

Carbon metabolic properties and functional diversity of airborne microbes in different regions of Lanzhou, China, in autumn

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Abstract - The Metabolic functional diversity of airborne microbial communities and its relationship with environmental factors in different regions [Scenic Area (SA), Main Traffic Line (MTL), Public Service Area (PSA), Culture and Education Area (CEA)] in Lanzhou were analyzed using Biolog ECO technique and redundancy analysis (RDA) method. Results showed that average well color development (AWCD) values of different regions were at stationary phase after 192h of cultivation and showed significant regional differences in the overall utilization of carbon source, PSA>SA>MTL>CEA. The primarily used carbon sources by airborne microbes in Lanzhou are esters and amino acids. There are 13 types of carbon sources that are significantly correlated to principal component 1, of which amino acids are the main carbon sources responsible for the regional difference experienced by Lanzhou airborne microbes. Environmental factors are closely related to metabolic function of airborne microbes. Temperature and wind speed were the leading factors responsible for the difference that was noticed at different functional regions.

Key Words: airborne microbe, metabolic characteristics, functional diversity, Biolog

1. INTRODUCTION

Air microorganisms are an important part of the ecosystem. The composition of microbes present in the air is relatively unstable. Presently, about 1200 species of bacteria and actinomycetes and 40000 species of fungi are known to exist in the air. Species diversity are found in soil, water, plants, animals, human, animal feeds and sewage treatment [1-2]. Air microorganisms have direct effects on the ecological balance and other life phenomenon, and play a very important role in the life cycle of nature [3]. At present, the study of urban air microbes mainly focuses on the characteristics, size, distribution and community structure of the population, and their health risk [4-6]. Study concerning the diversity of the microbial community's metabolic function and relationship with environmental factors in urban ecosystems is relatively small [7]. The use of Biolog micro plates to determine the microbial community's ability to utilize different carbon sources through microbial metabolic fingerprinting to characterize microbial communities in a more general multidimensional form has provided enormous carbon metabolism information on microbial functional diversity. Biolog technology has been widely applied in environmental microbial community. The present study used Biolog-ECO technology and redundancy analysis (RDA) to investigate the functional

diversity of air microbial community in different districts of Lanzhou, China. The microbes' different functional metabolism was compared in relation to environmental factors (wind speed, temperature, humidity and UVB) in order to accurately understand the city's air microbial community and ecological functions. The findings will provide a scientific basis for the evaluation of air microorganism in relation to the influence it has on environmental and human health.

2. MATERIALS AND METHODS

2.1 Research site

Four different regions in Lanzhou city were selected for this study: scenic area (SA), main traffic line (MTL), public service area (PSA) and culture and education area (CEA). From these areas, Xing Long Mountain, Lanzhou Railway Station, provincial hospital and Lanzhou Jiaotong University were respectively chosen. Lanzhou Jiaotong University was used as the monitoring point. Xing Long Mountain is a tourist scenic site with little or no surrounding buildings and has a low human influx rate. It has no traffic congestion problems and has more than 95% green vegetation. The Lanzhou Railway Station is the MTL with a lot of surrounding hotels, banks, cars, businesses and construction sites, as well high human population. It has no green vegetation. The provincial hospital is a public place that has frequent outgoing and incoming patients and visitors. The hospital has surrounding residential buildings and a research center. It has low influx rate of cars and low green vegetation. Lanzhou Jiaotong University is a cultural and educational site surrounded by a library, and dormitory and teaching buildings. It has a small number of cars and up to 40% of green vegetation. The human population in the school premises depends on the time of day and term time. The human population is comparatively high when the school and classes are in session and vice visa

2.2 Sample collection

A liquid impact sampler was used for air microorganism sample collection. The sampling time was mid October 2016; monitoring time was 9:00, 13:00 and 17:00 GMT. Each sample was collected thrice and every

sampling was repeated twice. The sampling flow of each air sample was 12.5L/min at a sampling time of 1h. Samples were collected using 15ml PBS buffer in the absence of light and stored at 4°C. During sampling, the wind speed was measured by AR866 anemometer; the ambient temperature and humidity were measured by TES1360A temperature hygrometer; the UVB intensity was measured by UV-B UV radiometer (Table1).

