International Journal of Engineering Science Invention (IJESI)
ISSN (Online): 2319 – 6734, ISSN (Print): 2319 – 6726
www.ijesi.org ||Volume 7 Issue 12 Ver. III || Dec 2018 || PP 72-81

Analysis of Microbial Diversity in different periods of Nihewan Using High-throughput Sequencing

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Abstract: *Microbial are widely distributed in various ecological environments and play essential roles in the carbon and nitrogen cycles in natural ecosystems. Nihewan site group is wide distribution, rich in content, and covers many subjects of ancient anthropology, paleontology, paleomagnetism, paleoclimatology, etc. At present, domestic and foreign research are concentrated mainly in the Nihewan archaeological value, geological structure, animal and plant community structure. Most community studies have been focusing on settlement of plants and animals but less on microbial succession. In this study, soil samples were collected from different geological Nihewan archaeological strata, and microbial communities were analyzed by HTS technology to reveal bacterial composition. To explore microbial groups in the history of evolution, we collected two soil samples in Nihewan which represent different periods. A total of 1159 OTUs were detected, which could attributed to 197 genera, 167 families, 127 orders, 86 classes and 34 phylums of bacteria in NHW1. A total of 905 OTUs were detected, which could attributed to 178 genera, 153 families, 117 orders, 77 classes and 33 Phylums of bacteria in NHW2, and the bacterial communities were different among the two samples.*

Keywords: *Nihewan, Diversity , Separation of species,evolution*

Date of Submission: 13-12-2018 Date of acceptance: 28-12-2018

I. Introduction

As an important component of terrestrial ecosystems, soil microbial communities are directly or indirectly involved in almost all soil ecological processes, including material cycles, energy conversion, and pollutant transformation.(He J Z et al.2015)Soil microbial community structure, diversity and changeis is a sensitive indicator of change of climate and soil environmental conditions, to a certain extent, reflects the quality of the soil. Understanding the community composition, structure and evolution of microbial is not only fundamentally important for studying their functions to ecosystem, but also conductive to protecting microbial resources.

As the most abundant biological habitat on the planet, soil ecosystem has the greatest biodiversity, and closely associated with the above-ground ecosystem. Along with the rapid economic development, disturbance by humans has become increasingly serious, and the soil biodiversity suffered significant threat. Under the background of mass extinction and the limit knowledge of soil biodiversity, it is extremely urgent for human to protect the soil biodiversity. (LI B et al.2015)Bacteria, a large domain of the prokaryoticmicroorganisms, have a very close relationship with human society. Thus, faced with the threat of microbial diversity interference, we have to carry out research in bacteria evolution to explore the emerging mechanisms, which would provide a basis for the preotecting and controlling of bacterial degradation.Besides, bacteria are ubiquitous in naturalenvironment. This planet would range from one million to one billion. We can study the genetic basis and evolutionary mechanisms associated with the bacterial diversity, which would lay a foundation for the study of life origin and evolutionary laws.As is well known, the genome carries all the genetic information of a species. The research based on genome sequences would present a more realistic evolutionary process. Fortunately, the development and popularity of sequencing technology provide rich genomic data. Thus, we can carry out the research of bacterial genome evolution, which will play a positive role in understanding the genetic traits, functional characteristics and evolutionary mechanismsof Bacteria.

Nihewan basin is located in the east sanggan rivervally of yangquan county in hebei province, more than one million years of history. It is one on behalf of the site calibrated by the calibration of the Quaternary Genomics and Applied Biology (Liu Y et al. 2012). Due to its large time span and complex geological structure, making the region in the study of geology, biology, archeology valuable(Jia Z X et al. 2011). It's well known because of its thicker sedimentary, rich mammalian fossils and human remains. Magnetic group parameters shows that and NHW1 which has 1.66 million - year history deposit under the condition of normal gravity differentiation, and

the deposition are not obvious later disturbance. So the soil environment of microbial is also stable relatively in the stratum.

In order to determine the distribution of the microbial species in soils from Nihewan basin, we investigated the species diversity of edaphon in different depths of soil layer. Microbial species were isdated from soil samples by dilution plate method. The pure cultures of microbial species were determined by morphological observation, physiological and biochemical characterisation as well as 16SrDNA sequencing. The study is the first time to isolate and identificate the microbial species in different depths of soil layer from in Nihewan basin. The study is an important part of archaeological study of Nihewan, providing scientific data for us to further understand the prehistory is ecological environment.(LV C S et al.2017)Our two samples, NHW2 is the representative of modern and contemporary topsoil, NHW1 is on behalf of the soil samples of 1.66 million years ago. The modern and contemporary topsoil is influenced by various environmental factors of comprehensive, thus formed the corresponding microbial community; Soil samples, 1.66 million years ago, exist in deep formation, long-term by stability change of the role of some environmental factors, evolved into the corresponding community. This is different from permafrost.Glaeier and frozen soil are the special complex ecosystem. It should be considered as a stabilizing factor for sustaining for organisms and biological macromolecular due to its property of low temperature and scant nutrition.

