http://ansinet.com/itj

ITJ

ISSN 1812-5638

INFORMATION TECHNOLOGY JOURNAL

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Information Technology Journal 12 (21): 6430-6439, 2013
ISSN 1812-5638 / DOI: 10.3923/itj.2013.6430.6439

Effect of Engineering Disturbance on the Culturable Soil Bacterial Community along the Qinghai-Tibet Highway, China

[1]Long Haozhi, [3]Wang Yilin, [1]Liu Guangxiu, [2]Chen Tuo, [1]Zhang Wei, [1]Wang Yun, [1]Wu Xiukun, [1]Tai Xisheng, [1]Zhang Baogui and [1]Sun Likun
[1]Key Laboratory of Desert and Desertification,
[2]State Key Laboratory of Cryospheric Sciences,
Cold and Arid Regions Environmental and Engineering Research Institute,
Chinese Academy of Sciences, 730000, Lanzhou Gansu, China
[3]School of Chemical and Biological Engineering, Lanzhou Jiaotong University, 730070,
Lanzhou Gansu, China

Abstract: The variation of the soil physicochemical properties and culturable bacterial community structure along the Qinghai-Tibet Highway (QTP) were studied. The soil Water Content (WC), Total Organic Carbon (TOC) and Total Nitrogen (TN) contents in the Original Land (OL) were higher compared with the corresponding Disturbed Land (DL). The soil pH was neutral to alkaline for almost all of samples and the values for the DL were generally higher. The number of culturable bacteria ranged from 0.25×10^5 to 1.61×10^8 and was larger in OL than in DL. A total of 64 isolates was identified and fell into the phyla Actinobacteria, Bacteroidetes, Firmicutes and Proteobacteria, with a dominance of *Arthrobacter*. Interestingly, the effects of disturbance on bacterial phylotype number were more significant in the southern slopes than in the northern slopes of the Tanggula Mountains. Statistical analysis showed that the number of bacteria in a beef extract-peptone medium were affected by TN in OL and influenced by the plant coverage in DL. The number of bacteria in Gause's No. 1 medium had no correlation with any parameters while was affected by the pH in DL and the number of functional bacteria were affected by the vegetation coverage in OL and were affected by the TOC and TN after the disturbance. The bacterial phylotype number in OL was influenced by soil Water Content (WC), whereas the bacterial phylotype number was affected by the plant diversity in DL.

Key words: Qinghai-Tibet highway, engineering disturbance, culturable bacteria

INTRODUCTION

The Qinghai-Tibet Plateau (QTP) is the highest and most extensive plateau in the world. Many studies have demonstrated and suggested that the QTP could be one of the most sensitive areas to global change (Liu and Chen, 2000). During the last decades, it had become evident that such extreme habitats harbor a unique community of microorganisms. Microbiological investigations of different Tibetan plateau soils have shown that these environments can harbor a large bacterial number of up to approximately 10^7 colony forming units CFU g^{-1} (Zhang *et al*., 2007a) which is comparable to the bacterial number in a Canadian high arctic region and Antarctic region (Steven *et al*., 2007; Xia *et al*., 2007). Moreover, an increasing number of studies have focused on unraveling the structure and function of these microbial communities (Zhang *et al*., 2007a, 2007b; Xiong *et al*., 2012).

The environment of the QTP is very fragile and the recovery process will be very slow after a disturbance (Yang *et al*., 2004). Several engineering construction endeavors on the Tibetan plateau have formed an engineering corridor (Jin *et al*., 2008), such that the ecological environment of the QTP has faced many challenges (Qin and Zheng, 2010). In particular, vegetation was destroyed during construction (Zhang *et al*., 2008b) which has had a direct influence on the composition of soil microbial communities through the provision of organic matter (Ganzert *et al*., 2011). To date, many studies have focused on the effects of a disturbance on the community structures of plants

Corresponding Author: Liu Guangxiu, Key Laboratory of Desert and Desertification,
Cold and Arid Regions Environmental and Engineering Research Institute, Chinese Academy of Sciences,
730000, Lanzhou Gansu, China Tel: 86-09314967525 Fax: 86-09314967518

(Zhu *et al*., 2006; Cui and Graf, 2009) and animals (Xia *et al*., 2007; Zhang *et al*., 2009a and Ge *et al*., 2011) on the QTP, whereas the effects of engineering disturbances on the microbial community are rarely studied.

In this study, we presented data for the soil biochemical properties and diversity of bacteria cultured from the Original Land (OL) and Disturbed Land (DL) along the QTH. The results could lead to a better understanding of the effect of engineering disturbances on the culturable soil bacteria communities. Our aims were (1) To explore the differences in the physicochemical factors and bacterial community between OL and DL and (2) To determine which parameter was driving the culturable microbial community after an engineering disturbance.

MATERIALS AND METHODS

Soil sampling: During the austral summer period of 2011, soil samples were collected along the QTH at Shengeligong Mountain (SGL), Amdo (AD), Wenquan (WQ), Tuotuohe (TTH) and Fenghuoshan (FHS). Altogether, ten soil samples (each site was divided into OL and DL, DL sample sites were the land that disturbed by engineering construction and each OL sampling site was more than 120 meters away from highway) were excavated for soil chemical, physical and bacterial screening (Table 1). Subsamples for the soil chemical and physical analyses were transferred into sealable plastic bags and stored at 4°C until further processed. Subsamples for the microbiological analysis were placed in a sterile soil box and kept frozen on ice during transport to the laboratory and analyzed then immediately.

