International Journal of Engineering Science Invention (IJESI)
ISSN (Online): 2319 – 6734, ISSN (Print): 2319 – 6726
www.ijesi.org ||Volume 7 Issue 12 Ver. IV || Dec 2018 || PP 01-10

The Diversity of Actinobacteria in Nihewan Basin

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***Abstract**: Actinobacteria diversity was investigated in Nihewan basin by using two pair of Actinobacteria-specific primers for 16S rDNA clone library. The 8 different longitudinal archaeological sites we choose from Nihewan vary from 1,660, 1,640, 1,400, 1,320, 1,100, 900, 200 to 100 thousands year. We obtained 937 Actinobacteria positive clone, and 733 positive clone has the taxonomic status of Actinobacteria, distributed in 4 subclasses, 5 orders, 9 suborders, 25 families and 45 genera, other 204 clone are in the unclassified status, accounted for 21.8% of the total number of clone. Comprehensive analysis based on Species richness, Shannon index and Simpson index, demonstrating the species abundance among 8 sample, and the highest Actinobacteria diversity were in sites of 1,400 thousands year while lowest in 1,660 thousands year. Furthermore, Nocardioides, Corynebacterium and Dietzia decreased from 1660 thousands year ago to 100 thousands year ago. While Aciditerrimonas, Conexibacter increased from 1660 thousands year ago to 1320 thousands year ago, however, the abundance decreased from 1320 thousands year ago to 100 thousands year ago, excepting the site of 200 thousands year ago. It is been studied that, the structure of microorganism may be linked with the environmental change.*

***Keywords:** community structure; 16S rDNA clone library; species diversity*

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

I. Introduction

Actinobacteria is an important and functionally diverse group of organisms that are known to be involved in carbon cycling and nutrient transformation in soils[24]. Nowadays, the microbial community composition is among the most important factors to consider, as they are responsible for most transformations and drive the development of stable and labile carbon and nutrient pools in soil(Schulz S, et al. 2013). Evidence is accumulating that microbial communities are fundamental difference in the composition of soil[28 30 38], marine[8 12], forest[13], freshwater[11 16], permafrost[43 40] and grassland [29 23] . One conspicuous group among the all the microbial, Actinobacteria is a group of gram positive bacteria with high DNA G+C content over 55%. More than 70% of bioactive compounds produced by Actinobacteria have the potential to be applied in pharmacy, industry, agriculture, and environment [9]. This role in sustaining soil processes reflects their metabolic diversity, their ability to produce secondary metabolites and their mycelial growth habit which, like soil fungi, makes it possible to explore the bulk soil in search of water and nutrients [25]. Members of Actinobacteria are also able to maintain metabolism and DNA repair at a low temperature and degrade complex organic matter in soi [5 42], The unique capabilities of Actinobacteria explain their prevalence in our study area. Furthermore, significant improvement in the resolving power of molecular tools have helped the reemergence of the field of microbial biogeography. Molecular genetic[14] evidence collected primarily over the past decade has shown that the relative abundances of most microbial taxa vary rather predictably with habitat, environmental conditions, and seasons.

To meet a better understanding about Actinobacteria requires a better understanding of how environmental factor function and ultimately, the ability to engineer diversity: function relationships so as to maintain and even enhance key system processes. The first paradigm in microbial biogeography, "everything is everywhere, but, the environment selects" was offered by Baas Becking (1934) more than 70 years ago [37] and his adage continues to be cited in nearly every recent publication on microbial biogeography. More specificly, studies showed that, pH[22], Organic matter [36], TN [29] and various other factors [27] may affect community composition of Actinobacteria in different environment. While, all in all, these studies focused more on Actinobacteria diversity among space changes, few studies learn about it adding the factor of time.

Nihewan, located in the east of Yangyuan country, Zhangjiakou city, Hebei province, and the north shore of the Sanggan River. This site could almost be paralleled with Olduvai gorge where known as the origin of human and located in the east Africa. The sample we collected from the different longitudinal archaeological sites separately, vary from 1,660, 1,640, 1,400, 1,320, 1,100, 900, 200 to 100 thousands year. The soil is

aggregated as stone under the pressure of gravity which means all microbial communities were in their dormancy and maybe does not influenced by climate dynamics outside after a few years' deposition. As special as our sample, the wide diversity of physical and chemical conditions that exist in Nihewan provides the possibility of riches for diversity microorganisms and novel Actinobacteria. All communities here that shared a common history with a given long time durable influence when compared to communities in other habitat. Based on the special soil condition in Nihewan Basin, the composition of microbial in this site has gone through thousands of years' continuous impact and never change.

Discriminative ecological factors also explained a large amount of the variation within Actinobacteria [7]. It is complex interactions of several abiotic factors rather than any singal factor that is responsible for microbial community. Here, the links between the measured ecological factors and the relative abundances within Actinobacteria categories at family level were investigated through RDA. In this study, we obtained 937 Actinobacteria positive clones by using Actinobacteria-specific primers for gene clone library from Nihewan basin, we investigated temporal dynamics of Actinobacteria composition to delineate the patterns in Nihewan, that's it, how Actinobacteria communities vary along diverse environmental gradients in special environments in Nihewan.