In this study, 2 stratums, were selected in NHW, which is located in yangquan county of hebei. Then, using 16S rDNA high-through put sequencing, the composition and evolution distribution patterns of soil microbial communities were analyzed. The bacterial communities were significantly different between the two samples, according to detrended correspondence analysis (DCA). Through analysis of bacterial α-diversity of the two samples, the number of OTUs ranged from 905 to 1159, and the Shannon index ranged from 3.61 to 3.76. Both of the measures exhibited strong negative correlations with the evolution, indicating that the environmental conditions of evolution may not benefit to the survival of bacterial species.

In this study, we adopted Illumina high-throughput sequencing technology to analyze the characteristics of soil bacteria in Nihewan Natural Reserve. To understand soil microbial community stability and the response to different stratum in Nihewan, a large soil microbial community experiment was conducted in the sampling locations. The aims of our study were to determine: microbial groups in the history of evolution.

II. Materials and methods

Study sites and sample collections

Soil samples were collected from various locations in the Nihewan Basin on October, 2011. Descriptions of soil collection sites are presented in Table 1. In each of the 2 stratums, there were 3 randomly selected 30 m×30 m replicate plots 100-150 m apart. In each plot, we collected 10 soil samples using a 2.5 cm diameter soil core, which extended to a depth of 10 cm. The 2 soil samples in each plot were composited and passed through a 2-mm sieve in the field. By pooling the 10 soil cores, we aggregated spatial heterogeneity at the scale of individual plots. The 3 soil plot samples were combined into a representative sample for each stratum. From the sieved composite sample, a 5.0-g sample was removed for DNA extraction. This was done to allow a characterization of the bacteria community at the scale of the entire Nihewan Basin,and to explore regional trends in community similarity that may have been structured by environmental factors. Soil samples was collected from Nihewan Basin and preserved Inhebei university. Details of the synthetic feed stock solution are shown in Table 1.（Tab.1）

Illumina sequencing analysis of 16S rRNA gene amplicons

Microbial genomic DNA was extracted from 5 g of well-mixed soil for each sample. The primers 520F：(5-AYTGGGYDTAAAGNG-3) 802R：(5- TACNVGGGTATCTAATCC -3) target-ing the V4 hypervariable regions of microbial 16S rRNA genes were selected.

Tab. 1 The source information of the soil samples

Number	Site	Geologic Age (thousands year)	Soil Type	Climate Type
NHW1	Majuangou2	1660	Silt and sand (salowness and celadon clayey silt）	Warm and humid
NHW2	Donggutuo	now	Yellowish brown sandy soil	Warm and humid

High throughput sequencing analysis of microbial diversity

The project soil samples were sent to Paisennuo biological technology limited company in Shanghai for high-throughput sequencing analysis. The approaches were used assess the bacterial composition in the samples; partial sequencing of cloned 16S rRNA fragments V4 region. Raw data were separated to samples according to same barcode sequence. Adapters, low quality and ambiguous reads were trimmed by Btrim (Y

Konget al.2011), and the forward and reverse reads were integrated into a whole sequence by FLASH. Sequences were divided into groups according to the similarity of each other, and operational taxonomic units(OTUs) were formed at 97% similarity level by using UCLUST (R.C et al.2010). Random resampling was processed with 15,000 sequences per soil sample.

III. Results

Surface sampling and study sites

At both 2 samples were collected in NHW. An overview of the sampling site is shown in Tab 1. Both of the samples were collected on the north site of NHW were the soil was highly variable covering.

Obtain High quality sequence

In order to ensure the accuracy of the results of the High-throughput sequencing analysis, it is necessary to further filter effective sequence and removing chimera sequence which generated from the process of PCR amplification, get the final quality sequence for subsequent analysis. After initial quality control 113521 high quality sequences were obtained. On average, 56761 sequences were obtained per sample. The result of sequence analysis is as follow. The proportion of effective sequences of the two samples were all above 98%, data show that the two samples can be analyzed for next. (Tab 2).

Tab. 2 sample sequence number of the two samples

Sample	Effective sequence	High quality sequence	proportion
NHW1	65863	64718	98.26%
NHW2	49686	48794	98.20%
Total	115549	113521	98.24%

Bacterial Alpha diversity analysis

Using NCBI online comparison to analysis NHW1's bacteria species diversity and phylogenetic relationships among species in Nihewan. Through using Illumina high-throughput sequencing technology, a total of 1159 OTUs were detected at 97% similarity level, which attributed to 197 genera, 167 families, 127 orders , 86 Classes and 34 Phylums of bacteria in NHW1, other 43 clones are in the unclassified status. We were obtained 905 OTU, distributed in 33 Phylums, 77 Classes, 117 orders, 153 families and 178 Genus, other 17 clones are in the unclassified status, accounted for 21.8% of the total number of clones in NHW2. The methods is showed in table 3. We have learned that NHW1 have the bigger number of bacteria species in all classify level than NHW2.