Soil characteristics and bacterial isolation: The soil water content was determined using a weight loss method after 24 h at 90°C in a drying oven. The soil pH was measured using a digital pH meter (PT-10, Sartorius) in a 1:2.5 soil-deionized water slurry that was shaken for 1 h. The Organic Carbon (TOC) and Total Nitrogen (TN) contents were quantified using a total C/N analyzer (Multi-N/C 2100S, Analytik Jena AG, Germany) after air-drying and grinding to allow passage through a 70 mesh sieve.

All of the samples were thawed at 4°C and 2.0 g soil sample was added to 18 mL 0.85% NaCl solution and suspended by shaking adequately. The suspensions generated were then spread on the surface of a culture medium after serial dilution. In this study, all of the samples were cultivated on beef extract-peptone medium for bacteria (DSMZ medium 1289, http://www.dsmz.de), Gause's No. 1 medium for Actinobacteria (DSMZ medium 1048, http://www.dsmz.de), Ashby medium for nitrogen-fixing bacteria (Ashby, 1907) and CMC-Congo Red medium for cellulose-decomposing bacteria (Kasana *et al*., 2008) at 20°C for one week to form colonies. The CFU were calculated and the data were expressed as CFU per gram dry weight of each soil sample. The isolates were consecutively re-streaked on a fresh plate three times.

Amplified ribosomal DNA restriction analysis: The bacterial DNA was extracted using the TIANamp Bacteria DNA Kit (Tiangen-Biotech, China) according to the manufacturer's instructions. The 16S rRNA genes were amplified with bacteria-specific primers, 27f and 1492r (Lane, 1991). The amplification conditions consist of an initial denaturation step at 94°C for 4 min, followed by 30 cycles of 94°C for 1 min, 56°C for 1 min and 72°C for 1.5 min, with a final elongation step of 20 min at 72°C. A 5 μL aliquot of each PCR mixture containing approximately 1.5 μg of amplified 16S rDNA was digested with 1.5 U of restriction enzymes *Hae*III and *Alu*I in a total volume of 20 μL at 37°C for 16 h. The reaction products were separated on a 2.5% agarose gel.

Sequence and phylogenetic analyses: Based on the Amplified Ribosomal DNA Restriction Analysis (ARDRA) of the isolates, one representative strain of each group

Table 1: Sampling site description

				Vegetation details			
Sample	Elevation (m)	Latitude (°N)	Longitude (°E)	Vegetation coverage (%)	Species richness	Shannon index	Vegetation type
SGL-OL	4892.3	32°10′	91°42′	95	8	1.33	Kobresia meadow
SGL-DL				85	8	1.16	
AD-OL	4790.3	32°23′	91°43′	98	7	1.49	Carex meadow
AD-DL				23	11	2.10	
WQ-OL	4864.6	33°13′	91°51′	68	11	1.35	Gramineae meadow
WQ-DL				22	6	1.70	
TTH-OL	4602.3	34°10′	92°24′	85	6	1.49	Kobresia meadow
TTH-DL				22	6	1.47	
FHS-OL	4975.4	34°40′	92°55′	40	10	1.53	Kobresia meadow
FHS-DL				22	11	1.88	

was selected for 16S rRNA gene sequence determination. Two primers were utilized for sequencing: 27F, 5'-AGAGT TTGATCCTGGCTCAG-3' and 1492R, 5'GGTTACCTTG TTACGACTT-3' (Lane, 1991). Nearly complete 16S rDNA nucleotide sequences were determined. The 16S rRNA gene sequences were aligned with representative reference sequences of the most closely related members obtained from the National Center for Biotechnology Information (NCBI) database (http://www.nvbi.nlm.nih.gov) using the multiple-alignment CLUSTALW 1.81 software package. The method of Jukes and Cantor (1969) was used to calculate the evolutionary distances. Phylogenetic dendrograms were constructed using the neighbor-joining method and the tree topologies were evaluated by performing bootstrap analyses of 1,000 data sets with the MEGA 4.1 package, as described by Zhang *et al*. (2012).

Statistical analysis of the data: The correlation coefficients (R) with their p-values were calculated according to Pearson and the cluster analysis was performed with the statistical program IBM SPSS (IBM Corporation, Armonk NY, USA). Data presented are the means of at least three independent experiments and expressed as the Mean±SE. The data including bacteria number, phylotpye number and soil parameters and vegetation details after log transformation (Y' = ln(A×Y+B), where A = 1.000 and B = 1.000) were for cluster and correlation analysis by using program SPSS.

Nucleotide sequence accession numbers: The 16S rDNA sequences of the representative strains isolated in this study were deposited in the GenBank database under the following Accession No.: JX827178-JX827241.

RESULTS

Soil characteristics and number of culturable bacteria: The soil characteristics of ten soil samples from the QTH were analyzed with regard to soil water content, pH, TOC and TN content (Table 2). The values of the soil pH in most of these soil samples were above 7.00 while the pH was 6.92 in SGL-OL; each DL pH value was higher than that in OL. The water content of the investigated soils ranged from 5.3-37.1%, with differences depending on their site characteristics. The TOC and TN content values ranged from 1.22-3.85 and 0.07-0.47%, respectively. Furthermore, the values of the soil water content, TOC and TN of each DL were lower than that of the corresponding OL. Of the four media used, we found that the number of culturable bacteria were different depending on the medium, with values ranging from 1.347×10^5 to 271.560×10^5 (beef extract peptone), 0.511×10^5 to 33.064×10^5 (Gause's No.1), 2.083×10^5 to 1611.600×10^5 (Ashby) and 0.248×10^5 to 814.400×10^5 (CMC-Congo Red), respectively (Table 2). The number of culturable bacteria in each medium was also lower in DL than that in OL.