Table-1: Environmental factors of sampling sites

Regions	Wind speed (ms ⁻¹)	Temperature (°C)	Humidity (%)	UVB (µWc m ⁻²)
Scenic Area (SA)	0.753	18.5	52.7	134.8
Main Traffic Line (MTL)	0.237	20.5	58.4	51.5
Public Service Area (PSA)	0.356	16.2	64.5	24.4
Culture and Education Area (CEA)	0.118	14.0	75.5	17.9

2.3 operational use of Biolog-ECO board

The Biolog-ECO plate was used to analyze the metabolic diversity of the air microbial community in different areas of Lanzhou. For each monitoring time, sampling liquid mixtures were inoculated onto the ECO plate (inoculation volume, 150 µl/hole). The inoculated micro plate was placed in an incubator at 30°C for 24h. The optical density values were measured at 590nm and 750nm using Biolog instrument. This measurement was continuously performed for 10 days.

2.4 Data analysis

The metabolic activity of microbial communities was measured by average well color development (AWCD) [8]:

$$AWCD = \sum(C_i - R) / n$$

Where: C_i is the optical density of each culture medium well; R is the optical density of the control well; N is the culture medium carbon source (this study = 31).

After 168h of culturing, principal component analysis (PCA) was used to analyze the Biolog data, functional diversity index and the calculation of different carbon source utilization rate.

The Shannon index (H) was used to assess the species richness of the community while the McIntosh index (U) measures the average degree of community species [9]:

$$H' = -\sum(P_i \cdot \ln P_i)$$

$$U = \sqrt{\sum n_i^2}$$

Where: P_i is the ratio of the difference in the optical density on each culture and control medium well to the total difference in the optical density on the whole reaction medium well, i.e. $P_i = (C_i - R) / \sum (C_i - R)$,

$$n_i = C_i - R.$$

Therefore, we were able to attain standardization variables and eliminate influence of the absorbance change in the operational process and culturing.

The Simpson index (D), also known as the dominance index, is used to evaluate the dominance of the most common species. It is used to express the diversity of carbon sources, but focuses more on describing the differences that exist between them [10-11]:

$$D = 1 - \sum P_i^2$$

Statistics and analysis of data was performed using Excel 2007 software. Origin8.5 was used for plotting graphs for graphical representation of data. PCA and one-way ANOVA were performed using SPSS 20 software, while, RDA analysis was carried out by Canoco software.

3. RESULTS

3.1 metabolic carbon activities of microbial communities in the air

The AWCD change rate revealed the functional diversity of microbial communities at the metabolic level, which reflects their overall utilization rate of the 31 kinds of carbon sources (Figure 1). With the extension of incubation time, there was an increasing trend in the utilization of carbon sources in the air at different regions, as well as significant spatial differences ($P < 0.01$). The results show that, after 24h of culturing, the AWCD of different regions entered logarithmic growth phase, and at 192h a stable growth phase was attained. Metabolic carbon activity of PSA and SA was significantly higher than that of MTL and CEA ($P < 0.01$), and there was significant difference between SA and PSA ($P < 0.05$). The trend in the ability to use carbon sources generally showed $PSA > SA > MTL > CEA$.

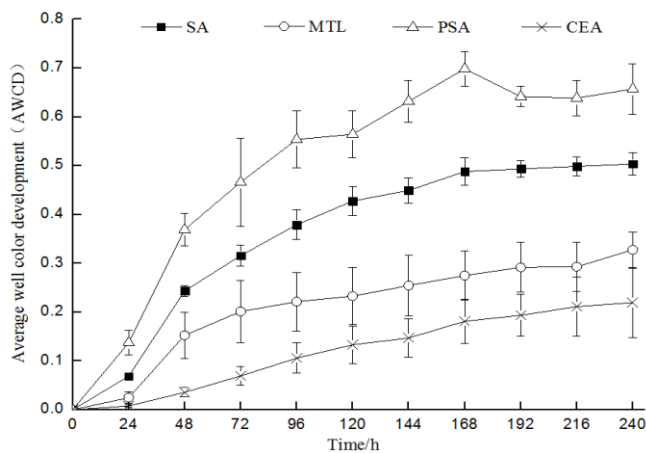


Figure 1. Average well color development changes of airborne microbes at different regions of Lanzhou. SA, scenic area; MTL, main traffic line; PSA, public service area; CEA, culture and education area.