High-throughput sequencing was applied to analyze the microbial diversity of the soil samples to investigate the effect of the enrichment of the bacteria community. Goods coverage of two samples were greater than 99% , indicating that the result represented the microbial components of the soil samples. Richness estimators Chao1 and Ace were used to estimate the total number of OTUs. A higher value shows greater richness. Diversity indexes Shannon and Simpson were used to reflect species diversity. A higher value indicates greater diversity. So on the study, choose the four indexes selected in each classification level advantage bacterium group as the research object. The methods is showed in table 4. We can clearly know that the four index of NHW1 are all higher than NHW2's, so NHW1 have higher value to indicates greater diversity.

Tab.3 The number of bacteria species of the two soil samples

Sample	Phylum	Class	Order	Family	Genus	Unclassified
NHW1	34	86	127	167	197	43
NHW2	33	77	117	153	178	17

Tab.4 Bacterial diversity index of the two samples

samples	chao	Ace	simpson	shannon
NHW1	1365.8756	1373.0578	0.090801	3.760402
NHW2	1125.6243	1171.0771	0.093344	3.619194

The sample classification

According to the result of OTU and classification position , each sample can be obtained in each classification level , including the types of bacteria and the proportion of bacteria in sample, can make us more intuitive to understand the composition of bacteria in the sample. Classified relative abundance is more than 1.0% of the Phylum, Class, Order, Family respectively andthe relative abundance is less than 1.0% recorded in the other; classified relative abundance is more than 0.5% of the Genusandthe relative abundance is less than 0.5% recorded in the other. The number 1 represent NHW1 and 2 represent NHW2.

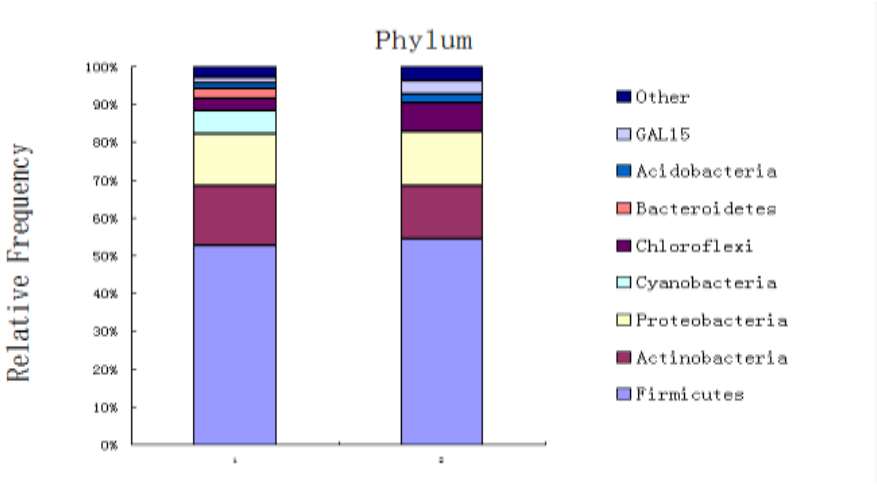


Fig.1 The distribution of the two soil samples bacteria at the level of Phylum

A large part of OTUs belonged to the Phylum of Firmicutes, which accounted for >50% relative of the abundances in the two samples. And some other OTUs belonged to the Phylum of Actinobacteria, Proteobacteria and Chloroflexi.Comparing the soil sample which is 1.66 million years ago in NHW1 with NHW2, analysis the advantageous species of the two samples and the proportion of them at the level of Phylum. At the phylum level, a total of 34 phylum were identified from NHW1(Fig. 1). The 8 most abundant genera,containing more than 97% of the total sequences, were Firmicutes, Actinobacteria, Proteobacteria, Cyanobacteria, Chloroflexi, Bacteroidetes, Acidobacteria, GAL15.Atotal of 33 phylum were identified from NHW2(Fig. 1). The 6 most abundant phylum, containing more than 96% of the total sequences, were Firmicutes, Actinobacteria, Proteobacteria, Chloroflexi, Acidobacteria, GAL15.The figure shows that there three relative frequency in high level of both the two soil samples in the classification level of phylum are Firmicutes, Actinobacteria, Proteobacteria respectively. Firmicutesis the highest phylum in both of the two sample, all above 50%. The ratio ofFirmicutesin NHW2 is higher**,** accounting for 54.5%; It has a low proportion in NHW1, which accounts for 52.8%. The ratio of Actinobacteria and Proteobacteria in NHW1 are higher, accounting for 15.8% and 13.6% respectively; Actinobacteria and Proteobacteria account for 13.9% and 14.4% respectively. Cyanobacteria, Chloroflexi and Bacteroidetes in NHW1 are also higher elative frequency, while Chloroflexi is lower in NHW2 relatively, and relative frequency of Bacteroidetes, Cyanobacteria are low extremely, lower than 1%. The study found that there was a difference in the number of microbial species in the soil sample of the two different geologic time scale, and as a whole the species composition is consistent. There areCaldithrix, AD3, Fibrobacteresand Lentisphaerae in sample NHW1, but no in sample NHW2; SC4, WPS-2 and WS4 only exists in sample NHW1.