Phylogenetic analyses of culturable bacteria: All of the 64 isolates were obtained from the ten soil samples. The strains from the QTH soils were affiliated to phyla Actinobacteria, Bacteroidetes, Firmicutes and Proteobacteria (Fig. 1, Table 3), with genus *Arthrobacter* as the dominant member (Fig. 1). Strains related to *Arthrobacter* were also present in all of the soil samples and were the most prominent at site FHS. Strains affiliated with *Pseudomonas* were detectable in almost all of the soil samples (except site TTH), being the major group at sites SGL and AD (Fig. 1). Members of *Bacteroidetes* were present at SGL, AD and WQ. Genera *Frondihabitans* and *Pedobacter* were only found in the SGL soil samples and *Sanguibacter* and *Stenotrophomonas* were only found at AD. In addition, *Amycolatopsis*, *Kribbella*, *Rathayibacter*, *Sporosarcina* and *Rhizobium* were only found at WQ, *Agromyces*, *Rhodococcus* and *Planomicrobium* were only found at TTH and *Labedella*, *Mycetocola* and *Bosea* were only found at FHS.

Table 2: Descriptions of the sampling sites and number of culturable bacteria (g^{-1}dry soil) of each medium

						No. of culturable bacteria			
Site	Land type	WC (%)	pH	TOC (%)	TN (%)	Beef extract peptone	Gause's No.1	Ashby	CMC-congo red
SGL	OL	22.4	6.92	2.96	0.47	271.56±9.99^a	6.77±0.61^a	19.29±1.13^a	24.32±0.16^a
	DL	11.6	7.81	2.06	0.31	19.99±0.94^b	4.19±0.09^b	4.24±0.96^b	2.14±0.47^b
AD	OL	37.1	7.76	3.85	0.46	62.82±16.71^a	28.02±1.58^a	11.05±0.72^a	14.92±0.81^a
	DL	31.6	8.18	3.10	0.31	5.773±1.79^b	0.76±0.18^b	2.08±0.26^b	0.25±0.09^b
WQ	OL	14.3	8.07	1.82	0.18	4.81±1.19^a	1.81±0.03^a	81.64±8.75^a	37.61±0.87^a
	DL	5.3	8.14	1.68	0.15	1.35±0.44^b	0.74±0.16^b	20.57±1.08^b	15.39±0.34^b
TTH	OL	16.8	8.12	1.79	0.15	2.51±0.62^a	0.61±0.05^a	22.71±0.60^a	11.33±0.55^a
	DL	8.2	8.22	1.54	0.07	1.44±0.00^b	0.51±0.21^a	13.31±0.210^b	9.71±0.69^a
FHS	OL	12.8	7.22	2.33	0.26	44.85±18.30^a	33.06±7.09^a	1611.60±57.4^a	814.4±143.4^a
	DL	11.2	8.11	1.22	0.08	2.44±0.82^b	2.25±0.66^b	43.64±0.85^b	30.69±4.22^b

WC: Water content, TOC: Total organic carbon, TN: Total nitrogen. No. of culturable bacteria results are given as Means±SE($\times10^5$). Different suffix letters indicate OL values that are significantly different from DL (independent-samples T-test, $p<0.05$)

