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Spatial Pattern of Bacterial Community Functional Diversity in a Drinking Water Reservoir, Shaanxi Province, Northwest China

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In this work, BIOLOG community-level physiological profile (CLPP) technique was employed to explore the bacterial community functional diversity from different depth (0.5 m, 30 m and 65 m) and sites (site A, site B and site C) of the JIN PEN drinking reservoir, China. The results showed that the highest bacterial community activities expressed as average well color development ($AWCD_{590nm}$) was observed in 30 m, the lowest was observed in 65 m. The significant "sites" and "depth" indicates that the $AWCD_{590nm}$ varied among the sites within the depth ($P<0.01$). Whilst, species richness diversity (R) was significantly higher in 30 m than that of 0.5 m and 60 m, but there were no significant two-way interactions for R and Shannon's diversity index ($P>0.05$). Meanwhile, Principle component analyses (PCA) of BIOLOG profiles indicated that bacterial community functional diversity was changed in different depths and sites. Carbon substrates with highest significant correlation coefficients for PC1 and PC2 were hydroxy butyric acid and mannitol. Overall the findings increased our knowledge about the aquatic bacterial community diversity in drinking water constitute a potential health risk, because increased functional microbial communities may influence drinking water quality.

Key words: Drinking water reservoir, carbon sources, bacterial community diversity, Community-level physiological profiles (CLPPs).

Microbial communities are ubiquitous inhabitants in various aquatic environments, contributing substantially to critical geological and ecological processes in water ecosystem, including breakdown of complex organic carbon substances, circulation of nitrogen, and modification of water aesthetic properties [1]. In the past few decades, several functional microbes harbored in water environmental conditions have been investigated [2-3]. Compared with the massive literatures on sea [4-6], lake [7-8], river [9-10], wetland [11-12], and spring [13-14], research on drinking water reservoir is limited, which represent only the sediment associated microbial community [15-16].

Drinking water reservoir is the important source of drinking water supply for the urban regions in arid and semiarid areas with lower groundwater stocks, northwest China [17]. Drinking water quality can be evaluated and quantified base on examination of physical, biochemical, or microbial parameters. To determine the raw water quality of reservoir, several reports has been paid to taste, odour and color [18], phosphorus absorption and release [19], heavy metals [20], organic contaminants [21] and cyanobacterial blooms and microcystins [7], fewer work is focused on the microbial community living in this oligotrophic water body. In particular, detailed spatial sampling for bacterial community functional diversity has not been generally understood.

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Drinking sources water microbial community as indicators of water quality [22]. Microbe growth can lead to the development of taste, odour and color in the drinking water. In drinking water reservoir ecosystems, a number of water microbial parameters such as microbial metabolic activity have the potential for use as diagnostic bioindicators of drinking water quality. Wilhelm *et al*.[7] reported the relationships between water nutrients and the structure of microbial communities in Lake Tai. Pereira *et al*.[2-3] also examined the temporal variations in the filamentous fungal and yeast populations in three drinking water sources, including surface water, spring water, and groundwater. Recently, Lautenschlager *et al*. [23] determined the effect of overnight stagnation of drinking water in households' taps on the structure of bacterial community and growth. However, water bacterial functional communities change over spatial in the drinking water reservoir is poorly evaluated.

The objective of the present study was, therefore, to explore the spatial pattern of bacterial community functional diversity in a drinking water reservoir, northwest China using community level physiological profiles (CLPPs), thus providing an experimental evaluation of the aquatic bacterial community diversity in drinking water body constitute a potential health risk.

MATERIALS AND METHODS

Study site description

The experiment was conducted in the JIN PEN reservoir, located in Zhouzhi County, Xi'an City, Shaanxi Province, northwest China (E108°112, N34°022), is a large drinking water reservoir with a maximal depth of 90-105 m, average depth of 60-80 m [19], area of 4550 m^2, and has a water volume of 2×10^8 m^3, serves as a municipal and domestic water supply sources for Xi'an city. Daily water supply capacity is 8×10^5.

Water sampling procedure

The polyethylene bottles (1L) were carefully sterilized using ethanol disinfection for 5 min and rinsed with sterile distilled water three times before sampling. In order to evaluate the spatial pattern of microbial community functional diversity in JIN PEN drinking water reservoir, water samples were collected from different three sampling sites (site A, site B, and site C) (Table 1 GPS position) on July 6, 2012. In each site, three different depths (0.5 m, 30 m, and 65 m) were selected. All water samples were immediately put on ice, and transported back to the lab within 5 h after collection. For BIOLOG analysis, each water samples (50 mL) were stored at 4° for less than 24 h until BIOLOG profiles were analyzed, to minimize any changes in bacterial communities.