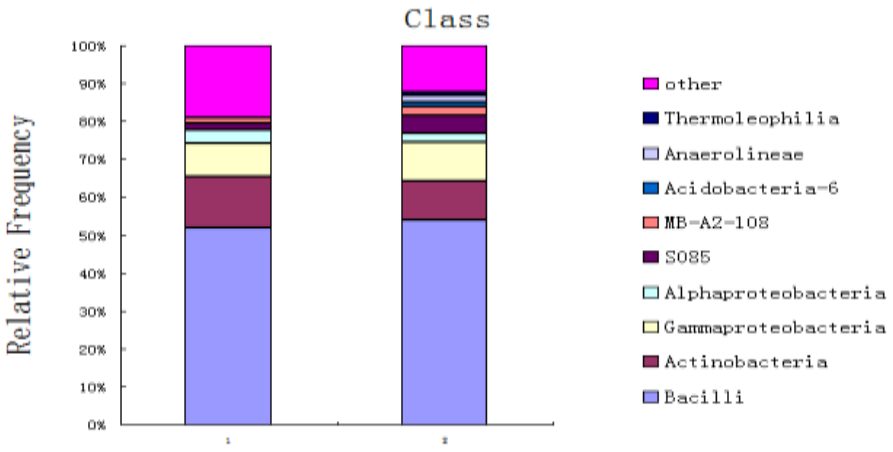


Fig.2 The distribution of the two soil samples bacteria at the level of Class

Comparing the soil sample NHW1 with the NHW2, analysis the advantageous species of the two samples and the proportion of them at the level of Class. At the class level, a total of 93 class were identified from NHW1 and 88 class were identified from NHW2 (Fig. 2). The figure shows that there three relative frequency in

high level of both the two soil samples in the classification level of Class are Bacilli, Actinobacteria, Gammaproteobacteria respectively. Bacilliis the highest class in both of the two sample, all above 50%. The ratio ofBacilli and Gammaproteobacteriain NHW2 are higher,accounting for 53.9% and 10.1% respectively; Those have a lower proportion in NHW1, accounting for 52.0% and 8.6% respectively. The ratio of Actinobacteriain NHW1 is higher,accounting for 13.4%. It has a low proportion in NHW2, which accounts for 10.3%. Acidobacteria-6,Anaerolineae, Thermoleophilia in NHW2 are also higher elative frequency, while those are lower than 1% in the NHW2. The study found that there was a difference in the number of microbial species in the soil sample of the two different geologic time scale, and as a whole the species composition is consistent.

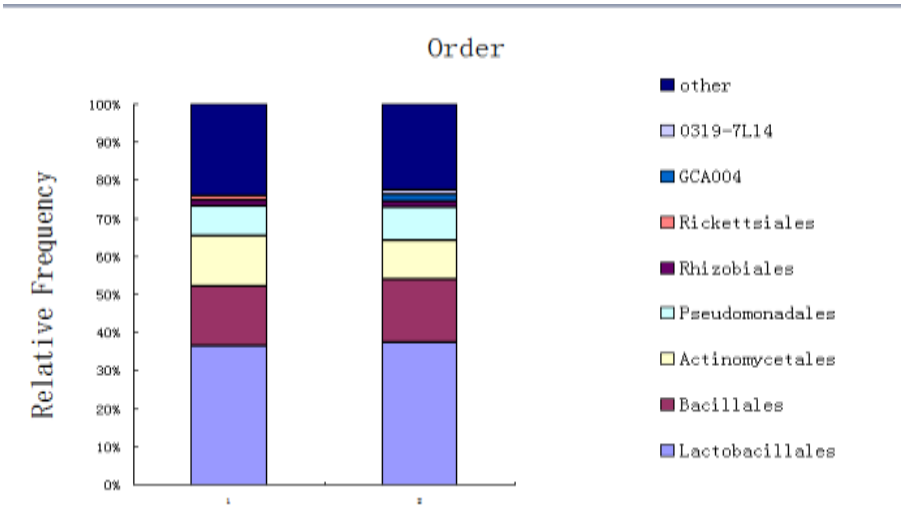


Fig.3 The distribution of the two soil samples bacteria at the level of Order

Comparing the soil sample NHW1 with the NHW2, analysis the advantageous species of the two samples and the proportion of them at the level of Order（Fig.3）. The figure shows that there three relative frequency in high level of both the two soil samples in the classification level of order are Lactobacillales, Bacillales, Actinomycetales respectively. Lactobacillalesis the highest order in both of the two sample,all above 35%. The ratio ofLactobacillales and Bacillalesin NHW2 are higher,accounting for 37.3% and 16.5% respectively; Those have a lower proportion in NHW1, accounting for 36.5% and 15.6% respectively. The ratio of Actinomycetalesin NHW1 is higher, accounting for 13.4%. It has a low proportion in NHW2, which accounts for 10.3% GCA004, 0319-7L14 in NHW2 are also higher elative frequency, while those are lower than 1% in the NHW2; Rickettsiales is also above 1% in NHW1 but not in NHW2. The study found that there was a difference in the number of microbial species in the soil sample of the two different geologic time scale, and as a whole the species composition is consistent.