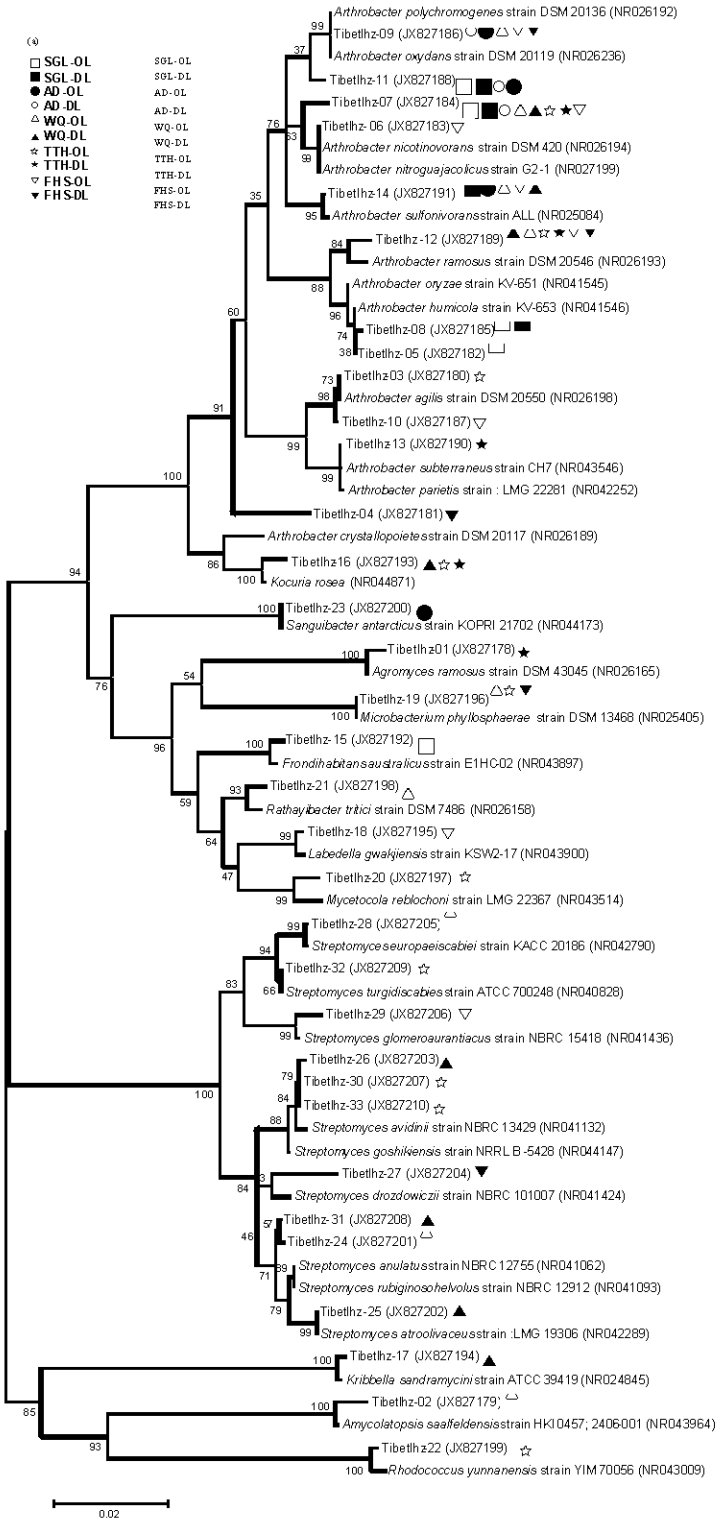

Fig. 1(a-c): Continue

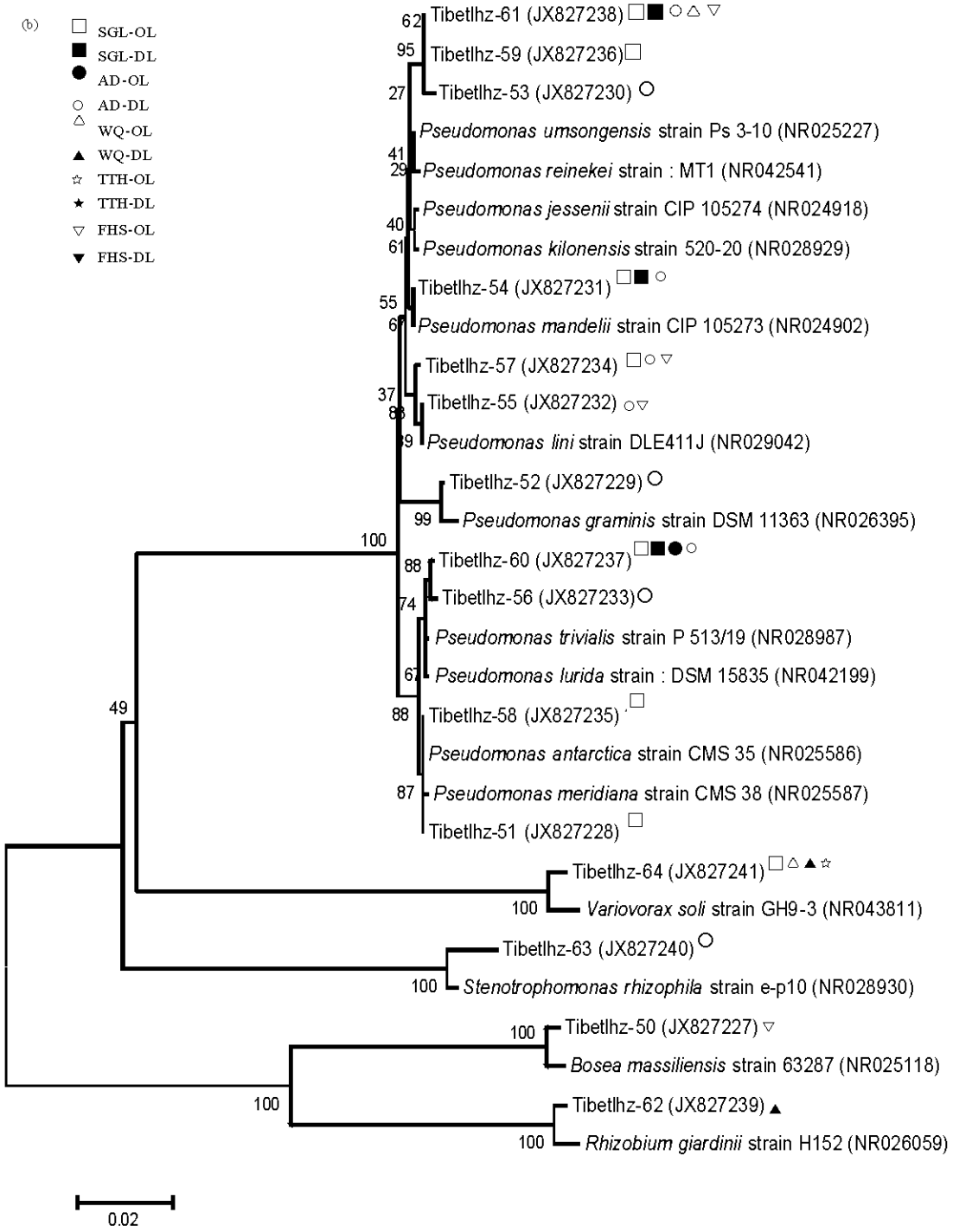

Fig. 1(a-c): Continue

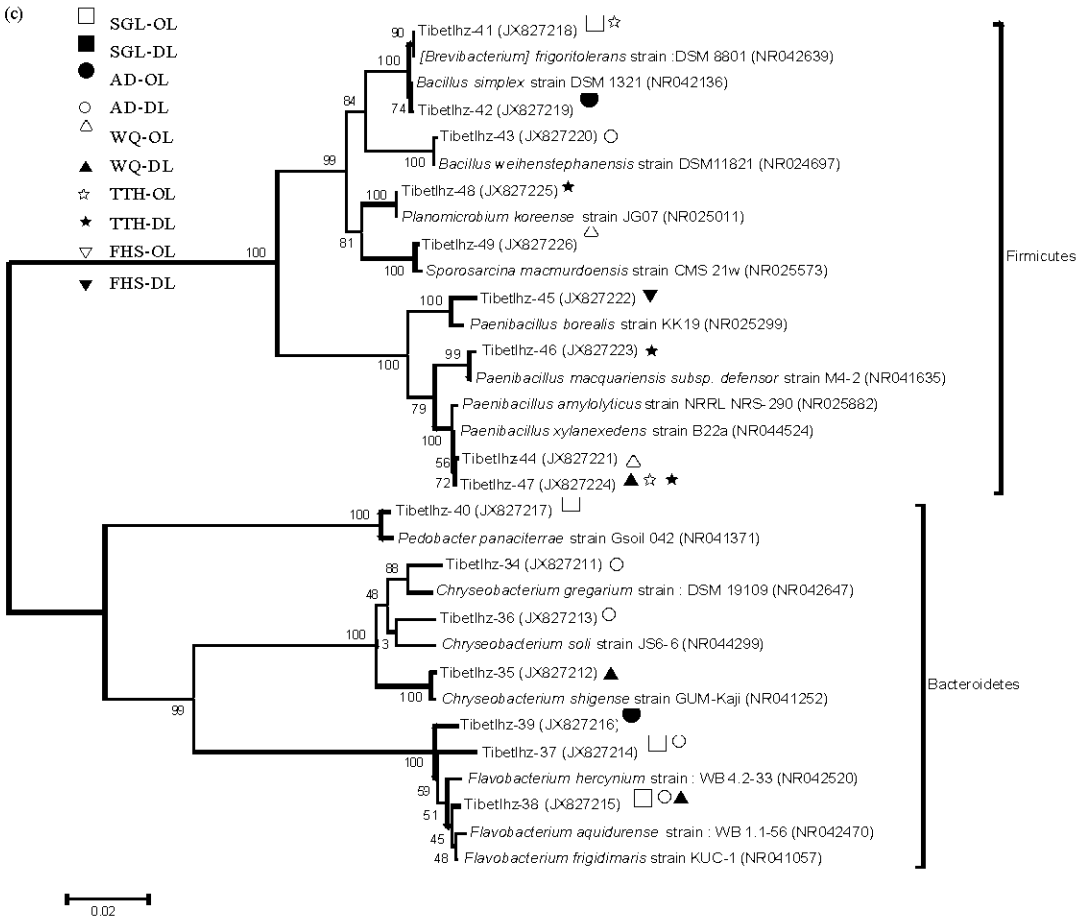

Fig. 1(a-c): Phylogentic dendrogram based on a comparison of the 16S rDNA sequences of the isolates (a) Actinobacteria, (b) Proteobacteria and (c) Firmicutes and Bacteroidetes) from the QTH and their closest phylogenetic relatives. Different symbol represent the sampling site. The tree was created using the neighbor-joining method. The numbers on the tree indicate the percentages of bootstrap sampling derived from 1000 replications

Table 3: Phylotype number of each single soil sample

	Sampling site and soil type									
	SGL		AD		WQ		TTH		FHS	
Phylogenetic affiliation	OL	DL	OL	DL	OL	DL	OL	DL	OL	DL
Actinobacteria	4	5	3	4	10	8	9	6	8	6
Bacteroidetes	3	0	4	1	0	2	0	0	0	0
Firmicutes	1	0	1	1	2	1	2	3	0	1
Proteobacteria	7	4	9	1	2	2	1	0	4	0