Community-level physiological profiles (CLPPs) determination

To examine the water bacterial community functional diversity, BIOLOG ECO plate technique was used to explore the carbon source utilization pattern (functional diversity) of bacterial community harbored in the water body [24]. BIOLOG ECO plate contains 31 different carbon sources, including ten carbohydrates, two phenolic compounds, four polymers, seven carboxylic acids, two amines, and six amino acids (Table 2). As described by Choi and Dobbs [24] and little modification, in the laboratory, water samples were added with eight channel pipette (Bio-Rad, USA) to each well of the ECO micro-plate with 150 µl. All of the inoculated ECO plates were put in to the polyethylene bag, incubating at 25±2° in the dark for 144 h. The absorbance at 590_{nm} was measured every 12 h interval, and optical density (OD_{590nm}) values that were negative were set to zero [25].

Bacterial community activity in BIOLOG ECO plate was expressed as average well color development (*AWCD*), and diversity index as Species richness (*R*) [24-25]. 96 h values were used to calculate *AWCD* and functional diversity index. Bacterial community activity in ECO plate was expressed as *AWCD*, and was assessed using the formula:

$$AWCD = \Sigma(A\text{-}A_0)/31 \quad ...(1)$$

where, the raw OD values were transformed: $A\text{-}A_0$, where A_0 was the mean of the control (water blanks) per plate and A was the mean of the same three wells.

Bacterial functional diversity was expressed as species richness (*R*) and Shannon's diversity (*H*). *R* was the number of oxidized carbon substrates in the BIOLOG-ECO plate. *H* was calculated as formula:

$$H' = -\sum_{i=1}^{S} Pi \ln Pi = -\sum_{i=1}^{S} (Ni/N)\ln(Ni/N) \quad ...(2)$$

where, *Pi* was proportional color development of the *i*th well over total color development of all wells of a ECO plate.

Statistical analysis

All data were analyzed with SPSS 16.0 (SPSS Inc., USA). The data expressed by the mean and standard errors (S.E) (n=3). A parametric two-way analysis of variance (ANOVAs) test, followed by Tukey-Kramer HSD test, was employed to evaluate the significant differences in the microbial properties of among different sampling sites and depth at 5% level ($\boldsymbol{P}$<0.05). Principle component analyses (PCA) were used to analyze the water bacterial community using the SPSS version 16.0 software package (SPSS Inc., USA) for windows. Graphical work was carried out using Sigma Plot (Version 10.0) software packages.

RESULTS AND DISCUSSION

Average Well Color Development and functional diversity index

Water bacterial community activity was expressed as Average Well Color Development ($AWCD_{590nm}$), $AWCD_{590nm}$ curve was shown in Figure 1, $AWCD_{590nm}$ was increased steadily during the cultural periods from 12 h to 144 h. 96 h values were used to calculate *AWCD* and functional diversity index. As shown in Figure 2, The $AWCD_{590nm}$ varied significantly and was the highest $AWCD_{590nm}$ (1.60±0.12) in 30 m of Site A, and lowest (0.71±0.01) in 65 m of Site B. In each of three different sampling sites, the $AWCD_{590nm}$ in the middle of drinking water reservoir (30 m) were higher than that of in the bottom (65 m) (Figure 2A). The highest species richness (R) was 28±1.76 in 30 m of Site A, and the lowest R was 16±1.15 in 65 m of Site B (Figure 2B). Meanwhile, Shannon diversity index (H) was determined for all samples, the Shannon's diversity (H) in 30 m of Site A was 3.26±0.06, which was significantly higher than that of 65 m in Site A with 2.68±0.06 (P<0.05) (Figure 2C). These results revealed that the ability to utilize sole carbon substrates and functional diversity (metabolic diversity) for drinking water bacterial community were stronger in 30 m than that in 65m. $AWCD_{590nm}$ varied both sites and depth as indicated by MANOVA (Table 3). The significant "sites" and "depth" indicates that the $AWCD_{590nm}$, R and H index varied among the sites within the depth (P<0.01). There was significant two-way interactions for $AWCD_{590nm}$ (P<0.05). However, there were no significant two-way interactions for R and H index (P>0.05).