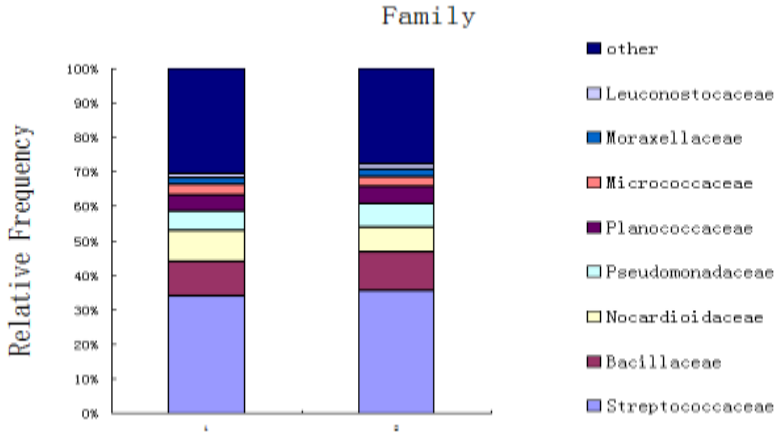


Fig.4 The distribution of the two soil samples bacteria at the level of Family

Comparing the soil sample NHW1 with the NHW2, analysis the advantageous species of the two samples and the proportion of them at the level of Family（Fig.4）. The figure shows that there three relative frequency in high level of both the two soil samples in the classification level of family are Streptococcaceae, Bacillaceae, Nocardioidaceae,Pseudomonadaceae, Planococcaceae respectively. Streptococcaceaeis the highest order in both

of the two sample, all above 30%. The ratio ofStreptococcaceae, Bacillaceae, Pseudomonadaceae and Planococcaceae in NHW2 are higher, accounting for 34.6%, 10.7%, 6.6%, 4.8% respectively; Those have a lower proportion in NHW1, accounting for 33.8%, 10.1%, 5.6% and 4.6% respectively. The ratio of Nocardioidaceaein NHW1is higher, accounting for 9.1%. It has a low proportion in NHW2, which accounts for 7.1%. The study found that there was a difference in the number of microbial species in the soil sample of the two different geologic time scale, and as a whole the species composition is consistent.

