>Desulfotomaculum guttoideum</i> strain DSM 4024 (NR_026409)	99	4
Nor11/11		Oscillospiraceae bacterium NML 061048 (EU149939)	99	6
Nor1/9	Nitrospira	<i>Nitrospira</i> sp. (AF035813)	99	2
Nor1/13	Alphaproteobacteria	<i>Amaricoccus veronensis</i> strain Ben102 (NR_029203)	96	1
Nor1/18	Betaproteobacteria	<i>Acidovorax</i> sp. 4_C16_23 (EF540489)	99	4
Nor11/5		<i>Achromobacter xylosoxidans</i> strain M66 (HQ676601)	99	3
Nor11/10		<i>Comamonas testosteroni</i> strain F4 (FJ967838)	100	4
Nor11/12		<i>Comamonas testosteroni</i> strain F4 (FJ967838)	99	3
Nor1/19		Uncultured Comamonadaceae bacterium clone LrhB76 (AM159380)	99	3
Nor1/1		<i>Naxibacter haematophilus</i> strain Ag15 (EU554441)	99	1
Nor1/15		Uncultured bacterium clone SAV07F01 (EU542355)	98	5
Nor1/16		Uncultured bacterium clone WW1_b49 (GQ264246)	99	1
Nor1/11	Deltaproteobacteria	Uncultured bacterium clone BaMNPk2RC24 (FR751122)	98	3
Nor12/20	Gammaproteobacteria	<i>Citrobacter</i> sp. CST-5.1 (HQ123576)	99	2
Nor12/3		<i>Klebsiella variicola</i> strain C109 (HQ407284)	99	4
Nor12/23		<i>Klebsiella variicola</i> strain C109 (HQ407284)	99	3
Nor11/17		<i>Pseudomonas</i> sp. BCB05 (EU140959)	99	2

and 99%. In Gammaproteobacteria, three genus were identified including *Pseudomonas*, *Klebsiella* and *Citrobacter*. Clone Nor11/17 belongs to genus *Pseudomonas*. The genus *Pseudomonas* contains more than 140 species; most *Pseudomonas* strains known to cause disease in humans are associated with opportunistic infections. It is a ubiquitous free-living bacterium and is found in most moist environments, although it seldom causes disease in healthy individuals. Clone Nor11/17 is not related with opportunistic pathogen *Pseudomonas* strain. So they should be normal soil *Pseudomonas* stains exist in arable soil. Clone Nor12/3 and Nor12/23 were affiliated with *Klebsiella variicola* strain C109 with 99% sequence identity which usually found in the roots of plants such as wheat, rice, and corn, where they act as nitrogen-fixing bacteria. Clone Nor12/20 was affiliated with *Citrobacter* sp. CST-5.1 which usually found almost everywhere in soil, water, wastewater and human intestine.

3.3 Bacteria community diversity of arsenic and sulphate polluted soil sample

For phylogenetic analysis of Sul-1 soil bacteria community, 19 unique clones from total 44 clones were selected for sequencing. The 19 unique clone sequences were classified into 2 bacterial phylum including Firmicutes (29.5%, 13/44), and Proteobacteria (70.5%, 31/44) (Table 3). In phylum Proteobacteria, the major clones also belong to Betaproteobacteria (64.5%, 20/31) and Gammaproteobacteria (29%, 9/31). But there is dramatic different bacteria community structure of Sul-1 soil compared with bacteria community of Nor-1 soil. In Betaproteobacteria, the majority of clones were affiliated with uncultured bacteria with sequence identity of between 97 and 99%. While clone Sul1/2, Sul1/7 and Sul1/12 were affiliated with *Methyloversatilis universalis* strain 500 with 98% sequence identity. Those clones represented the most abundant composition of Sul-1 soil bacteria commu-

