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Characterization of microbial communities of soils from gold mine tailings and identification of mercury-resistant strain

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ABSTRACT

To enrich the understanding of the complex environmental system of soil and microorganisms in gold tailings, we studied the effects of environmental factors on the microbial community diversity in gold mine tailing soil in Beijing, and the strains screened from the soil with serious mercury (Hg) pollution. The results showed that microbial diversity and community composition varied among sites, and at varying depths, soil microbes were significantly affected by soil environmental factors such as lead (Pb), Hg, pH, and total organic carbon (TOC). Pb and Hg negatively affected soil microbial diversity, and less-polluted soil showed increased microbial diversities and complex community structure. Community composition analysis showed that *Firmicutes*, *Proteobacteria* and *Actinobacteria* were the dominant microorganisms. Moreover, Hg-resistant bacterial species isolated from soil samples were identified as *Pseudomonas plecoglossicida* with a high Hg tolerance efficiency. This study is important in understanding the microbial diversity and function in gold mine tailing soils and can widen the application for bioremediation process.

1. Introduction

Anthropogenic activities such as mining (Pavilonis et al., 2017), smelting (Fröhlichová et al., 2018; Yun et al., 2018), and production of chemicals and electronics (Luo et al., 2018; Su et al., 2018) have caused damage to the environment because of the heavy metals that they produce. Tailing is one of the pathways in which these metals enter into the environment (Bech et al., 2016). The heavy metals in tailing are taken up by organisms in the biogeochemical cycle and food chain, and then cause serious potential risks of food and ecological safety to residents. Gold (Au) tailings contain large amounts of toxic substances such as mercury (Hg) and cyanide (CN), which are used in mining (Huang et al., 2012). Hg is always used in the process of Au extraction because pure Au and volatile Hg can be obtained when heating Au-Hg alloy (Chamba et al., 2017). The toxicity of Hg, a hazardous and persistent environmental pollutant, leads to a number of clinical conditions in humans (Jia et al., 2017; Mahbub et al., 2016).

Soil microbial community does not only contribute to soil fertility but can also serve as an indicator of the survival to changes in the microenvironment conditions (H. Xiao et al., 2017). The soil microbial community can respond to changes in soil ecological mechanisms and environmental stress, and bring about the change of community structure. It plays a key role in the operation of soil ecosystems by establishing diversity of their structure and/or activity (Pignataro et al., 2012). Therefore, soil microbial community is considered as an early warning and sensitive indicator of changes in the soil ecosystem and has an important directive function to soil quality change (Zhang et al., 2011). With the growth of soil ecology research, interest on microbial community and metal-resistant strains has become increasingly important in ecological sciences. The soil microbial community is the most important living ingredient in soil (X.Y. Xiao et al., 2017). Soil characteristics (horizontal distribution, vertical distribution) and environmental factors (total organic carbon (TOC), pH, heavy metal and relative humidity) affect the abundance of soil microorganisms (Guo et al., 2016). However, with respect to mining-related pollution risk assessment, such interactions between environmental factors and soil organisms are still less studied and understood.

Studies related to the ecological environment of mine tailings have shown that the diversity and abundance of microorganisms in reclaimed soil significantly affects the diversity and stability of vegetation in that area (Mendez et al., 2008; Sherriff, 2005). The presence of heavy metals in soil environment will result in low density of microorganisms. Therefore, the mine tailing soil is one of the main receivers of mineral discharged wastes, and the presence of gold mine tailing inevitably

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causes pollution in the soil ecosystem as well as change in microbial community structure (Simonovicova et al., 2017). Analyzing the effects of environmental factors, especially heavy metals, on microbial community in gold mine tailing can guide the restoration of mining-related contaminated soil (Bararunyeretse et al., 2017). In addition, exploring the microbial community structure in contaminated soil can be conducive to obtain resistant strains for the study of heavy metal resistance genes (Guo et al., 2017).

Recently, considerable attention has been paid to the effects of Hg pollution on humans (Buch et al., 2017), and finding an effective solution has become the key to current research. The method of controlling Hg pollution by biology has long been a subject of interest in recent studies (Mahbub et al., 2017). Microorganisms can be used not only to indicate Hg contaminated soil but also for remediation (Geesey et al., 2016). Because of their adaptability to the environment, many microorganisms can resist heavy metals such as Hg. These Hg-resistant microorganisms play an important role in the remediation of environmental Hg pollution. In the field of microbial remediation on Hg-polluted soil, the reported strains with anti-Hg properties include *Escherichia coli*, *Proteus* and *Saccharomyces* (Chen et al., 2018; Rodríguez et al., 2016; Zhao et al., 2005). Microorganisms in soils contaminated with Hg may have evolved a complete set of mechanisms for Hg tolerance (Rahman and Singh, 2016). Screening Hg-resistant bacteria and performing detailed research can provide a clear idea and direction for Hg pollution remediation in mining areas.