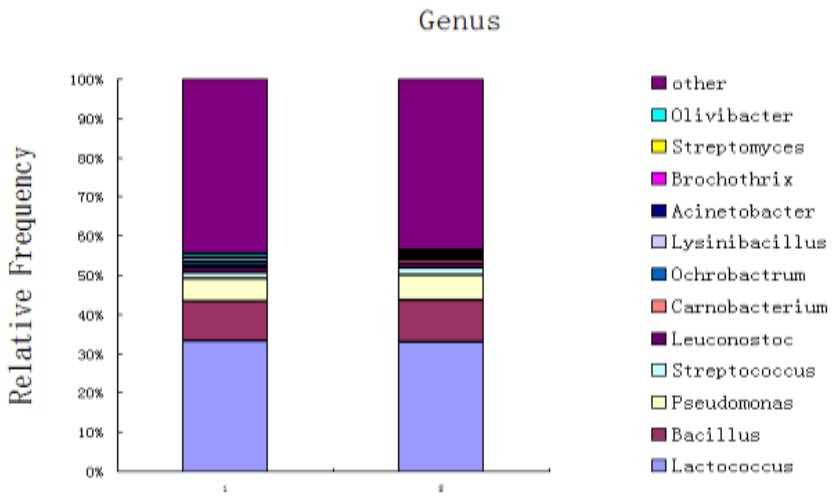


Fig.5 The distribution of the two soil samples bacteria at the level of Genus

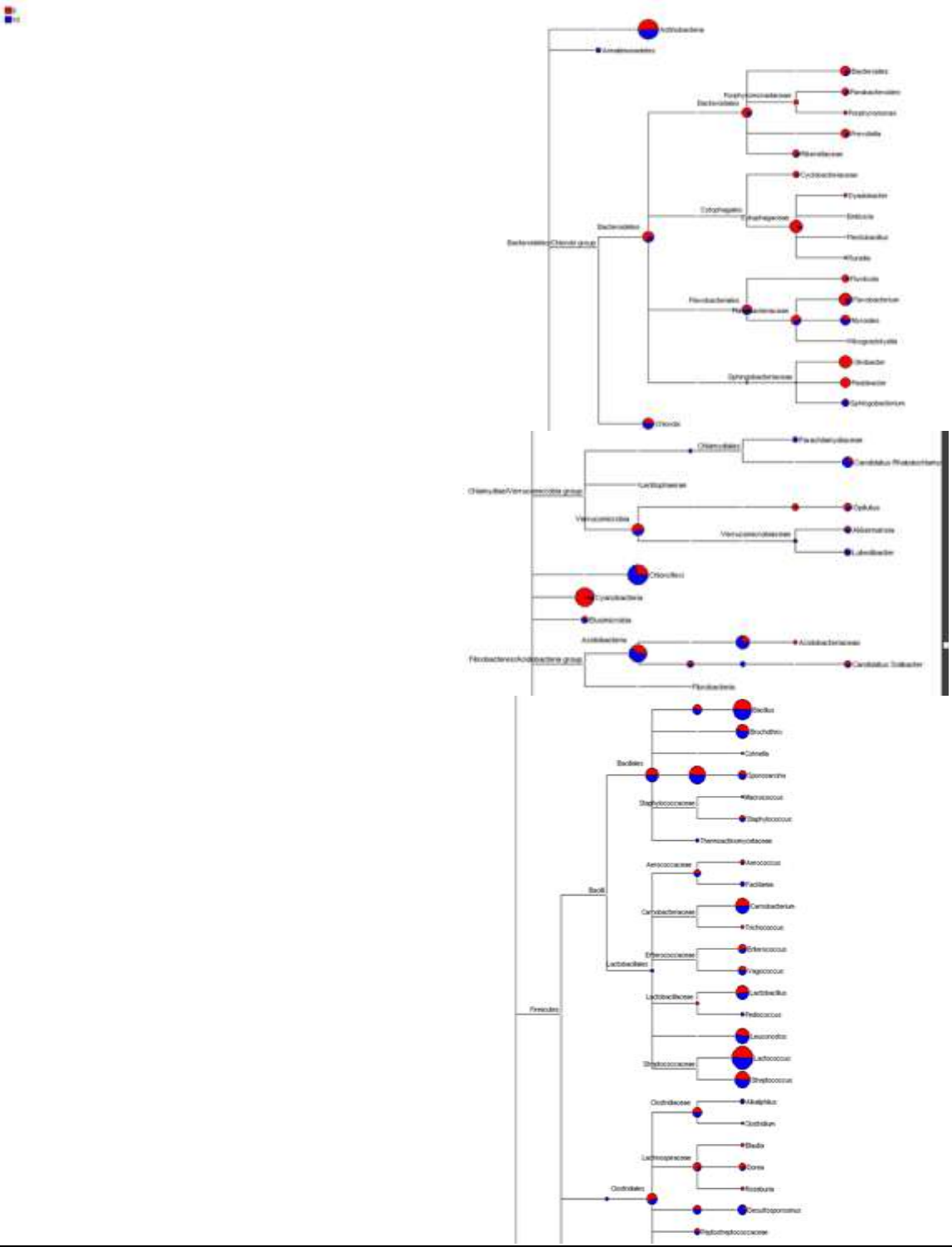
At the genus level, a total of 197 genus were identified from NHW1(Fig. 5). The 10 most abundant genus, containing more than 54% of the total sequences, were Lactococcus, Bacillus, Pseudomonas, Streptococcus, Leuconostoc, Carnobacterium, Ochrobactrum, Lysinibacillus, Streptomyces, Olivibacter.A total of 178 genus were identified from NHW2(Fig.5). The 10 most abundant genus,containing more than 56% of the total sequences, were Lactococcus, Bacillus, Pseudomonas, Streptococcus, Leuconostoc, Carnobacterium, Ochrobactrum,Lysinibacillus, Acinetobacter, Brochothrix.The figure shows that there five relative frequency in high level of the two soil samples in the classification level of Genus are Lactococcus, Bacillus, Pseudomonas, Streptococcus, Leuconostoc respectively over 1%,Above all of the five families in NHW2 are higher than NHW1; Carnobacterium, Ochrobactrum, Lysinibacillus are lower. Acinetobacter and Brochothrixin NHW2 are higher relative frequency than 0.5%, but not in NHW1. while those are lower than 1% in the NHW1; Rickettsiales is also above 1% in NHW1 but not in NHW2. Streptomyces and Olivibacter are opposite NHW1. There are Olivibacter, Pseudoxanthomonas, Promicromonospora,Glycomyces,Rhizobium, Sphingopyxis, Aquicella, Dyadobacter, HyphomonasandMarinobacter in sample NHW1, but no in sample NHW2; Rhodobacter, Novosphingobium, Oleibacter, Frigoribacterium, Cohnella, Megasphaera, Roseococcus, Tepidimonas, Tatlockiaand Arenimonasonly exists in sample NHW1.

According to analysis of the time pattern of five classification levels, the result indicated that theyhad different varying trends along the time. For example, the relative abundance of Cyanobacteria was higher in NHW1, but itsrelativeabundancewasdecreased in NHW2. The relative abundance of Chloroflexi showed a monotonous increase along the evolution, and Acidobacteria exhibited amonotonous increase with increase in evolution. Because of similar relative abundance of FirmicutesorProteobacteria in NHW1 and NHW2, their varyingtrends along the time were not obvious.To further determine the distribution pattern of bacteria diversity along the evolution, the alpha diversity was calculated by thenumber of OTUs and the Shannon-Wiener index. Betweentwo soil sample, the richness index ranged from 1125 to 1365, and the Shannon-Wiener index ranged from 3.61 to 3.76. The regression analysis indicated that both richness index and Shann on indexs how edadecliningtrendin evolution.