nity. Interestingly, Clone Sul1/3 was affiliated with uncultured bacterium clone EJ10-Ash91-53 with 99% sequence identity, and also was affiliated with *Thiobacillus plumbophilus* strain DSM 6690 with 97% sequence identity. Because of high concentration of sulphate in Sul-1 soil sample, clone Sul1/3 may represent major reducing sulphate bacteria which have important role in Sul-1 soil. In Gammaproteobacteria, three genus were identified including *Acinetobacter*, *Arenimonas*, and *Enterobacter*. Clone Sul11/15, Sul12/7 and Sul12/11 were affiliated with genus *Acinetobacter* which are important soil organisms where they contribute to the mineralization of, for example, aromatic compounds. And some *Acinetobacter* have ability of degradation of arsenic contaminants. Clone Sul1/1 was affiliated with *Arenimonas malthae* strain CC-JY-1 with low identity of 92% may represent a new *Arenimonas* genus strains. Clone Sul1/5 was affiliated with *Enterobacter aerogenes* strain C1111 which is generally found in the human gastrointestinal tract and does not generally cause disease in healthy individuals. It has been found to live in various wastes, hygienic chemicals, and soil.

3.4 Comparatively phylogenetic relationship between Nor-1 soil and Sul-1 soil bacteria community

To investigate the phylogenetic relationship between Nor-1 soil bacteria community and Sul-1 soil bacteria community, we constructed a comprehensive phylogenetic tree using the available related 16S rRNA gene sequences (Fig. 1). Clone sequences of Nor-1 soil 16S rRNA gene library were classified into 5 bacterial phyla including Firmicutes, Nitrospira, Actinobacteria, Acidobacteria and Proteobacteria, while Clone sequences of Sul-1 soil 16S rRNA gene library were only classified into 2 bacterial phylum including Firmicutes and Proteobacteria. This result suggested that the high concentration of arsenic and sulphate influenced the bacterial populations of Sul-1 soil

Table 3 Taxonomic affiliation of 16S rRNA gene sequences from an arsenic and sulphate polluted soil (Sul-1) according to their ARDRA patterns

表 3 根据 ARDRA 分析受砷及硫酸盐污染土壤样品(Sul-1)中 16S rRNA 基因序列的分类位置

Clone 克隆子	Phylogenetic assignment 系统进化关系	Closest relative strain/clone and GenBank accession number 最高亲缘关系菌株/克隆及其序列号	Identity 相似度(%)	Number 数量
Sul11/9	Firmicutes	<i>Lysinibacillus</i> sp. 210_61 (GQ199763)	100	3
Sul12/1		Uncultured bacterium clone G35_D8_L_B_E12 (EF559159)	96	2
Sul12/8		<i>Clostridium butyricum</i> strain IDCC 5101 (EF533982)	94	1
Sul12/6		<i>Clostridium</i> sp. BG-C131 (FJ384387)	99	4
Sul12/9		<i>Clostridium</i> sp. BG-C131 (FJ384387)	99	3
Sul1/4	Alphaproteobacteria	<i>Sphingomonas</i> sp. PW-1 (AB255386)	100	2
Sul1/10	Betaproteobacteria	Uncultured bacterium clone BSS150 (HQ397479)	99	3
Sul1/13		Uncultured bacterium clone BSS150 (HQ397479)	99	3
Sul1/11		Uncultured bacterium clone PP4-54 (EU148976)	99	1
Sul1/2		<i>Methyloversatilis universalis</i> strain 500 (DQ923115)	98	6
Sul1/7		<i>Methyloversatilis universalis</i> strain 500 (DQ923115)	98	1
Sul1/12		<i>Methyloversatilis universalis</i> strain 500 (DQ923115)	98	2
Sul1/14		Uncultured bacterium clone DF5IPCant18c04 (GQ921433)	97	1
Sul1/3		Uncultured bacterium clone EJ10-Ash91-53 (HQ900305)	99	3
Sul11/15	Gammaproteobacteria	<i>Acinetobacter calcoaceticus</i> strain GWRVA20 (EU921458)	99	3
Sul12/7		<i>Acinetobacter</i> sp. C-4 (HQ896038)	99	1
Sul12/11		<i>Acinetobacter</i> sp. SeaH-As2w (FJ607348)	99	2
Sul1/1		<i>Arenimonas malthae</i> strain CC-JY-1 (DQ239766)	92	1
Sul11/5		<i>Enterobacter aerogenes</i> strain C1111 (AB244467)	100	2