II. Methods

Sample Collection

Soil samples were collected from various locations in the Nihewan, October 9 to 10, 2013. Descriptions of soil collection sites are presented in Table 1. By setting up geological profiles, the geological layer was selected from the sites according the representative of the ancient site of the typical characteristics. In each of the 8 sites, there were 3 randomly selected 20 m × 20 m replicate plots and 20 soil samples collected randomly from each geological layer, which extended to a depth of 1 m and the 20 soil samples were composed together. 1 kg soil removed from the composited soil sample immediately and placed on dry ice, shipped back to the laboratory preserved at - 80 ℃. By pooling the 20 soil cores, we aggregated spatial heterogeneity at the scale of individual site. The methods for determination of soil physical and chemical properties showed in table 2

Number	Site	Geologic Age （thousands year）	Soil Type	Climate Type
Nhw1	maquangouⅢ	1,660	Silt （tan silty sand）	Warm and humid
Nhw2	maquangou Ⅱ	1,640	Silt and sand （ sallowness and celadon clayey silt）	Warm and humid
Nhw3	maquangou	1,550	loess （yellowish gray soil）	Cool and cool semi-humid
Nhw4	Banshan	1,320	Silt （Gray clay）	Cool and cool semi-humid
Nhw5	Tusijiao	1,100	Silt and clay （Grayish yellow clay silt）	Mild and humid

Table 1. The source information of the soil samples

physical and chemical factors	methods
pH	Potentiometric method
Organic matter(OM)	Potassium dichromate volumetric method
Total nitrogen(TN)	Potassium Dichromate - Sulfuric Acid Digestion
Hydrolyzable nitrogen(HN)	Alkali diffusion method
Total phosphorus(TP)	Sulfuric acid - perchloric acid digestion method
Available phosphorus(AP)	Sodium bicarbonate method

Table 2. The Methods for determination of soil physical and chemical properties

DNA Extraction Methods

We design a kind of soil pretreatment method to improve the proportion of Actinobacteria DNA. Air dried 8 g soil sample were treated by 120℃ for 2 hour. After pretreatment of soil samples, and centrifugal washing two times with TENP buffer（50mmol/L Tirs, 20mmol/L EDTA, 100mmol/L NaCl, 0.01g/mL PVP, pH 10）, The total soil DNA was extracted by SDS-phenol chloroform extraction method,The yield and integrity of the environmental DNA obtained were confirmed through electrophoresis in 1.5% agar gel.

Construction of 16S rRNA Gene Libraries

The purified DNA was used as a template to specifically amplify 16S rRNA gene fragments, a ~1500bp region using the bacteria-specific primers (Lane, 1991): 27F (5-AGAGTTTGATCC/ATGGCTCAG-3) and 1525R (5-AAGGAGGTGA/TTCCAA/GCC-3). The size and quality of the resulting PCR products was confirmed by agar gel electrophoresis (2.0 % agar). They were then cloned into the pMD18-T linear plasmid vector (Takara Bio Group, Code D101A) and then into E. coli DH5 a competent cells(Takara Bio Group). Grown overnight after the transformation, single-clone colonies were picked up with sterile toothpicks and transferred into LB broth containing ammonium (100μg/mL). Cultured at 37℃, 180 r/min cultured for 6-8 h。 Picked 1,500 ~ 2,500 clones randomly to construct 16S rDNA gene library.

Primer	Sequence (5'-3')
Act-20	CGCGGCCTATCAGCTTGTTG
Act-19	CCGTACTCCCCAGGCGGGG
Com2xf	AAACTCAAAGGAATTGACG
Ac1186	CTTCCTCCGAGTTGACCC

Table 3. Ribosomal primers used in this study

Amplification and Sequencing of Actinobacteria 16S rRNA Genes

The primer Act-20, Act-19 Com2xf, Ac1186 (Table 3) was designed to target all sequences in the phylum Actinobacteria[35 31]. The success of PCR reactions were determined by subjecting the amplified products to 1% agar gel electrophoresis. All positive clones full sequenced using (Aokedingsheng Biological Technology Co., Ltd, Beijing). The comparison of the 16S rDNA sequence and the GenBank database was carried out to compare the species diversity analysis and comparison in each clone library.

Phylogenetic Analyses

The 16S rRNA gene sequences were taxonomically assigned using the Naïve Bayesian rRNA classifier of the Ribosomal Database Project II (RDP; [41]. Sequences from this study were subsequently aligned using the ClustalW multiple alignment tool from BioEdit v7.0.5.3. The program DNADIST v3.5c in BioEdit was used to compute a distance matrix from the aligned nucleotide sequences. The distance matrix was input into the DOTUR program (v1.53) to assign the sequences to operational taxonomic units (OTUs) using the furthest-neighbor clustering algorithm[32] at 97, 95, and 90% identities. Sequences from each clone library were aligned separately, and OTUs were identified at 97% identity. One representative sequence was selected for each OTU. Representative sequences from each OTU (97%) in 8 libraries determined in this study were deposited in the NCBI database under accessions No. KT905437-KT905868. The OTUs were used to depict the similarities and differences between different communities at 8 samples. Rarefaction curves were produced by standard calculations by comparing the total number of clones obtained to the number of clones representing unique OTUs. Alpha diversity Shannon index, Simpson's diversity index, as well as species evenness and species richness for the clone libraries were calculated using the DOTUR program, summarized in Table 4. The observed species richness is affected not only by the number of individuals but also by the heterogeneity of the sample. Species richness [17] is the number of different species represented in an ecological community, landscape or region And the index is simply a count of species, does not take into account the abundances of the species or their relative abundance distributions. Species diversity takes into account both species richness and species evenness. Principal coordinate analysis (PCoA), which was used to measure dissimilarity at genus distances based on Uni Frac analysis, was performed with QIIME and visualized using KING[21]. PCoA based on weighted (PC1 and PC2 represented 81.1 % and 14.9 % of the variance) (Figure 2) and unweighted (PC1 and PC2 accounted for 24.8 % and 14 % of the total variance) Uni Frac values showed that the bacteria communities of NFYM largely overlap between two different sampling groups. However, MANOVA based on the principal coordinates of the unweighted Uni Frac metric showed that the difference of bacterial community structure between the two groups was significant ($P < 0.05$). The unweighted pair-group method using arithmetic means(UPGMA) (Figure 2) with the Uni Frac analysis indicated there was a discriminative trend in NFYM from the two sampling groups, although some samples could not be distinctly separated.