Neighbor-joining tree

The bacterial clone libraries obtained from samples NHW1 and NHW2 showed high diversity as is presented in a neighbor-joining tree of sequences in Fig. 6. Neighbor-joining trees of sequences from the 16S rRNA clone libraries, databases showing phylogenetic relationships. The main performanceis 11 Phylum categories, respectively Actinobacteria,Armatimonadetes,Bacteroideles,Chlamydiae, Chloroflexi, Cyanobacteria, Elusimicrobia, Fibrobacteres, Firmicutes, Gemmatimonadetes, Nitrospira, Plantomycetes, Proteobacteria, Spirochaetes, Candidatus and Caldithrix. Chlamydiales and Verrucomicrobia form a cluster; Acidobacteria and

Fibrobacteria form a cluster. Bacteroidetes, Pyadobacter, Emticicia, Runella, Winogrodskyella, Sphingbacteriaceae, Lentisphaerae, Lactobacillaceae, Blautia, Roseburia, Faeclibacteriam, Oscillospira, Ruminococcus, Achromobacterand Marinobacterare increasing in the NHW1,as it evolved in NHW2, it eventually disappeared, which exhibited strong negative correlations with the evolution, indicating that the environmental conditions may not benefit to the survival of bacterial species. Bacteroides, Parabacteroides,Porphyromonas, Sphingbacterium,Verrucomicrobia, Lactobacillus, Pediococcus, Agrobacterium, Roseococcus, Alcaligenes,have evolutionary trends. In particular, Flectobacillus, Clostridium, Hyphomicrobium, Roseococcus, Dechloromonasis only found in region NHW2 not found in NHW1, which exhibited strong positive correlations with the evolution, indicating that the environmental conditions may benefit to the survival of bacterial species. The number 9 represent NHW1 and 10 represent NHW2.



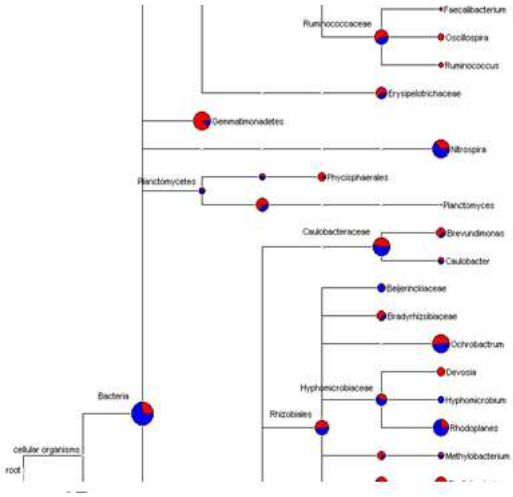


Fig.6 The neighbor-joining trees of the two soil samples bacteria

Clone libraries of the bacterial 16S rRNA genes in sample NHW1 and NHW2 and their closest known relatives are presented in a neighbor-joining trees of sequences which is summarized in Fig. 6 , databases showing phylogenetic relationships.

IV. Discussion and conclusions

In this study, westudy the microbial community composition and species diversity characteristics in different Nihewan archaeological. In order to clarify microbial community evolution.

A total of 113521 high-quality sequences were obtained from samples at the two stratums; the average number of reads per sample was 56761, and the read counts were greater than those in previous studies in NHW. Moreover, according to good's coverage index of each sample, the modified sequences were comprehensive enough to cover most bacterial diversity.

In this study, we taking soil samples in different geological Nihewan archaeological strata as the research object, using HTS technology to analysis the diversity of soil samples. Using NCBI online comparison to analysis the microbial community composition and species diversity characteristics in different Nihewan archaeological strata. Results show that we were obtained 1159 OTU, distributed in 34 Phylums, 86 Classes, 127orders, 167 families and 197 Genus, other 43 clones are in the unclassified status in NHW1; we were obtained 905 OTU, distributed in 33 Phylums, 77 Classes, 117orders, 153 families and 178 Genus, other 17 clones are in the unclassified status, accounted for 21.8% of the total number of clones in NHW2.Comprehensive analysis based on species Richness, Shannon index and Simpson index, demonstrating the there index of NHW1 are all higher than NHW2's, so NHW1 have higher value to indicates greater diversity.

At the phylum level, a total of 34 phylum were identified from NHW1 (Fig. 1). The 8 most abundant genera, containing more than 97% of the total sequences, were Firmicutes, Actinobacteria, Proteobacteria, Cyanobacteria, Chloroflexi, Bacteroidetes,Acidobacteria, GAL15. The two samples have similar advantage bacterium in the level of phylum. The fiture show that there three relative frequency in high level of the two soil samples are Firmicutes, Actinobacteria, Proteobacteria respectively, Firmicutesin NHW2 is higher; Cyanobacteria, Chloroflexi, Bacteroidetes in NHW1 are also higher elative frequency, while Chloroflexi is relatively lower in the NHW2 , and relative frequency of Bacteroidetes, Cyanobacteria are extremely low; In classification level of Genus, the figure shows that there five relative frequency over 1% in high level of the two soil samples are Lactococcus, Bacillus, Pseudomonas, Streptococcus, Leuconostoc respectively, Above all of the five families in NHW2 is higher than NHW1; Carnobacterium, Ochrobactrum, Lysinibacillus is lower. Acinetobacter and Brochothrixin NHW2 are higher relative frequency than 0.5%, but not in NHW1. while those are lower than 1% in the NHW1; Rickettsiales is also above 1% in NHW1 but not in NHW2. Streptomyces and Olivibacter are opposite in NHW1. There are Caldithrix, AD3, Fibrobacteres and Lentisphaerae in sample NHW1, but no in sample NHW2; SC4、WPS-2 and WS4 only exists in sample NHW1. The study found that there was a difference in the number of microbial species in the soil sample of the two different geologic time scale, and as a whole the species composition is consistent. Advantage bacterium group is roughly similar, their ability to cope with growth environment change is stronger, therefore, they can be more stable in the soil. And some of microbial is weak to cope with environment changes which can affect their survival. So there may be some factors which exist in the environment for a long term that affect the evolution of microbial, the ability of adapting the changing environment, creating a variety of microbial.