3.2 Characteristics of different types of carbon sources used by airborne microbes

Air microorganisms in the different functional regions of Lanzhou city were found to make use of six different kinds of carbon source. These carbon sources comprises of seven forms of sugar, four forms of esters, three forms of alcohols, three forms of amines, and eight forms of acids. The relative utilization rate, as shown in Figure 2, shows that SA and PSA air microorganisms using different carbon sources were higher than that of the other two regions. Analysis of variance showed that there were significant differences in the rate at which the six different kinds of carbon sources were utilized by microbes of different regions ($P < 0.01$). In Lanzhou, the most important used carbon sources were esters and amino acids. Esters had the highest metabolic rate, while amino acids had the lowest level of metabolism, which was significantly different at the various regions ($P < 0.01$). There were also differences in the utilization of different carbon sources at the same region.

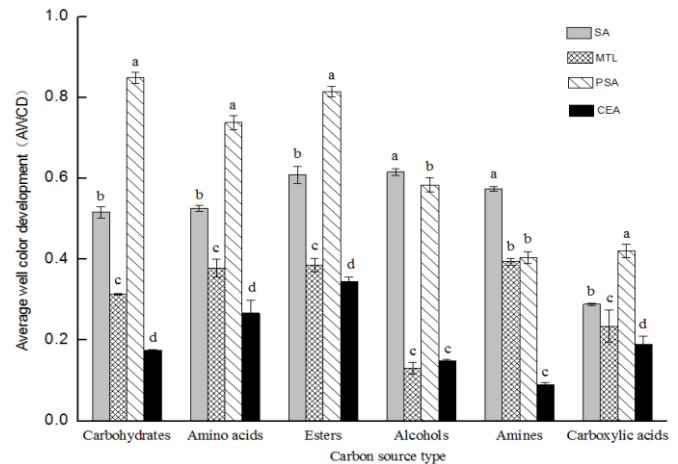


Figure 2. Relative utilization ratio of different carbon sources by airborne microbes in different regions of Lanzhou. Different letters for the same carbon source means a significant difference among different functional regions at $P < 0.05$. SA, scenic area; MTL, main traffic line; PSA, public service area; CEA, culture and education area.

3.3 Principal component analysis of metabolic function of microbial community

Principal component analysis (PCA) was used to analyze the metabolic function of microbial communities of different regions of Lanzhou (Figure 3). The contribution rate of principal component 1 (PC1) was 55.467%, and the contribution rate of principal component two (PC2) was 28.264%. The PC1 load is greater than 0.85 with 13 kinds of matrix, including amino acids (4), other form of acids (2), esters (2), sugars (3), amines (1), and alcohol (1). The PC2 load was also greater than 0.85 with 4 kinds of matrix, including acids (2), amino acids (1), and esters (1) (Table 2). The analyses showed that CEA and MTL air microbes were similar and close, indicating that there were no significant differences in their microbial community and carbon metabolism rate; however SA, PSA and CEA regions were relatively scattered and far apart. The distance between them shows diversity among the three groups of air microbial community as well as differences in carbon metabolism rate and function. The type of matrix and air microorganism present was significantly correlated with the PCI capability of using carbon sources. The high PCI load and the metabolic functions of the microbial communities present in the different regions of Lanzhou city define its uniqueness and significance.