Phylotype number	15	9	17	7	14	13	12	9	12	7

Statistical analyses: For both OL and DL (Fig. 2), the SGL and AD sites were all clustered while sites WQ, TTH and FHS were grouped together after disturbance. Table 4 and 5 showed that the phylotype number of bacteria was significantly positively correlated with the water content ($p<0.05$) in OL, whereas the plant species diversity had the greatest effect ($R = -0.854$, $p = 0.065$) in DL. The number of CFUs using beef extract-peptone medium was significantly correlated with pH ($p<0.05$) and TN ($p<0.05$) in OL but with the plant coverage ($p<0.05$) in DL. The number of CFUs on Gause's No. 1 medium have no correlation with any parameters in OL and was positively correlated with the pH in DL ($p<0.05$). The number of CFUs using Ashby medium was

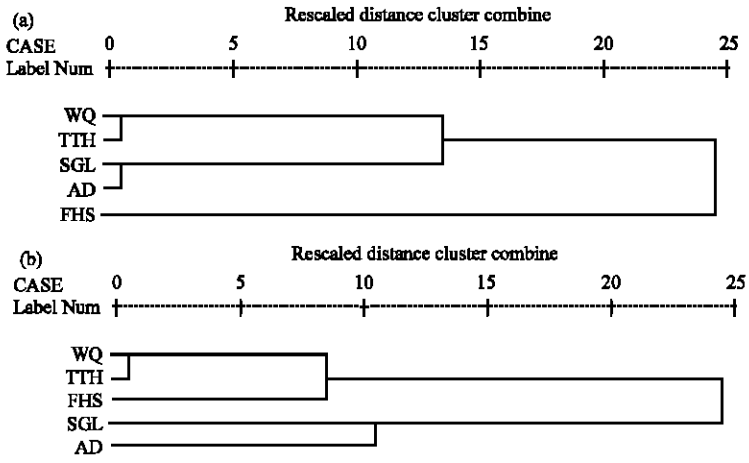

Fig. 2(a-b): Hierarchical cluster analysis of OL and DL from each sampling site along QTH (a) OL and (b) DL