Carbons sources utilization

Carbon substrates were utilized by drinking water bacterial community. The utilization of substrates of carbohydrates, polymers and carboxylic acids was higher in 30 m than in 65 m, but that of phenolic compounds was similar between 30 m and 65 m (data not shown). F-values for two-way ANOVAs of the variables for different carbon sources were shown in Table 3, The significant "sites" and "depth" indicates that the carbon sources varied among the sites within the depth (P<0.001). However, there were no significant two-way interactions in carboxylic acids, polymers and phenolic compounds (P>0.05). Thus, carbon

Table 1. 31 different sole carbon sources used in this study[25]

Carbohydrates	Carboxylic acids	Amino acids	Polymers	Phenolic compounds	Amines
D,L-a-Glycerol	Pyruvic acid methyl ester	Arginine	Cyclodextrin	4-Hydroxy benzoic acid	Phenyl ethylamine
a-D-L-lactose	g-Hydroxy butyric acid	Threonine	Glycogen	2-Hydroxy benzoic acid	Putrescine
b-Methyl-D-glucoside	D-Galacturonic acid	Serine	Tween40		
phosphate	a-Ketobutyric acid	Phenylalanine	Tween80		
i-Erythritol	D-Glucosaminic acid	Asparagine			
D-Cellobiose	D-Malic acid	Glycyl-L-glutamic acid			
D-Mannitol	Itaconic acid				
D-Xylose					
Glucose-1-phosphate					
N-Acetyl-D-glucosamine					
D-Galactonic acid lactone					

Table 2. Location (longitude and latitude) and water depth of three sampling sites (site A, site B, site C) in the JIN PEN drinking water reservoir, Shaanxi Province, northwest China

Sampling sites	Longitude (E)	Latitude (N)	Water depth (m)
Site A	34°02' 13.70"	108°11' 33.92"	0.5
Site A	34°02' 13.70"	108°11' 33.92"	30
Site A	34°02' 13.70"	108°11' 33.92"	65
Site B	34°02' 39.44"	108°11' 52.86"	0.5
Site B	34°02' 39.442"	108°11' 52.86"	30
Site B	34°02' 39.44"	108°11' 52.86"	65
Site C	34°02' 39.81"	108°12' 20.55"	0.5
Site C	34°02' 39.81"	108°12' 20.55"	30
Site C	34°02'2 39.81"	108°12' 20.55"	65

Table 3. Two Way-ANOVA significance levels for the main and interaction effects of sites (S) and depths (D) on water bacterial community diversity of JIN PEN drinking water reservoir, Shaanxi Province, northwest China

Parameters of water bacterial community diversity	Site (S) (*F*-value)	Depth (D) (*F*-value)	Interaction (S*D) (*F*-value)
$AWCD_{(590nm)}$	17.73***	10.28**	3.48*
Species richness (*R*)	24.37***	9.64**	1.78ns
Shannon's diversity (*H*)	30.95***	12.06***	2.55ns
Carbohydrates	20.13***	13.27***	3.12*
Carboxylic acids	31.78***	10.11**	1.96ns
Amino acids	21.62***	9.32**	3.05*
Polymers	29.93***	13.01***	2.43ns
Phenolic compounds	25.11***	8.54**	1.82ns
Amines	17.62***	10.17**	3.39*

ns non-significant $P > 0.05$, *$P < 0.05$, ** $P < 0.01$,*** $P < 0.001$.

Table 4. Carbon substrates with significant correlation coefficients (Pearson's correlation coefficient) for PC1 and PC2 in principle component analyses of water bacterial community functional diversity patterns for each sampling sites. Carbon substrates with the $r \leq 0.5$ are shown ($P < 0.05$)

PC1	*r*	PC2	*r*
a-Cyclodextrin	0.785	Glucose-1-phosphate	0.697
Glycogen	0.800	Phenyl ethylamine	0.633
a-D-L-lactose	0.874	Phenylalanine	0.755
i-Erythritol	0.748	D-Mannitol	0.873
D-Glucosaminic acid	0.854	Glucose-1-phosphate	0.838
D,L-a-Glycerol	0.767	Tween40	0.779
g-Hydroxy butyric acid	0.849	a-Ketobutyric acid	0.842
Itaconic acid	0.667		
Serine	0.777		
D-Cellobiose	0.805		