Number	Geologic Age（thousands year）	OTU	positive clone	Ratio of uncalssified clones	Diversity index			
					Shannon_H	Simpson_D	Species evenness	species richness
Nhw1	1,660	34	131	3.0	2.795	0.888	0.481	6.727

Nhw2	1,640	39	99	5.1	3.246	0.948	0.658	7.525
Nhw3	1,400	46	70	49.3	3.580	0.962	0.780	9.133
Nhw4	1,320	41	95	18.1	3.249	0.944	0.628	8.145
Nhw5	1,100	45	84	29.4	3.375	0.946	0.649	9.207
Nhw6	900	28	62	26.2	3.081	0.936	0.778	6.094
Nhw7	200	26	66	26.7	3.046	0.940	0.809	5.556
Nhw8	100	34	78	22.0	2.981	0.900	0.579	7.166

Table 4 Actinobacteria sequencing statistics and alpha diversity measures of soil samples of Nihewan

Environmental Variables and Multivariate Statistical Analysis

Environmental characteristics contain some index: a biogeochemical data set composed of factors, The biogeochemical data matrix included soil pH and total nitrogen (TN); total phosphorus (TP); available phosphorus (AP); available potassium (AK); organic matters (OM). The phylogenetically divergent lineages coexisting in the community may give diverse responses to the local environmental characteristics. To test this, Redundancy Analysis (RDA) was conducted with the software Canoco 4.5[3], exploring the relationships between relative abundances and environmental variables. Furthermore, it is a choice of method for identifying important environmental variables to determine community structures in ecological data sets, assuming linear species response to the underlying environmental gradient[26]. Here, it was carried out to determine the relationship between the Actinobacteria community and the environmental factors.

	pH	OM	TN	TP	AN	AP	AK
pH	1						
OM	-0.4213	1					
TN	-0.2775	0.4772	1				
TP	0.2379	-0.5363	-0.2067	1			
AN	0.0815	-0.013	0.3901	-0.4008	1		
AP	0.007	-0.3805	0.4266	0.5646	0.243	1	
AK	-0.1743	-0.1728	-0.5241	0.1363	-0.3272	-0.2597	1

Table 5. The relevance of eight Nihewan soil chemical and physical factors

(a)TP: total phosphorus (b) AP: available phosphorus (c) TN: total nitrogen (d) AK: available potassium (e) OM: organic matter (f) AN: Available nitrogen

III. Result

Testing of an Actinobacteria Primer System

The Actinobacteria specific primer system detected 937 positive clones from the 16S rDNA clone library among the 8 samples. And the results show in table 4. To determine the validity and specificity of the primer system, all clones in the library were sequenced and classified. 733 positive clones were Actinobacteria belong to 45 known genera and 204 unclassified clones.

Soil Actinobacteria community structure at Station in the Nihewan

Soil samples from 8 stations were treated at 120℃ for 2 h, then centrifugal washing two times with TENP buffer then the bacteria 16S rDNA clone library was constructed. We randomly selected 1000-1500 clones (for sufficient Actinobacteria coverage) from each station to detect Actinobacteria using two Actinobacteria-specific primer sets. From the 1000-1500 clones generated, the positive clones showed in table 4. Depending on the result, the number of OTUs defined positive clone of Actinobacteria among total clones varied from 62 to 131 grouped at the 97％ similarity level. A total of 293 OTUs were presented in the 8 clone libraries. The highest number of OTUs was found in the Nhw3, while the lowest OTUs was found in the sample Nhw7(table 4).

Rarefaction curves for (a) observed Actinobacteria clone and (b) OTUs for Actinobacteria are shown in Figure 1. Although the rarefaction curve is not parallel with the x-axis, the Simpson diversity index reached saturation, suggesting that some additional phenotypes could be added with additional sequencing, but the great majority of Actinobacteria diversity was captured. Meanwhile, the Shannon diversity and observed species indices were applied to measure Actinobacteial sequence abundance and diversity among 8 samples.

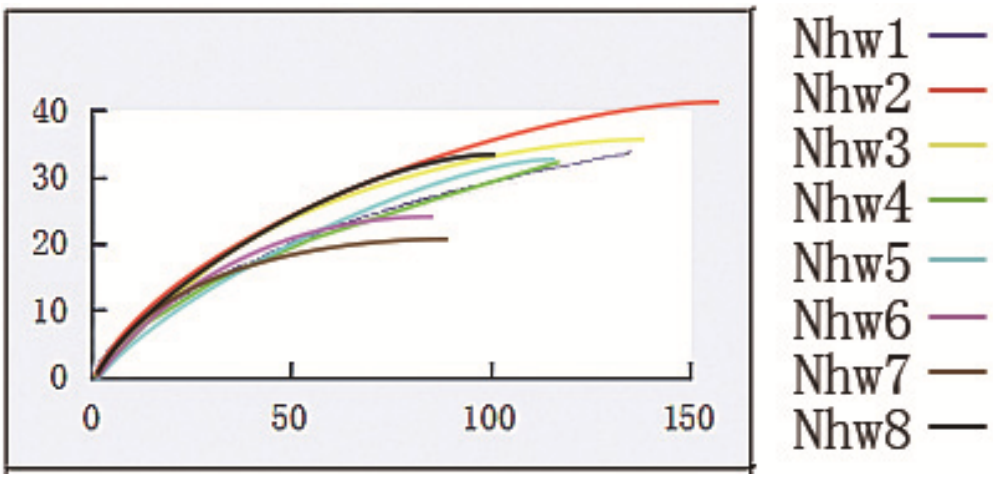

Figure1. Rarefaction curves for clone and culture libraries

To further determine the distribution pattern of Actinobacteria diversity along the different Nihewan archaeological strata for thousands of years, the alpha diversity was calculated by the number of OTUs and the Shannon-Wiener index[18]. The proportion of unclassified positive clones varies from 3.0 to 49.3. Percentage coverage showed that Nhw3 had potentially the most undetected OTUs (49.3% coverage). The Shannon indices collected in different sites of geological age ranged from 2.795 to 3.580(Table 4) and the Simpson indices ranged from 0.888 to 0.962. Diversity estimates, Shannon_H and Simpson_D, indicated that Nhw3 were more diverse than the other sites, while the abundance of Actinobacteria in Nhw1 contains the lowest OTUs. Species Richness index indicated Nhw3 covers sufficient Actinobacteria community.