Table 4: Correlations analysis between the numbers and phylotype number of culturable bacterial and enviroumental factors as well as vegetation conditions in OL

B	G	A	C	PN	WC	pH	TOC	TN	PLTS	PLTC	PLTH
B	0.683	-0.052	0.180	0.615	0.625	-0.897*	0.790	0.916*	-0.198	0.182	0.046
G	1.000	0.362	0.553	0.310	0.594	-0.558	0.730	0.624	0.424	-0.275	0.197
A		1.000	0.971**	-0.649	-0.489	-0.258	-0.364	-0.380	0.356	-0.991**	0.671
C			1.000	-0.482	-0.300	-0.443	-0.141	-0.148	0.351	-0.932*	0.657
PN				1.000	0.913*	-0.239	0.811	0.847	-0.449	0.701	-0.111
WC					1.000	-0.219	0.945*	0.866	-0.056	0.546	-0.178
pH						1.000	-0.457	-0.649	0.185	0.128	-0.195
TOC							1.000	0.944*	0.075	0.453	-0.250
TN								1.000	-0.194	0.489	-0.167
PLTS									1.000	-0.367	-0.325
PLTC										1.000	-0.676

B: CFUs of Beef extract peptone medium, G: CFUs of Gause's No.1 medium, C: CFUs of CMC-congo red medium, PN: Bacteria phylotype number, PLT-S: Plant species number, PLT-C: Plant coverage, PLT-H: Plant diversity. *Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed)

Table 5: Correlations analysis between the numbers and diversity of culturable bacterial and environmental factors as well as vegetation conditions in DL

B	G	A	C	PN	WC	pH	TOC	TN	PLTS	PLTC	PLTH
B	0.763	-0.674	-0.665	-0.286	0.214	-0.857	0.511	0.825	-0.468	0.895*	0.355
G	1.000	-0.037	-0.033	-0.222	-0.308	-0.917*	-0.124	0.348	-0.526	0.823	0.344
A		1.000	0.994**	0.208	-0.696	0.271	-0.936*	-0.880*	0.106	-0.434	-0.175
C			1.000	0.284	-0.748	0.234	-0.959**	-0.888*	0.009	-0.383	-0.275
PN				1.000	-0.678	-0.050	-0.219	-0.135	-0.370	0.044	-0.854
WC					1.000	0.317	0.755	0.443	0.461	-0.181	0.523
pH						1.000	-0.071	-0.541	0.712	-0.967**	-0.076
TOC							1.000	0.869	0.220	0.186	0.310
TN								1.000	-0.100	0.597	0.332
PLTS									1.000	-0.794	0.544
PLTC										1.000	-0.019

B: CFUs of Beef extract peptone medium, G: CFUs of Gause's No.1 medium, C: CFUs of CMC-congo red medium, PN: Bacteria phylotype number, PLT-S: Plant species number, PLT-C: Plant coverage, PLT-H: Plant diversity. *Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed)

significantly negatively correlated with the plant coverage ($p<0.01$) in OL but was correlated with the TOC ($p<0.05$) and TN ($p<0.05$) in DL. The number of CFUs on the CMC-Na medium was significantly correlated with the plant coverage ($p<0.05$) in OL but was correlated with TOC ($p<0.01$) and TN ($p<0.05$) in DL.

DISCUSSION

The studies of QTP permafrost habitats revealed a large bacterial abundance and diversity and some novel species have been isolated (Zhang *et al*., 2008a, 2009b; Shen *et al*., 2012). Microbial communities could indicate the condition of the soil ecosystem and be affected by

many environmental factors, such as the soil physicochemical properties and geographical factors (Harris, 2009; Xiong *et al*., 2012). In the present study, we screened the culturable bacteria in OL and DL soils along the QTH and identified the variation of the soil parameters and culturable bacteria community structure after an engineering disturbance.

Although, disturbance of the QTP is quite rare because of the extreme climate conditions, the fragile ecosystem is facing many challenges with the several construction projects on the plateau. The soil samples from OL and DL revealed a heterogeneity with regard to sample-specific properties (e.g., water content, TOC, TN, pH and vegetation conditions) and culturable bacterial community structure. The soil pH value was generally above 7.00 and was higher in DL compared to OL; the soil TOC, TN and soil water content were higher in OL. In this study, the number of CFUs in each medium was higher in OL than DL which might result from the destruction of vegetation producing chemical components, include organic acids (Shi *et al*., 2011). The plant coverage was lower in DL than that in OL (Table 1). In addition, higher carbon and nitrogen values coinciding with a high number of culturable heterotrophs were observed (Table 2); this result suggests that the quantity and availability of organic compounds derived by plants decreased after the engineering disturbance (Ganzert *et al*., 2011).

Sequencing of the 16S rRNA and the phylogenetic analyses indicated that the 64 isolates fell into 4 phyla: Actinobacteria, Bacteroidetes, Firmicutes and Proteobacteria. Zhang *et al*. (2007a) obtained similar results from QTP; conversely, Steven *et al*. (2007) and Hinsa-Leasure *et al*. (2010) cultured bacteria from the Canadian high Arctic and Siberian permafrost and found that there were no *Bacteroidetes* which may play an important role in cold habitats by degrading polymeric organic matter and providing low molecular compounds (Ganzert *et al*., 2011). The genus *Arthrobacter* was predominant in our study (Table 3), similar to the permafrost from the Tianshan Mountains and Antarctic regions (Bai *et al*., 2006; Xiao *et al*., 2007). In addition, after an engineering disturbance, the phylotype number decreased in almost all of the sampling sites and the effect of disturbance on the bacterial phylotype number on the southern slopes (SGL and AD) was more significant than that on the northern slopes (WQ, TTH and FHS) of the Tanggula Mountains (Table 3). These results may be due to changes in the environmental parameters, including the soil type, soil pH, soil moisture and soil nutrient quality and vegetation conditions which can influence the microbial community by affecting individual microbial metabolic activities and, thus, the microbial community (Haichar *et al*., 2008; Djukic *et al*., 2010; Rousk *et al*., 2010; Ganzert *et al*., 2011; Brockett *et al*., 2012).