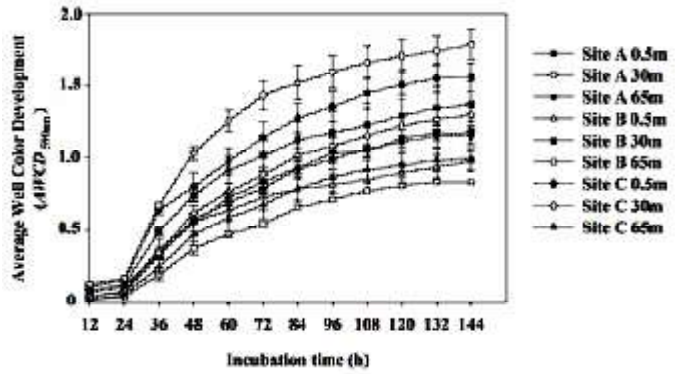

Fig. 1. Average Well Color Development ($AWCD_{590nm}$) of bacterial community in the water collected from different sampling sites (Site A, B, C) and depth (0.5m, 30m, 65m) of the JIN PEN drinking water reservoir. The data expressed by the mean and standard errors (n=3)

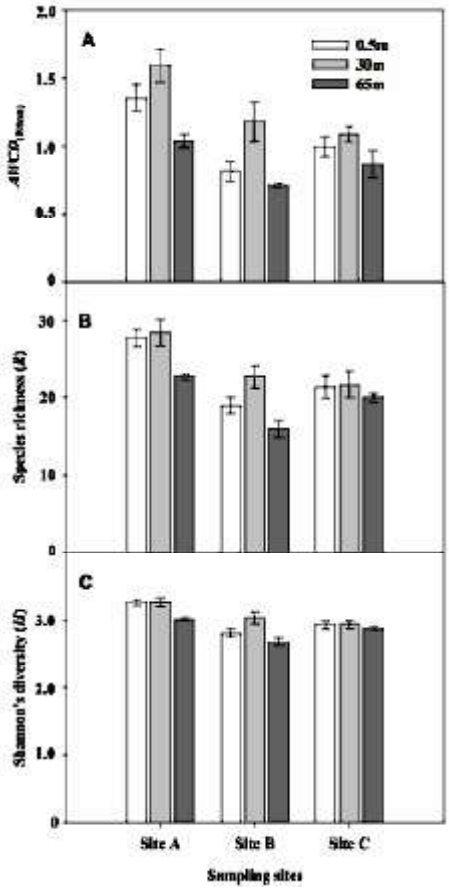

Fig. 2. $AWCD_{590nm}$, species richness (R) and Shannon's diversity (H) of bacterial community functional diversity in the water of collected from different sampling sites (Site A, B, C) and depth (0.5m, 30m, 65m) of the JIN PEN drinking water reservoir. The data expressed by the mean and standard errors (n=3)

sources utilized by water bacterial community showed strong variations within depth in drinking water reservoir. We found out that in the surface water, carbohydrates were easily utilized, while in the lower layers 65m, amino acids were utilized (data not shown).

Principal components analysis of BIOLOG profiles

To analyze the BIOLOG profiles, principal components analysis (PCA) was used. PCA ordination reveals differences in water bacterial community functional diversity among samples, with 32.96% of the variability explained by PC1 and 23.22% of the variability explained by PC2. Site B was located in the top of graph. PCA showed that the water bacterial communities changed significantly throughout the Site depending on depth. Carbon substrates with significant correlation coefficients for PC1 were glucosaminic acid, L-lactose, hydroxy butyric acid, etc. D-mannitol, ketobutyric acid, glucose-1-phosphate had higher significant correlation coefficients for PC2 (Table 4).