leading to construction of obviously specific bacteria community. The majority of sequences identified as belong to Beta and Gammaproteobacteria from Sul-1 soil 16S rRNA gene library also have different phylogenetic relationship with those from Nor-1 soil 16S rRNA gene library. The majority of clone sequences from Sul-1 soil 16S rRNA gene library which belong to Betaproteobacteria were clustered into one phylogenetic clade, while the majority of clone sequences from Nor-1 soil 16S rRNA gene library were clustered into another phylogenetic clade. In bacteria phylum Firmicutes which were distributed to a large number of phylotypes in both Nor-1 and Sul-1 soil 16S rRNA gene library show more complicate phylogenetic relationship. In our study, Acidobacteria, Actinobacteria, Nitrospira, Alphaproteobacteria and Deltaproteobacteria could be considered as rare phyla of Nor-1 soil bacteria commu-

nity with only one to four sequences. And those phyla except Alphaproteobacteria even cannot be detected in Sul-1 soil 16S rRNA gene library.

4 Discussion

The long time arsenic and sulphate contaminant into the studied soil has induced a shift of the community structure in response to perturbation. The phylum of Proteobacteria is the most abundant bacteria in soil libraries^[23]. The Proteobacteria encompass enormous morphological, physiological and metabolic diversity, and are of great importance to global carbon, nitrogen and sulfur cycles. In Both Nor-1 and Sul-1 soils bacteria community, our results are in agreement with most studies showing the importance of the Proteobacteria, especially the Beta and Gamma division. Analyses of the total community 16S rRNA gene sequences of