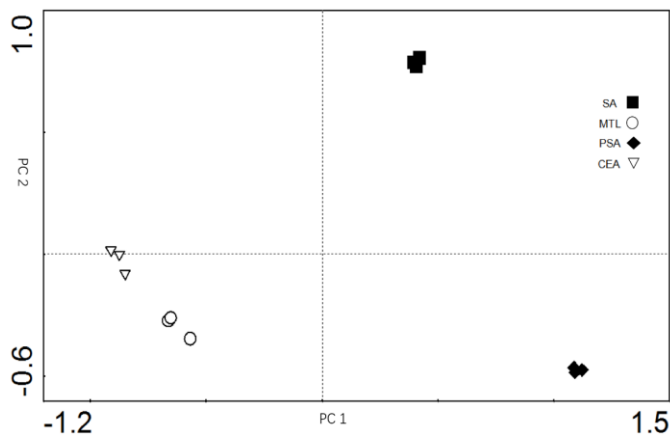


Figure 3. Principal component analysis of carbon utilization rate of airborne microbes in different regions of Lanzhou. SA, scenic area; MTL, main traffic line; PSA, public service area; CEA, culture and education area.

Table-2: Main carbon sources significantly correlated with PC1 and PC2 in principal component analysis.

Type	Carbon source	Principal component	
		PC1 (r)	PC2 (r)
Carbohydrates	Glycogen	0.940	
	β-Methyl-D-Glucoside	0.958	
	Glucose-1- Phosphate	0.928	
Amino acids	L- Phenylalanine		-0.857
	Glycyl-L-Glutamic Acid	0.997	
	L-Arginine	0.956	
	L-Asparagine	0.868	
	L-Threonine	0.856	
Esters	40 Tween 40	0.981	
	D-Galactonic Acid γ-Lactone	0.918	
	Pyruvic Acid Methyl Ester		0.899
Alcohols	D-Mannitol	0.895	
Amines	Phenylethyl- amine	0.896	
Carboxylic acids	γ-Hydroxybutyric Acid	0.994	
	2-Hydroxy Benzoic Acid	-0.853	
	Itaconic Acid		-0.909
	D-Malic Acid		0.932

3.5 Spatial variance of microbial community diversity and its correlation with environmental factors

Studies have shown that the functional diversity of the microbial community was related to environmental factors [12-13]. In Lanzhou City, the function of the air microbial communities and their diversities in different regions (carbon metabolism and functional diversity index) in relation to environmental factors is shown in Figure 4. The results show that the diversity and functions of air microorganisms in different regions are

significantly different: PSA and SA are located at the positive side of the first ordinal axis while the MTL and CEA are located on the negative side; the PSA and MTL are located on the positive end of the second ordinal axis while the SEA and SA are on the negative side. The first ordinal axis was positively correlated with temperature (0.1125), wind speed (0.5631) and UVB (0.2144), and negatively correlated with humidity (-0.4424). The second ordinal axis was positively correlated with temperature (0.7341), humidity (-0.3274) and UVB (-0.1858). The Shannon index and Simpson index were positively correlated with wind speed, humidity and UVB, and negatively correlated with temperature. The McIntosh index was positively correlated with temperature, UVB and wind speed, and was negatively correlated with humidity. Comprehensive analysis showed that there were significant differences in microbial functional diversity at different regions in Lanzhou. The main factors that affected the spatial diversity of air microbial diversity was temperature and wind speed.

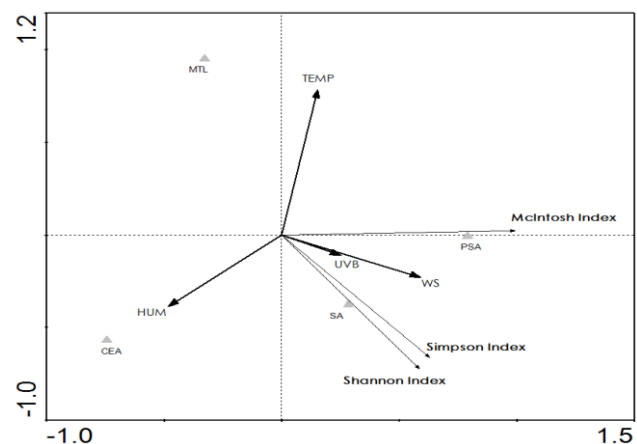


Figure 4. Redundancy analysis of spatial variation of carbon metabolic function of airborne microbes in correlation with environmental factors. TEMP, temperature; HUM, humidity; WS, wind speed; SA, scenic area; MTL, main traffic line; PSA, public service area; CEA, culture and education area.