Water microbial community structure acts as a vital role in defining drinking water quality and health, such as carbon, nitrogen and phosphorus cycling, drive organic matter transformation [3, 13, 15, 22]. However, significant gaps remain in our understanding of spatial pattern of bacterial community functional diversity in a drinking water reservoir. This work provides substantial new insights into the bacterial community functional diversity in the drinking water reservoir. Water bacteria are important drivers for several biogeochemical cycles in aquatic

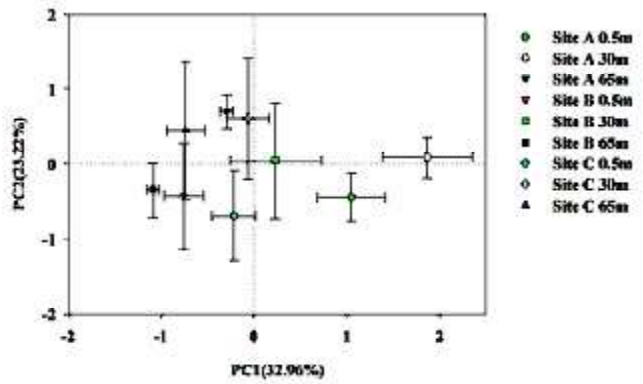

Fig. 3. Principal components analysis (PCA) of carbon utilization profiles of water bacterial community collected from different sampling sites (Site A, B, C) and depth (0.5m, 30m, 65m) of the JIN PEN drinking water reservoir. PC1 explains 32.96% of the variance of the data and PC2 explains 23.22% of the variance in the data. The data expressed by the mean and standard errors (n=3)

ecosystems and play a critical role in most nutrient transformations in water and sediment [16]. Several techniques were used for determine microbial community, such as BIOLOG, PLFA, PCR-DGGE, and pyrosequencing. In this survey, a cultural dependent BIOLOG method was used. BIOLOG method is based on explaining profiles of sole carbon sources utilization represented by color development in a 96 well plate [24]. In the previous reports, BIOLOG has been widely employed to determine microbial community functional diversity from various environmental conditions, including soil [25], air [26], wetland [27], and so on.

Choi and Dobbs [24] compared the abilities of BIOLOG ECO and GN plates to distinguish the water bacterial communities from ballast water, chesapeake Bay, tidal creek, oceanography pond, tony's pond, groundwater, and suggested that BIOLOG is a fast and useful technique to explore water bacterial community. The activity and structure of microbial communities affect water nutrient cycling. The main important reason was different water quality affects water bacterial community diversity. Physical properties of water such as temperature, dissolved oxygen and pH may be shape water bacterial community metabolic characteristic.

In this study, we found that water bacterial community activity and functional diversity were changed from the surface to the bottom of drinking water reservoir. The similar survey conducted by Comeau *et al.* [28] found that the markedly differences of water bacteria communities between the surface (2-12 m) and deeper (29-60 m) strata of a perennially stratified saline Arctic Lake using molecular method high-throughput 16S rRNA gene tag-pyrosequencing, because the strong different limnological conditions. Meanwhile, the same phenomenon was also observed other fresh water body. This result corresponded to a cultivation-in dependent study of Karlov *et al*. The distribution of water microbial community diversity in the upper (1.3 m) and bottommost horizons (367 m) of the Lake Radok water column was determined by Karlov *et al*. [29] and suggested that distinctly microbial stratification of the Lake Radok water column with different microbial community composition. Previous studies indicated that water properties such as pH value or temperature are important drivers of bacterial community structure. Water microbial community was dramatically driven by water physicochemical property, it is therefore that saline and limnological factors may also drive community composition.

Integrate cultural dependent method BIOLOG and cultural independent techniques such as qPCR and tag-pyrosequencing, temporal pattern of drinking water microbial community composition should be investigated, and the relationship between water quality and microbial community should be examined deeply in the future.

CONCLUSIONS

In this work, the spatial pattern of structural and functional diversity of microbial

communities in JIN PEN drinking water reservoir was evaluated. $AWCD_{590nm}$ was increased steadily during the cultural periods. The $AWCD_{590nm}$ varied significantly and was the highest $AWCD_{590nm}$ (1.60±0.12) in 30 m of Site A, and lowest (0.71±0.01) in 65 m of Site B. The Shannon's diversity (H) in 30 m of Site A was 3.26±0.06, which was significantly higher than that of 65 m in Site A with 2.68±0.06 (P<0.05). The significant "sites" and "depth" indicates that the $AWCD_{590nm}$, R and H varied among the sites within the depth (P<0.05). However, there were no significant two-way interactions (P>0.05). Principle component analyses (PCA) of BIOLOG profiles indicated that bacterial community functional diversity was varied in different depths and sites. Carbon substrates with highest significant correlation coefficients for PC1 and PC2 were hydroxy butyric acid and mannitol. Meanwhile, BIOLOG method can be used as a suitable and sensitive tool for determination of drinking water bacterial community functional diversity in the future.

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