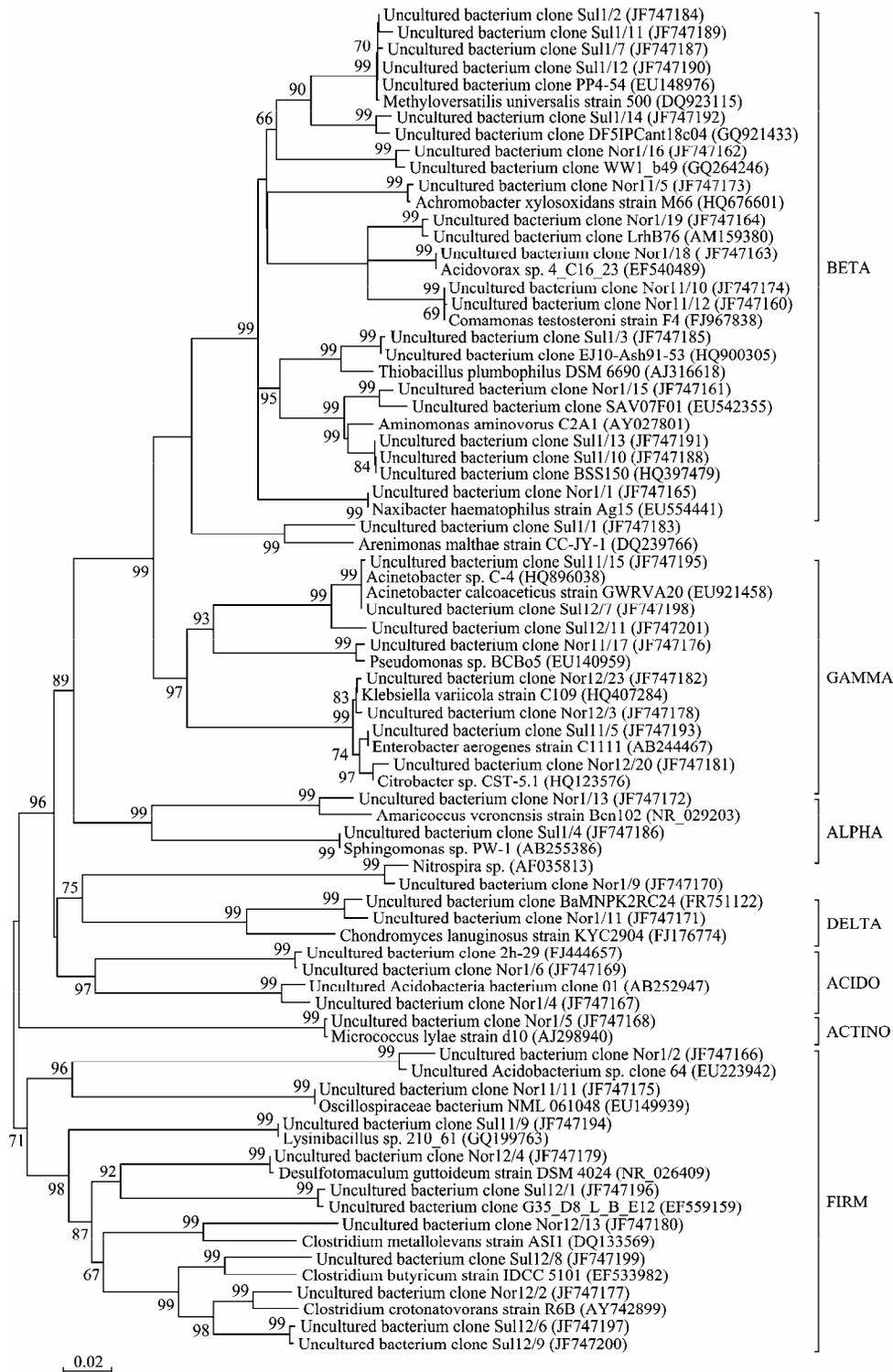


Fig. 1 A phylogenetic tree constructed from the 16S rRNA gene clone sequences from Nor-1 and Sul-1 soil samples

图1 利用来自Nor-1和Sul-1土壤样品的细菌16S rRNA基因序列构建系统发育树

Note: Numbers in parentheses represent the sequences accession number in GenBank. The number at each branch points is the percentage supported by bootstrap. Bar means 2% sequence divergence. The bold represents the 16S rRNA gene clone sequence from Nor-1 and Sul-1 soil sample. BETA, GAMMA, ALPHA, DELTA, ACIDO, ACTINO and FIRM mean Betaproteobacteria, Gammaproteobacteria, Alphaproteobacteria, Deltaproteobacteria, Acidobacteria, Actinobacteria and Firmicutes phylogenetic clade respectively.

Nor-1 and Sul-1 soil suggest that the site harbors a wide range of microorganisms that has yet to be isolated. It was also showed that the major population present in the Nor-1 soil belongs to phylum Proteobacteria particularly the Betaproteobacteria, considering the diversity of potential habitats and the great genetic diversity of microbes, the majority of the microbes from arsenic and sulphate polluted soil is still unknown and could be a potential source of novel bacteria strains which have ability of suffering high sulfuric acid and detoxifying arsenic. This study was the first attempt to analyze the microbial community of the arsenic and sulphate polluted soil site by using environmental DNA. An improved understanding of the microbial diversity in the environment of arsenic and sulphate polluted soil is needed if the ultimate goal is to discover novel bacteria strains for industrial and biotechnological applications of arsenic remediation. Future work should focus on detecting the presence of bacteria tolerance high concentration of arsenic and sulphate contaminants. Interestingly, a lot of *Acinetobacter* related sequences have been detected in Sul-1 soil bacteria community including clone Sul11/15, Sul12/7 and Sul12/11. Because *Acinetobacter* strains are often ubiquitous, exhibit metabolic versatility, those related strains may be good targets for exploiting novel arsenic detoxification bacteria.

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关于《微生物学通报》专题刊申请的通知

当前,随着生物技术的飞速发展,微生物学涵盖的领域越来越广,交叉学科的研究也越来越受到关注。除了已有的微生物学、病毒学、基因工程、细胞工程、酶工程、发酵工程之外,基因组学、代谢工程、纳米科学、生物炼制、生物质能等也逐步成为微生物学研究的热门领域。为了更加系统、集中地反映各个领域的研究成果,以及该领域学科的热点难点问题,充分发挥《微生物学通报》的学科引领和导向作用,促进学科发展,为某个领域的科研人员提供一个交流的平台,《微生物学通报》编委会决定自2008年起,每年出版一定数量的专题刊。专题刊将系统地反映微生物学相关领域或新学科生长点的最新进展,及时介绍国内外微生物相关前沿领域的突破性成果,以及面向国家和社会发展需要并具有重大应用前景的研究成果。真诚欢迎本领域各学科的学术带头人,申请并组织专题刊。申请得到编委会批准后,申请人将被邀请担任本专题刊的特邀编辑,负责组织稿件、确定审稿专家,并撰写专题刊序言。

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