A total of five different orders were detected in the complete data set, with the abundance of Acidimicrobiales, Actinomycetales, Gaiellales, Solirubrobacterales and Euzebyales varies in 8 samples. The dominant bacterial Actinomycetales accounting for 97.74%, 99.35%, 43.00%, 50.00%, 100%, 89.12%, 91.20% of the total sequence respectively, except Nhw7(35.00%). Interestingly, Euzebyales was specific exist in Nhw7.

At the genus level, members of the genera Rhodococcus, Microbacterium, Gaiella, Saccharopolyspora, Microbacterium, Amycolatopsis, Conexibacter and Amycolatopsis, were most frequently detected (49.18, 25.68, 28.57, 35.79, 27.38, 54.84, 39.39,and 56.41% of the sequences, respectively), not the universal Streptomyces. These findings provided data for resource exploitation.

More specificly, Agromyces, Ilumatobacter, Agrococcus and Microlunatus were the unique genera in Nhw1； Aeromicrobium, Jiangella, Brachybacterium, Cellulosimicrobium, Georgenia, Agromyces and Nocardia were specific genera in Nhw2; while Iamia and Mycobacterium were special genera in Nhw 3； Couchioplanes only exist in Nhw4； Mobilicoccus , Pseudoclavibacter and Plantibacter only found in Nhw5； Euzebya was only belong to Nhw7； Acidothermus and Alloactinosynnema derived from Nhw8.

The results on the relevance of soil physical and chemical factors in Nihewan calculated by CANOCO version 4.5.1, shown in Table 5. Organic matter (OM) was negatively correlated with total phosphorus (TP), available phosphorus (AP) and available potassium (AK), and was positively connected with total nitrogen (TN) , while the pH was negatively correlated with the content of organic matter (OM) and total nitrogen (TN) available potassium (AK).

We further used a phylogeny-based metric, Uni Frac, to assess the differences among microbial communities. Figure 2 shows the distribution of PC scores on the first two axes. PC1 explained 62% of the variance in Actinobacteria composition, while PC2 explained a further 11%. As shown in Figure 2, the Nhw1, Nhw3, Nhw6, Nhw7 and Nhw8 scattered on the left, while Nhw2 and Nhw4, Nhw5 gathered respectively on the right of the coordinate axis, furthermore, from the perspective of PCoA2, Nhw2, Nhw6, Nhw8 scattered on the above of the table respectively, while Nhw1, Nhw3, Nhw4, Nhw5 on the following one, more interesting one, Nhw7 just stand on the axis of PCoA2.

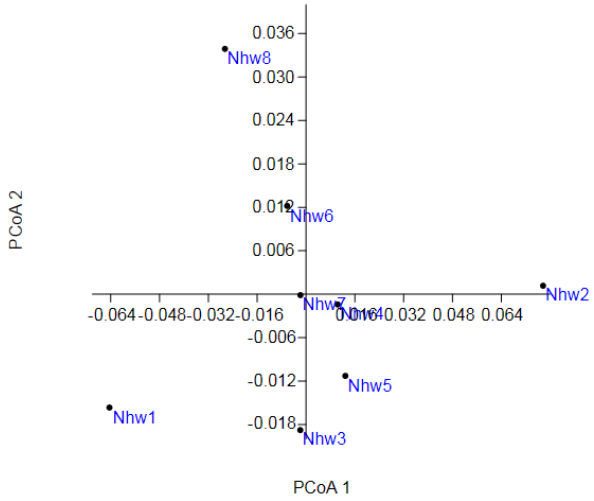

Figure 2. The principle coordinate analysis(PCoA) of Actinobacteria composition among eight samples in Nihewan sites.

Influence of environmental variables on specific lineages

A modified test based on some kinds of components was applied to study the effect of environmental variables on Actinobacteria community composition of the taxonomic groups listed in Figure 3. It revealed and confirmed dramatic differences in the abundance of specific Actinobacteria community members in response to environmental variables.

Our RDA examined the significance of pH, OM, TN, TP, AN, AP,AK in relation to Actinobacteria community structure in Nihewan. These 7 significantly correlated variables accounted for some specific Actinobacteria species in Nihewan. We divided them into 3 clusters. The first group was mostly positive affected by MT, TP and consisted of Rarobacteraceae, Actinospcaceae, Conexibacteraceae, Cryptosporangiaceae, Thermomonosporaceae, The physiological features of the second group, which included members of the family: Streptomycetaceae,Nakamurellaceae,Intrasporangiaceae, Nocardiaceae, Pseudonocardiaceae, Microbacreriaceae, Acidimicrobiaceae,Streptosporangiaceae,Nocardioidaceae,Micrococcaceae,Kineosporiaceae,Solirubrobacteraceae, Propionibacteriaceae, Acidothermaceae, Rubrobacteraceae, Micromonosporaceae, Geodermatophilaceae, were probably different from those of the first group, these family more related with pH, AP, OM and TN. Jiangellaceae, Promicromonosporaceae, Acidimicrobineae, Iamiaceae were devided into cluster 3. Specifically, available pH concentrations were highly positively correlated with the abundance of Streptomycetaceae, Nakamurellaceae and which had a negative correlation with Acidimicrobiaceae, Jiangellaceae, Promicromonosporaceae; OM was positively related with Propionibacteriaceae, whereas had a negatively related with Cryptosporangiaceae; TN was positively related with the Micromonosporaceae, Rubrobacteraceae and was negatively related with Rarobacteraceae, Conexibacteraceae, Actinospcaceae.