4. DISCUSSIONS

The Biolog ECO microplate is mainly used for the analysis of microbial community characteristics. This is done by using different carbon sources to characterize the metabolic and functional diversities of the microbes [14-15]. The micro plate has a certain concentration and activity requirements for the inoculation of microbial samples. This study used a liquid impact sampler for the collection of air microorganism samples. This is because it can effectively maintain the physiological activities of the collected microbial samples [16]. The results showed that there were significant differences in the overall utilization rate of carbon sources at different functional regions in

Lanzhou during autumn (Figure 1). PSA had the highest carbon source utilization ability. One of the main reasons for the increase in the concentration of airborne microorganisms in this area can be attributed to the fact that the wind speed was higher compared to that of ground dust particles. Also, the release of bacterial microbes, as a result of coughing, sneezing and breathing, as well as the release of those attached to skin and hair of people may be another reason for the high concentration of air microbes in this area.

Therefore, the air microbial community structure found in PSA is not the same as other regions, for example, SA is a scenic area and has rich vegetation. The presence of moisture in these areas (PSA and SA) also provides a good and conducive environment for air microbial growth. These features enable the air microbes in these functional areas to have a higher carbon metabolism rate. Carbon metabolism rates at MTL and CEA were relatively low, which was probably due to low wind speed and the presence of strong air resistance microorganisms, such as *Bacillus* in these regions [17].

The carbon sources that have the best air microbial metabolic advantage in the four functional areas studied at Lanzhou were esters and different types of amino acids. Principal component analysis showed that amino acids was mainly responsible for the Lanzhou city air microbial community metabolic function, as well as the differentiation experience at the different regions. The research by Chanjuan *et al.* [7] in Hangzhou city regarding air microbial community metabolism rate, also shows that carbohydrates and amino acids are the main type of carbon sources that are usually used. Wu *et al.* [12] found that the carbon metabolism process by air microorganisms in Qingdao city is of a different type: carboxylic acid. The findings of this study were quite different, which may be due to differences in regions and environmental factors. These differences lead to regional variations in carbon metabolism by air microorganisms. In relation to habitat environmental factors affecting air microbial community diversities and metabolic function, the RDA analysis showed that there is a good correlation between the functions of Lanzhou city air microbial communities at different regions. Environmental factors, such as temperature and wind speed, as well as carbon metabolism and functional diversity index showed greater correlation. The temperature maintains the microbial physiological activities of microbes [18], while wind speed can change the air microbial community composition and concentration [19]. Temperature and wind speed were the main factors to cause differences in the effects of spatial variance in Lanzhou air microbial functional diversity.

The Biolog ECO microplate is one of the major methods used to study the diversity of microbial communities in the environment by characterizing the diversity of microbial communities with different multidimensional data [20]. However, since the air microbial composition

varies, there exists a complex relationship between microbial community structure and functional diversity; the Biolog method can only determine microorganism functional diversity in relation to their differences in carbon source utilization degree and abilities. Future study should combined Biolog and molecular biology methods to effectively and accurately understand the city air microbial diversity and its ecological function.

5. CONCLUSIONS

In the present study, investigation on the functional diversity of the air microbe community in different regions of Lanzhou was performed. From our findings, firstly, we conclude that there were significant differences in the carbon source metabolic activity of the air microbial communities, as well as the carbon source metabolic activity of PSA and SA in the four regions of Lanzhou in autumn. Secondly, the primary used carbon source by air microbes in the four regions of Lanzhou were esters and amino acids. Different types of amino acids were utilized by the microbial communities located at the different regions in Lanzhou for their metabolic functions and activities. Lastly, temperature and wind speed were the main factors influencing the spatial diversity of air microbial diversity in Lanzhou.

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BIOGRAPHIES



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