Statistical analyses of the bacterial community and the environmental parameters showed a strong influence of the soil water content on the bacterial phylotype number of OL. However, the pH was not positively correlated with the bacterial phylotype number, even though it is known to have an influence on the composition of soil microorganisms (Lauber *et al*., 2009; Rousk *et al*., 2010; Xiong *et al*., 2012). This finding might be due to the narrow range of pH values among the study sites. Furthermore, the plant diversity was correlated with the bacterial phylotype number in DL and the number of CFUs in each medium was affected by different environmental factors between OL and DL which might be due to the lower soil nutrients in DL than OL because of the lack of vegetation after the engineering disturbance (Tables 1 and 2). The number of CFUs in the Ashby and CMC-Na media for OL were significantly negatively correlated with the plant coverage. However, the CFU number in the Ashby and CMC-Na media were more significantly negatively correlated with the soil TOC and TN in DL than in OL, indicating that the dominant factors were altered after the engineering disturbance.

Interestingly, the cluster analysis showed that the sampling sites on the northern and southern slopes of the Tanggula Mountains were different between OL and DL; the results showed that the phylotype number of bacteria was more significantly decreased after the disturbance on the southern slope (Table 3). This result might be due to the differing geographical conditions: The climate is different between the northern and southern slopes of the Tanggula Mountains. Indeed, as shown in Table 1, the vegetation coverage on the northern plateau was lower than on the southern because the annual precipitation on the southern slope of the Tanggula Mountains is generally much higher than that on the northern slope (Yang *et al*., 2003, 2007). Therefore, the effect of disturbance on the bacterial communities was more severe on the northern slope than that on the southern slope.

In conclusion, this report presented the data for the effect of an engineering disturbance on the soil physicochemical parameters and the influence on the soil culturable bacteria community along the Qinghai-Tibet Highway. The results showed the following:

- An engineering disturbance can change the properties of the soil, as the number and phylotype of the soil culturable bacteria were decreased after the disturbance

- The major factor that influences the microbial community is different between OL and DL: Bacterial phylotype number was affected by the soil water content in OL, whereas it was influenced by the vegetation diversity in DL

ACKNOWLEDGMENTS

This study was funded by National Basic Research Program (973) of China (No. 2012CB026105), International S&T Cooperation Program of China (No. 2011DFA32520), Natural Science Foundation of China (No. 31170465).

REFERENCES

Ashby, S.F., 1907. Some observations on the assimilation of atmospheric nitrogen by a free living soil organism: Azotobacter chroococcum of beijerinck. J. Agric. Sci., 2: 35-51.

Bai, Y., D. Yang, J. Wang, S. Xu, X. Wang and L. An, 2006. Phylogenetic diversity of culturable bacteria from alpine permafrost in the Tianshan Mountains, northwestern China. Res. Microbiol., 157: 741-751.

Brockett, B.F.T., C.E. Prescott and S.J. Grayston, 2012. Soil moisture is the major factor influencing microbial community structure and enzyme activities across seven biogeoclimatic zones in western Canada. Soil Biol. Biochem., 44: 9-20.

Cui, X. and H.F. Graf, 2009. Recent land cover changes on the Tibetan Plateau: A review. Clim. Change, 94: 47-61.

Djukic, I., F. Zehetner, A. Mentler and M.H. Gerzabek, 2010. Microbial community composition and activity in different Alpine vegetation zones. Soil Biol. Biochem., 42: 155-161.

Ganzert, L., A. Lipski, H.W. Hubberten and D. Wagner, 2011. The impact of different soil parameters on the community structure of dominant bacteria from nine different soils located on Livingston Island, South Shetland Archipelago, Antarctica. FEMS Microbiol. Ecol., 76: 476-491.

Ge, C., Z. Li and C. Huang, 2011. The effects on birds of human encroachment on the Qinghai-Tibet Plateau. Trans. Res. Part D: Trans. Environ., 16: 604-606.

Haichar, F.Z., C. Marol, O. Berge, J.I. Rangel-Castro and J.I. Prosser *et al*., 2008. Plant host habitat and root exudates shape soil bacterial community structure. ISME J., 2: 1221-1230.

Harris, J., 2009. Soil microbial communities and restoration ecology: Facilitators or followers? Science, 325: 573-574.

Hinsa-Leasure, S.M., L. Bhavaraju, J.L.M. Rodrigues, C. Bakermans, D.A. Gilichinsky and J.M. Tiedje, 2010. Characterization of a bacterial community from a Northeast Siberian seacoast permafrost sample. FEMS Microbiol. Ecol., 74: 103-113.

Jin, H., Q. Yu, S. Wang and L. Lu, 2008. Changes in permafrost environments along the Qinghai-Tibet engineering corridor induced by anthropogenic activities and climate warming. Cold Reg. Sci. Technol., 53: 317-333.

Jukes, T.H. and C.R. Cantor, 1969. Mammalian Protein Metabolism. Academic Press, New York, pp: 21-132.