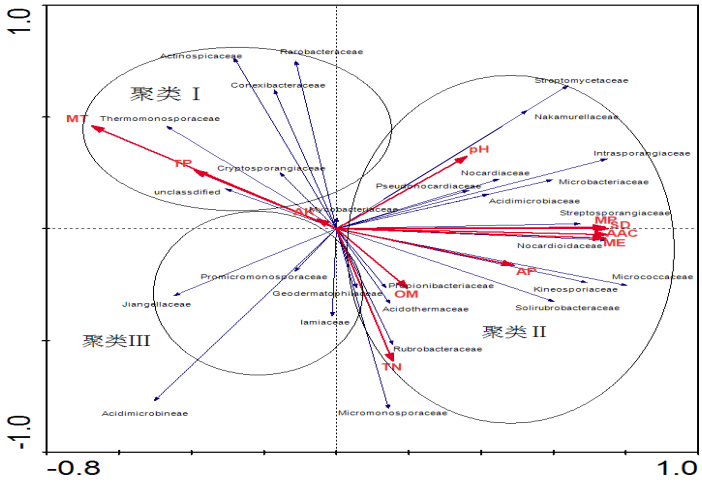

Figure 3. RDA diversity analysis ordination diagram of soil Actinomycetes

(a) TP: total phosphorus; (b) AP: available phosphorus; (c) TN: total nitrogen; (d) AP: available potassium; (f) OM: organic matter; (g) AN: Available nitrogen; (h)MP: annual mean precipitation; (i) SD: annual mean sunshine duration; (j) AAC: mean active accumulated temperature(>10℃); (k) ME: mean sea level elevation

IV. Discussion

At present, soil microbial diversity patterns along ecosystem gradient had been reported a lot, including agricultural soil[39], extreme environment[33], and marine microbial diversity[4]. However, only few studies focused on Actinobacteria changes along the different geological era in Nihewan basin. Existing studies have shown that the diversity of Actinobacteria determined that they could adapt any extremely environments, such as cold, anaerobic, poor nutrition, etc [2]. and is a kind of important organism mediating plant litter decay and the subsequent formation of soil organic matter in terrestrial ecosystems [6].

Whilst Actinobacteria are generally considered as soil-dwelling bacteria, it remains unclear what kind of environmental factors play a more part in determine their community structure. Understanding Actinobacteria distribution in the environment is important in understanding their ecological role and for pharmaceutical bioprospecting. In this study, Based on 16S rDNA full sequence alignment analyzed among 8 clone libraries found, at least 18.1％ unclassified sequence clone among each clone library besides in Nhw1 and Nhw2. The result demonstrates that composition of Actinobacteria exist in Nihewan, but also contain much untapped potential Actinobacteria resources in Nihewan.

1000-1500 positive clones of the 16S rRNA gene library of the Nihewan were analyzed, and the positive clones were amplified by two pair of Actinobacteria specific primers, obtained 937 positive clones. Based on the sequencing analysis of 8 full sequential clone library 733 positive clones has the taxonomic status of Actinobacteria, distributed in 4 subclasses, 5 orders, 9 suborders, 25 families and 45 genera, other 204 clones are in the unclassified status, accounted for 21.8% of the total number of clones.

These clones were clustered into operational taxonomic units (OTUs) at the 97% similarity level and analyzed using rarefaction curves to demonstrate the library size is large enough to cover all kinds of Actinobacteria community in 8 sites. In this paper, comprehensive analysis based on Species richness, Shannon index and Simpson index, demonstrating the species abundance of sample. The highest Actinobacteria diversity (Shannon_H index=3.580, Simpson index=0.962) was at Nhw3 whereas the lowest was measured at Nhw1(Shannon_H index=2.795,Simpson index=0.888). From the result above, the estimated coverage of each clone library were moderately high, ranging from 62 to 131. Different archaeological, sample's environments and that they are rich sources of both novel species and abundant of Actinobacteria.

From the perspective of era, result showed that, the number of Micrococcus and Microbacterium change massively in sample of Nhw2(1640 thousands year ago) and Nhw5(1100 thousands year ago), in other sample, decreased with the passage of time. Aciditerrimonas, Conexibacter increased from Nhw1(1660 thousands year ago) to Nhw4(1320 thousands year ago), which means the number of these two genera increased from Nhw1(1660 thousands year ago) to Nhw4(1320 thousands year ago), however, the abundance decreased from Nhw4 (1320 thousands year ago) to Nhw8 (100 thousands year ago), excepting Nhw7(200 thousands year ago). The number of Nocardioides, Corynebacterium and Dietzia decreased from Nhw1(1660 thousands year ago) to Nhw8(100 thousands year ago). So, what made these different? It is been studied that, the structure of microorganism may be linked with the environmental change. Ao H, et al, [1]demonstrated that, Nihewan, as the accepted standard of quaternary strata in the world, environmental change can be divided into four stages, including has not yet been established stable waters and mesomorphic classes-stage I, stabled water which contain a little salinity-stage II, the basin began to shrink, and the climate shifted from warm to semi-arid-stage III and the lake was is further salted, and that climate is further dehumidify by warm humidification to semi-arid development, and finally dries out-stage IV. To some extent, the dynamic environment may be the reason why the confused Actinobacteria change in era dimension.

Apart from environmental change, it can be seen from the soil type that, the soil type of Nhw3 is loess, high quality as it is, contains not only three layers including soil humiliation layer, leaching layer, deposition layer, but also the unique quality nutrition which other type of soil could not have. For example, the content of organic matter,carbonate minerals much more abundance comparing with other soil type. Above all, the soil samples of Nhw3 are more familiar with the modern soil type, which may be more suitable for the survival of Actinobacteria. While the soil type of Nhw1 is Silt（tan silty sand）which much more barren. Unclassified clones varied in 8 samples, from 4 in Nhw1 to 68 in Nhw3, The preliminary investigation of terrestrial soils from Nhw3 demonstrated that there is much untapped potential Actinobacteria in Nihewan. The vast majority of OTUs are composed of sequences from one or two samples, completely different as indicated results. While, some specific OTUs were presented in all samples. These observations may demonstrated, the Actinobacteria diversity in Nihewan needs to be learn, clear distinction between samples from each ample remain to be analyzed.