Microbial characteristics is the internal cause of affecting their distribution, environmental factors is

external factor, combination of internal and external causes that microorganism distribution in different environment has particular characteristics. Main environmental factors are associated microbial climate factors such as temperature, humidity, solar activity, organic matters such as PH, salinity, heavy metals , and environmental pollution caused by human activities and other interference events (such as flood, fire, etc.). These factors affect the microbial in different time and space scope, they plays a decisive role on microbial species and amount, and should not underestimate role in the eolution of microbial. So it is possible to study the relationship of environmental changes and the phenotype or its genotype. On a long scale, the evolution of microorganism can be linked to the larger scale of the environment. Lived in frozen soil environment, microorganisms have corresponding unique structure and biochemical characteristics to survive in the environment which absence of 'interference' for thousands of years or even millions of years. Relevant literature report: Forty-one representative bacterial bands were selected for sequeneing and phylogenetic analysis from permafrost. The phylogenetic trees placed these clones into seven major groups: Acidobacteria, Actinobaeteria,Gemmatimonadetes, Chloroflexi, Firmieutes, Proteobacteria and Baeteroidetes, including genera Gemmatimonas, Carnobaeterium, Bacillus, Acidobacterium, Arthrobacter, Pseudomonas, Rhodoplanes, Nordella, Herminiimonas, Deniratisoma, Ramlibacter, Flavobacterium,Thermoleiphilumand unidentified baeteria. Actinobaeteria, FirmieutesandProteobacteriaaremore stable in the soil, whether in theGlaeier, frozen soil or NHW, which are dominant bacteria. Other bacteria have their own characteristics under their respective environmental factors(Guozengwang et al. 2010).

The soil abiotic parameters have an important influence on bacteria community structure(Kmaresan et al. 2011). Showed that it is complex interactions of several abiotic factors rather than any singal factor that is responsible for microbial community in Nihewan(Kumaresanet al. 2011). Analysis of soil physical and chemical properties and species diversity of bacteria showed bacteria abundance at 5% significance level being driven by several abiotic factors, such as PH, OM, TN, TP, AN, AP, soil type and different geological age and so on(Meiruz,et al.). Different types of climate conditions such as temperature, rainfall, sunshine time, ultraviolet intensity all have influence on microbial. Temperature and water are the two most direct factors.Temperature affects soil microbial community composition directly or indirectly(Zhang W et al,2005).In recent years, many studies have shown that climate warming is the key factors of influencing ecological system and the important drivers of biodiversity loss; the temperature can cause the change of the microbial community(Hartley I P et al, 2008).the long-term warming can significantly reduce bacterial community(Deslippe J R et al, 2012). Soil pH is the key factor of biological geographical distribution of the soil bacteria(Baker K L et al, 2009; Jesus E D et al,2009; Jones R T et al, 2009; Lauber C L et al, 2009; Chu H Y et al, 2010). In recent years a large number of experiments have proved that the soil organic matter content is the main factors that influence the soil microbial community structure and distribution(Fierer N et al,2007; Philippot Let al, 2009; Goldfarb K C et al, 2011). Experiments show that soil bacteria diversity increases with altitude in the rocky mountain(Bryant J A et al,2008).

In the past, soil bacteria were mainly studied through conventional separating techniques and identification methods. With the rapid development of molecular technology, metagenomics technologies by directly extracting DNA from environment samples had been widely applied to study microorganism. Currently, the High-Throughput sequencing could be used to analyze the diversity, composition, structure and dynamics of microbial communitiesin different habitats. High-throughput sequencing technologies used in the study of environmental microbial community structure has obvious advancement and advantages, it can be read quickly and easily in a sample of complex microbial structure(LI Ret al,2010; YANG F et al, 2012). With the development of the high-throughput sequencing technique and bioinformatics, much progress has been made in observations of microbial diversity(Xiangzhen Li et al,2016).However, information is still lacking on the relationship between microbial community succession and multiple environmental changes resulting from climate change. The contrasting results require further studies to examine the effects of environment on the microbial community structure and the mechanisms involved.

conflict of interest

We declare that this article does not involve any conflict of interest

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Liping Zhang" Analysis of Microbial Diversity in different periods of Nihewan Using High-throughput Sequencing" International Journal of Engineering Science Invention (IJESI), vol. 07, no. 12, 2018, pp 72-81