Kasana, R.C., R. Salwan, H. Dhar, S. Dutt and A. Gulati, 2008. A rapid and easy method forthe detection of microbial cellulases on agar plates using gram's iodine. Curr. Microbiol., 57: 503-507.

Lane, D.J., 1991. 16S/23S rRNA Sequencing. In: Nucleic Acid Techniques in Bacterial Systematic. Stackebrandt, E. and M. Goodfellow (Eds.). John Whiley and Sons, New York, pp: 115-175.

Lauber, C.L., M. Hamady, R. Knight and N. Fierer, 2009. Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. Applied Environ. Microbiol., 75: 5111-5120.

Liu, X. and B. Chen, 2000. Climatic warming in the Qinghai-Tibet Plateau during recent decades. Int. J. Climatol., 20: 1729-1742.

Qin, Y. and B. Zheng, 2010. The Qinghai-Tibet Railway: A landmark project and its subsequent environmental challenges. Environ. Dev. Sustainability, 12: 859-873.

Rousk, J., E. Baath, P.C. Brookes, C.L. Lauber and C. Lozupone *et al*., 2010. Soil bacterial and fungal communities across a pH gradient in an arable soil. ISME J., 4: 1340-1351.

Shen, L., Y. Liu, N. Wang, T. Yao and N. Jiao *et al*., 2012. *Massilia yuzhufengensis* sp. nov. isolated from an ice core on Qinghai-Tibet Plateau. Int. J. Syst. Evol. Microbiol.,. 10.1099/ijs.0.042101-0

Shi, S., A.E. Richardson, M. O'Callaghan, K.M. DeAngelis and E.E. Jone *et al*., 2011. Effects of selected root exudate components on soil bacterial communities. FEMS Microbiol. Ecol., 77: 600-610.

Steven, B., G. Briggs, C.P. Mckay, W.H. Pollard, C.W. Greer and L.G. Whyte, 2007. Characterization of the microbial diversity in a permafrost sample from the Canadian high Arctic using culture-dependent and culture-independent methods. FEMS Microbiol. Ecol., 59: 513-523.

Xia, L., Q. Yang, Z. Li, Y. Wu and Z. Feng, 2007. The effect of the Qinghai-Tibet railway on the migration of Tibetan antelope *Pantholops hodgsonii* in Hoh-xil National Nature Reserve, China. Oryx, 41: 352-357.

Xiao, X., M. Li, Z. You and P. Wang, 2007. Bacterial communities inside and in the vicinity of the Chinese Great Wall Station, King George Island, Antarctica. Antarct. Sci., 19: 11-16.

Xiong, J., Y. Liu, X. Lin, H. Zhang and J. Zeng *et al*., 2012. Geographic distance and pH drive bacterial distribution in alkaline lake sediments across Qinghai-Tibet Plateau. Environ. Microbiol., 14: 2457-2466.

Yang, M., S. Wang, T. Yao, X. Gou, A. Lu and X. Guo, 2004. Desertification and its relationship with permafrost degradation in Qinghai-Xizang (Tibet) plateau. Cold Reg. Sci. Technol., 39: 47-53.

Yang, M., T. Yao, X. Gou, H. Wang and L. Hao, 2007. Comparison analysis of the summer monsoon precipitation between northern and southern slopes of Tanggula Mountains, Qinghai-Xizang (Tibetan) Plateau: A case study in summer 1998. Hyd. Proc., 21: 1841-1847.

Yang, X., C. Kamenik, R. Schmidt and S. Wang, 2003. Diatom-based conductivity and water-level inference models from eastern Tibetan (Qinghai-Xizang) Plateau lakes. J. Paleolimnol., 30: 1-19.

Zhang, G., F. Niu, H.J. Busse, X. Ma and W. Liu *et al*., 2008. *Hymenobacter psychrotolerans* sp. nov., isolated from the Qinghai-Tibet plateau permafrost region. Int. J. Syst. Evol. Microbiol., 58: 1215-1220.

Zhang, G., X. Ma, F. Niu, M. Dong, H. Feng, L. An and G. Cheng, 2007. Diversity and distribution of alkaliphilic psychrotolerant bacteria in the Qinghai-Tibet plateau permafrost region. Extremophiles, 11: 415-424.

Zhang, G., X. Ma, F. Niu, W. Liu and M. Dong *et al*., 2007. Phylogenetic diversity of bacteria isolates from the Qinghai-Tibet plateau permafrost region. Can. J. Microbiol., 53: 1000-1010.

Zhang, Q., L. Xia, J. Ma, P.W. Wu and Q.S. Yang, 2009. Effects of the Qinghai-Tibet railway on the community structure of rodents in qaidam desert region. Acta Ecol. Sin., 29: 267-271.

Zhang, T., T.H.W. Baker, G.D. Cheng and Q. Wu, 2008. The Qinghai-Tibet railroad: A milestone project and its environmental impact. Cold Reg. Sci. Technol., 53: 229-240.

Zhang, W., G. Zhang, G. Liu, Z. Li, T. Chen and L. An, 2012. Diversity of bacterial communities in the snowcover at tianshan number 1 glacier and its relation to climate and environment. Geomicrobiol. J., 29: 459-469.

Zhang, X., X. Ma, N. Wang and T. Yao, 2009. New subgroup of *Bacteroidetes* and diverse microorganisms in Tibetan plateau glacial ice provide a biological record of environmental conditions. FEMS Microbiol. Ecol., 67: 21-29.

Zhu, G., L. Tao and J. Ren, 2006. Evaluation of using land for constructing Qinghai-Tibet railway on native vegetation. Acta Agrestia Sinica, 14: 160-164, 180.