Then, data for the Actinobacteria community were analyzed at the order and genus levels. From the

statistics of the species and number in different Actinobacteria taxa in eight Nihewan clone libraries, Acidimicrobidae, Actinobacteridae, Rubrobacteridae, Solirubrobacterales, Nitriliruptoridae were detected in Nihewan site, but the above groups in the eight samples of the proportion is slightly different. Acidimicrobidae, Actinobacteridae, Rubrobacteridae, were judged to be major components of each library since they constituted a large proportion of the total clones of a given library. However, the number of Solirubrobacterales is much more abundance than Actinobacteridae in Nhw7. Nitriliruptoridae were only found in Nhw7. They were identified as contributing substantially to the relative abundance of Actinobacteria. Actinobacteridae was dominant and was found in each library.

Moreover, most of the order currently contain only a single family, most family contain only a single genus. This results reinforce the fact that enormous influence of environment on Actinobacteria, leading to the abundance varies. In different geological age sample, shows some specific factors influence the vary number of specific Actinobacteria.

The dominant genus varies in different samples. Rhodococcus was unique in Nhw1，accounting for 44.4%; Microbacterium was the dominant species in Nhw2 for 24.4%; Gaiella for 14.6% in Nhw3; Saccharopolyspora in Nhw4 occupying for 29.3%; Nhw5, unique species was Microbacterium for 19.3%; Amycolatopsis both in Nhw6 and Nhw8，accounting for 40.5% and 44.0%; Last one sample Nhw7, Conexibacter for 28.9%;

Soil pH was the best predictor of variability in diversity levels within each of dominant phyla[22]. The effects of soil pH on the relative abundance of Actinobacteria vary in different paper nowadays. Lauber C L, et al. demonstrated the abundances of Actinobacteria positively correlated with soil pH, while in this paper, the first group we analyzed had a strongly positive correlation with pH, completely contrast with the second group(Figure 3). then we can safely conclude that the influence of pH varies on Actinobacteria species. More specifically, In Nhw1, Streptomycetaceae were the main genotypes. The indices of pH, were the main driving factors for this genotypes. Therefore, Jiangellaceae and Micrococcaceae may be the main contributors to the Actinobacteria community structure of Nhw2 and may be driven by the factors of pH. Nocardiaceae is among all eight samples, had a positive correlation with pH. It can been seen, pH in particular have not only changed the community structure but also the taxonomic diversity of Actinobacteria in these soils.

Although pH has been found to be the prevailing environmental factor in shaping soil bacterial community compositions, other factors were also shown to mediate the geographic distribution of the microbial communities in various environments. Microbial biomass was also strongly correlated with OM. Furthermore, our study demonstrated that most Actinobacteria remain had a positive abundance with OM, the certain group(Thermomonosporaceae, Cryptosporangiaceae, Actinospcaceae and Conexibacteraceae) of relative abundance of Acidobacteria decreased with increasing OM, while the relative abundance of Acidobacteria(Propionibacteriaceae, Acidothermaceae and Rubrobacteraceae) increased with soil OM. Futhermore, Sasha Jenkins, et al. demonstrated Arthrobacter and Micrococcus more abundant in soils receiving organic inputs.

Consistent across nearly all soils, N addition decreased microbial respiration rates [29], with an average decrease of 11% over the year-long incubation, and decreased microbial biomass by 35%. Ramirez K S et al. found, N addition increased the relative abundance of Actinobacteria. In our study, from the family level of Actinobacteria, interestingly, while Acidimicrobinese increased with TN , Streptomycetaceae have no correlation with TN, while the abundance of Actinospicaceae totally negative with TN. This is different from the results of Jenkins S N, et al., who showed that TN with an increase in the relative abundance of acidophilic taxa such as Acidimicrobium, Streptomyces and Actinospica [19]. Corynebacteriaceae mainly existed in Nhw3, while negatively correlated with TN, maybe demonstrated that Corynebacteriaceae may be inhibited by a kind of substance in Nhw3 correlated with the contents of TN. TN were the diving factors for Micromonosporaceae, mostly discovered in Nhw7, whereas were strongly negatively with the first group mostly. While the first group mainly correlated with TP, AK and MT positively. Then we can safely conclude that, to some degree, the indices of TN were negatively correlated with TP, AK and MT.

These observation is unfamiliar with other studies partly, while still shows the chemical and physical factors to be generally correlated with the structure and composition of the microbial communities across geographic scale[20 34 15 10]. Above all, surveys nowadays must put mostly concentration on the Actinobacteria abundance from a low level, such as family.

Combined the above analysis, pH and OM were the common important environmental factors that impact Actinobacteria, and the environmental change during quaternary strata and its special soil type in 8 sites. The variation of soil nutrient contents may largely had impact on the community structure of Actinobacteria. Our study mainly analyzed results show that, the way by using two specific Actinobacteria primers to improve methods for detection and identification of Actinobacteria could reflect the community composition, to some extent, but still needs to fix for it could not cover all Actinobacteria in sample; on the other hand, although pH and OM appears to be a driver of many patterns in soil microbial diversity, the influence of other special

samples will need to be understood in order to develop a more completely model that accurately predicts soil microbial community structure across larger spatial scales. It highlights the need to address dynamics at the family levels to gain insights into the factors regulating distribution and composition of Actinobacteria.

Compliance with Ethical Standards

Policy and ethics: This article does not contain any studies with human participants or animals performed by any of the authors

Conflict of Interest: We declare that we have no conflict of interest.

Funding: This study was funded by National Natural Science Foundation of China (No. 31370061).

Reference

[1]. Hong Ao, Chenglong Deng, Mark J. Dekkers, Youbin Sun, Qingsong Liu, and Rixiang Zhu, 'Pleistocene Environmental Evolution in the Nihewan Basin and Implication for Early Human Colonization of North China', Quaternary International, 223 (2010), 472-78.

[2]. H. F. Arocha-Garza, R. Canales-Del Castillo, L. E. Eguiarte, V. Souza, and S. De la Torre-Zavala, 'High Diversity and Suggested Endemicity of Culturable Actinobacteria in an Extremely Oligotrophic Desert Oasis', PeerJ, 5 (2017), e3247.

[3]. Ter Braak, and P. N. Smilauer, 'Canoco Reference Manual and Canodraw for Windows User"S Guide: Software for Canonical Community Ord', Ithaca Ny Usa Www (2002).

[4]. M. V. Brown, M. S. Schwalbach, I Hewson, and J. A. Fuhrman, 'Coupling 16s-Its Rdna Clone Libraries and Automated Ribosomal Intergenic Spacer Analysis to Show Marine Microbial Diversity: Development and Application to a Time Series', Environmental Microbiology, 7 (2010), 1466-79.

[5]. B. J Campbell, S. W Polson, T. E Hanson, M. C Mack, and E. A. G Schuur, 'The Effect of Nutrient Deposition on Bacterial Communities in Arctic Tundra Soil', Environmental Microbiology, 12 (2010), 1842-54.

[6]. Kristen M Deangelis, Allgaier Martin, Chavarria Yaucin, Julian L Fortney, Hugenholtz Phillip, Simmons Blake, Sublette Kerry, Whendee L Silver, and Terry C Hazen, 'Characterization of Trapped Lignin-Degrading Microbes in Tropical Forest Soil', Plos One, 6 (2011), e19306.1-e06.9.

[7]. Guo Chun Ding, Yvette M. Piceno, Holger Heuer, Nicole Weinert, Anja B. Dohrmann, Angel Carrillo, Gary L. Andersen, Thelma Castellanos, Christoph C. Tebbe, and Kornelia Smalla, 'Changes of Soil Bacterial Diversity as a Consequence of Agricultural Land Use in a Semi-Arid Ecosystem', Plos One, 8 (2013), e59497.

[8]. G. A. Ellis, C. S. Thomas, S Chanana, N Adnani, E Szachowicz, D. R. Braun, M. K. Harper, T. P. Wyche, and T. S. Bugni, 'Brackish Habitat Dictates Cultivable Actinobacterial Diversity from Marine Sponges', Plos One, 12 (2017).

[9]. L. Z. Fang, X. C. Kun, Z. C. Song, X. J. Qin, H. Y. Qiu, D. C. Qun, and M. M. He, 'Fungistatic Intensity of Agricultural Soil against Fungal Agents and Phylogenetic Analysis on the Actinobacteria Involved', Current Microbiology, 62 (2011), 1152-9.

[10]. N Fierer, and R. B. Jackson, 'The Diversity and Biogeography of Soil Bacterial Communities', Proceedings of the National Academy of Sciences of the United States of America, 103 (2006), 626-31.

[11]. Swapnil Gaikwad, Yogesh S. Shouche, and Wasudev N. Gade, 'Microbial Community Structure of Two Freshwater Sponges Using Illumina Miseq Sequencing Revealed High Microbial Diversity', Amb Express, 6 (2016), 40.

[12]. Savvas Genitsaris, Sebastien Monchy, Jeremie Denonfoux, Stephanie Ferreira, Konstantinos Ar. Kormas, Telesphore Simengando, Eric Viscogliosi, and Urania Christaki, 'Marine Microbial Community Structure Assessed from Combined Metagenomic Analysis and Ribosomal Amplicon Deep-Sequencing', Marine Biology Research, 12 (2016), 30-42.

[13]. Kelly Hamonts, Andrew Bissett, Ben C. T. Macdonald, Philip S. Barton, Adrian D. Manning, and Andrew Young, 'Effects of Ecological Restoration on Soil Microbial Diversity in a Temperate Grassy Woodland', Applied Soil Ecology, 117-118 (2017), 117-28.

[14]. China A Hanson, M. Claire Horner-Devine, Jennifer B. H. Martiny, and Jed A. Fuhrman, 'Microbial Biogeography', Encyclopedia of Biodiversity, 33 (2013), 271-79.

[15]. Mn Hogberg, P Hogberg, and Dd Myrold, 'Is Microbial Community Composition in Boreal Forest Soils Determined by Ph, C-to-N Ratio, the Trees, or All Three?', Oecologia, 150 (2007), 590-601.

[16]. K Holmfeldt, C Dziallas, J Titelman, K Pohlmann, H. P Grossart, and L Riemann, 'Diversity and Abundance of Freshwater Actinobacteria Along Environmental Gradients in the Brackish Northern Baltic Sea', Environmental Microbiology, 11 (2010), 2042-54.

[17]. S. H. Hong, J Bunge, S. O. Jeon, and S. S. Epstein, 'Predicting Microbial Species Richness', Proc Natl Acad Sci U S A, 103 (2006), 117-22.

[18]. Thanitsara Inthasotti, and Wasu Pathom-Aree, 'Diversity of Actinobacteria Associated with Nostoc Commune Vaucher Ex Bornet & Flahault Macrocolonies', Annals of Microbiology, 65 (2015), 2229-40.

[19]. Sasha N. Jenkins, Ian S. Waite, Adrian Blackburn, Rebecca Husband, Steven P. Rushton, David C. Manning, and Anthony G. O'Donnell, 'Actinobacterial Community Dynamics in Long Term Managed Grasslands', Antonie Van Leeuwenhoek, 95 (2009), 319-34.

[20]. Ederson Da C Jesus, Terence L Marsh, James M Tiedje, and Fatima M De S Moreira, 'Changes in Land Use Alter the Structure of Bacterial Communities in Western Amazon Soils', Isme Journal, 3 (2009), 1004-11.

[21]. P. F. Kemp, and J. Y. Aller, 'Bacterial Diversity in Aquatic and Other Environments: What 16s Rdna Libraries Can Tell Us', Fems Microbiology Ecology, 47 (2010), 161-77.

[22]. 22 Christian L. Lauber, Micah Hamady, Rob Knight, and Noah Fierer, 'Pyrosequencing-Based Assessment of Soil Ph as a Predictor of Soil Bacterial Community Structure at the Continental Scale', Applied & Environmental Microbiology, 75 (2009), 5111.

[23]. Xiao Liu, Jing Cong, Hui Lu, Yadong Xue, Xiulei Wang, Diqiang Li, and Yuguang Zhang, 'Community Structure and Elevational Distribution Pattern of Soil Actinobacteria in Alpine Grasslands', Acta Ecologica Sinica, 37 (2017), 213-18.

[24]. And M Goodfellow, and S T Williams, 'Ecology of Actinomycetes', Ann.rev.microbiol, 37 (1983), 189.

[25]. Alan J. Mccarthy, and Stanley T. Williams, '17 Methods for Studying the Ecology of Actinomycetes', Methods in Microbiology, 22 (1990), 533-63.

[26]. Bruce Mccune, and James B Grace, 'Analysis of Ecological Communities', Mjm Software Design Gleneden Beach, 289 (2002), 448.

[27]. M. S. Meyerhof, J. M. Wilson, M. N. Dawson, and J. M. Beman, 'Microbial Community Diversity, Structure, and Assembly across Oxygen Gradients in Meromictic Marine Lakes, Palau', Environmental Microbiology, 18 (2016).

[28]. R Narendrulakotha, and K. K. Nkongolo, 'Microbial Response to Soil Liming of Damaged Ecosystems Revealed by Pyrosequencing and Phospholipid Fatty Acid Analyses', Plos One, 12 (2017), e0168497.
[29]. Kelly S Ramirez, Joseph M Craine, and Noah Fierer, 'Consistent Effects of Nitrogen Amendments on Soil Microbial Communities and Processes across Biomes', Global Change Biology, 18 (2012), 1918-27.
[30]. L. B. Salam, M. O. Ilori, O. O. Amund, Y Liimien, and H Nojiri, 'Characterization of Bacterial Community Structure in a Hydrocarbon-Contaminated Tropical African Soil', Environmental Technology (2017), 1.
[31]. J Schäfer, U Jäckel, and P Kämpfer, 'Analysis of Actinobacteria from Mould-Colonized Water Damaged Building Material', Systematic & Applied Microbiology, 33 (2010), 260-68.
[32]. P. D. Schloss, and J Handelsman, 'Introducing Dotur, a Computer Program for Defining Operational Taxonomic Units and Estimating Species Richness', Applied & Environmental Microbiology, 71 (2005), 1501-6.
[33]. M. M. Shinwari, S. A. Alharbi, I. Ara, M. Wainwright, and M. A. Khiyami, 'Gas Chromatography and Mass Spectrum Analysis of the Selective Diversity of Actinobacteria Isolated from Extreme Environments in Saudi Arabia', Journal Of Pure And Applied Microbiology, 7 (2013), 107-16.
[34]. Bk Singh, N Nunan, Kp Ridgway, J Mcnicol, J Peter, Wdaniell Young, Tj, Ji Prosser, and P Millard, 'Relationship between Assemblages of Mycorrhizal Fungi and Bacteria on Grass Roots', Environmental Microbiology, 10 (2010), 534-41.
[35]. J. E. Stach, L. A. Maldonado, A. C. Ward, M. Goodfellow, and A. T. Bull, 'New Primers for the Class Actinobacteria: Application to Marine and Terrestrial Environments', Environ Microbiol, 5 (2003), 828-41.
[36]. Woo Jun Sul, Stella Asuming-Brempong, Qiong Wang, Dieter M. Tourlousse, C. Ryan Penton, Ye Deng, Jorge L. M. Rodrigues, Samuel G. K. Adiku, James W. Jones, and Jizhong Zhou, 'Tropical Agricultural Land Management Influences on Soil Microbial Communities through Its Effect on Soil Organic Carbon', Soil Biology & Biochemistry, 65 (2013), 33-38.
[37]. Hui Tang, Xunxun Shi, Xiaofei Wang, Huanhuan Hao, Xiu Min Zhang, and Li Ping Zhang, 'Environmental Controls over Actinobacteria Communities in Ecological Sensitive Yanshan Mountains Zone', Frontiers in Microbiology, 7 (2016).
[38]. Qin Tian, Takeshi Taniguchi, Wei Yu Shi, Guoqing Li, Norikazu Yamanaka, and Sheng Du, 'Land-Use Types and Soil Chemical Properties Influence Soil Microbial Communities in the Semiarid Loess Plateau Region in China', Scientific Reports, 7 (2017), 45289.
[39]. V. Torsvik, and L. Ovreas, 'Microbial Diversity and Function in Soil: From Genes to Ecosystems', Curr Opin Microbiol, 5 (2002), 240-5.
[40]. D Wagner, S Kobabe, and S Liebner, 'Bacterial Community Structure and Carbon Turnover in Permafrost-Affected Soils of the Lena Delta, Northeastern Siberia', Canadian Journal of Microbiology, 55 (2009), 73.
[41]. Qiong Wang, George M. Garrity, James M. Tiedje, and James R. Cole, 'Naïve Bayesian Classifier for Rapid Assignment of Rrna Sequences into the New Bacterial Taxonomy', Applied & Environmental Microbiology, 73 (2007), 5261.
[42]. Etienne Yergeau, Hervé Hogues, Lyle G Whyte, and Charles W Greer, 'The Functional Potential of High Arctic Permafrost Revealed by Metagenomic Sequencing, Qpcr and Microarray Analyses', Isme Journal, 4 (2010), 1206-14.
[43]. Juanli Yun, Yiwen Ju, Yongcui Deng, and Hongxun Zhang, 'Bacterial Community Structure in Two Permafrost Wetlands on the Tibetan Plateau and Sanjiang Plain, China', Microbial Ecology, 68 (2014), 360-69.

Liping Zhang" The Diversity of Actinobacteria in Nihewan Basin" International Journal of Engineering Science Invention (IJESI), vol. 07, no. 12, 2018, pp 01-10