Most of the research on gold mining and tailings were focused on heavy metal sources, distribution, and risk assessment, and few had studied the microbial diversity and community structure in mining areas (Cai et al., 2017; Clifford, 2017; Gulley, 2017; Pavilonis et al., 2017). Furthermore, little attention has been paid to the contribution of Hg-resistant bacteria in multiple-heavy-metal resistance and monitoring of contaminated areas. In this perspective, the current survey aims to observe the impact of heavy metal pollution on microbial communities along with assessing Hg resistance in heavy metal-resistant bacteria isolated from surface soil. Experiments were conducted in gold mining area located in Huairou and Miyun districts, Beijing, China. Soils in the study area are contaminated with gold mine waste, resulting in high concentrations of heavy metals. Therefore, they are suitable for studying microbial communities under heavy metal-polluted soils. The objectives of this work are to (1) analyze the microbial diversity at varying soil depths of gold mine tailings, (2) study the types and characteristics of microbial community structure in soils with different depths of gold mine tailings, (3) evaluate the effects of soil environmental parameters on soil microbial diversity and activity, and (4) investigate isolated bacterial strain for heavy metal resistance and heavy metal removal ability. Studying the diversity of microbial communities and the characteristic of Hg-resistant bacterial strains in gold mine tailing soils will help us to have a better understanding of soil and microorganisms, which is a complex environmental system. Furthermore, it is helpful to provide a scientific basis for future research on resistance genes and the bioremediation of soil contaminated with Hg.

2. Material and methods

2.1. Study area

The study area (40°39′21″-40°48′55″N, 116°39′35″-117°00′27″E) is located in Huairou and Miyun districts, northeast of Beijing. The climate of the study area is a cool continental monsoon with annual precipitation between 400 mm and 700 mm and temperature of 9–13 °C (Han et al., 2017; Q. Li et al., 2014). The main types of soil are brown, cinnamon, tidal, and paddy soils (Yang et al., 2011). The nearby river basin belongs to the upper reaches of the Miyun Reservoir (Fig. 1). Many gold and iron mining areas are present in the upper reaches of the Miyun Reservoir, which are part of the Yanshan gold mineralization belt (Yang and Zhao, 1994), and several amounts of tailings are piled up near the gold mining area. The contaminants diffuse toward to the periphery through precipitation or acidification, and then pollute the nearby soil and water (Leiva and Morales, 2013; Shu et al., 2017; Zhao et al., 2012). In this study, the soil samples were collected.

2.2. Sample collection and storage

Soil samples were collected twice in September and November 2015. Vertical profile soil samples used for microbial community and diversity studies were collected around Xituogu gold tailing in Miyun district, Houanling gold tailing and Qidaoliang gold tailings in Huairou district (Fig. 1 and Table S1) in September 2015. Surface soil samples were collected near the Xituogu gold tailing (Fig. 1 and Table S1) in November 2015, which were heavily polluted. The surface soil samples with high concentration of Hg were screened to examine the response of microorganisms to Hg. The vertical profile soil sampling depth was 0–75 cm, and the profile stratification are 0–15 cm, 15–30 cm, 30–45 cm, 45–60 cm and 60–75 cm, respectively. Three subsamples of each soil layer collected from a circular area (50 cm in diameter) were mixed to obtain the composite soil samples. Random sampling method was used to collect the topsoil (0–20 cm) of the study area in November. GPS (Garmin GPS 72H) was used to identify the sample locations. A total of 35 soil samples were collected in the research area (Fig. 1), which included 6 sets of soil profile (A, C-G) and 5 surface soil samples (S1–S5). The information of sampling are shown in Table S1. All soil samples were packed in polyethylene bags and stored in a refrigerator.

Naturally air-dried soil sample was sieved (2 mm × 2 mm mesh size) to remove stones, debris, and other large particles. Then, it was sealed in plastic and preserved for measurement. A portion of the soil sample was cryopreserved in 10 mL sterile centrifuge tube for sequencing and microorganism culture experiments

2.3. Analytical method

The pH of the soil sample was measured in a beaker using combined glass calomel electrode in a 1:2.5 mixture (soil/water ratio, w/v) (Abbas et al., 2018), and total organic carbon (TOC) content was determined using the Total Organic Carbon Analyzer (Elemetar, Germany). Mixed acid digestion (5:4:4:2, v/v/v/v, $HCl + HF + HNO_3 + HClO_4$) of soil samples was conducted before the determination of total concentrations of Cr, Cu, Zn, Ni, and Pb in the soil (Jiang et al., 2018; Wu et al., 2018). Briefly, a 0.5 g soil sample was digested with 10 mL of HCl, 8 mL of HF, 8 mL of HNO_3, and 4 mL of $HClO_4$ in a closed Teflon vessel on an electric hot plate at ~ 190 °C until no black material remained and the silicate minerals had completely disappeared. The digested solution was then diluted to 50 mL with deionized water for subsequent measurement using an ICP-MS (PerkinElmer NexlON 300Q, USA). The concentration of Hg was determined by atomic fluorescence spectroscopy (AFS-920; Beijing Jitian Instrument Co., Beijing, China). In detail, 0.5 g of soil was wetted with a small amount of deionized water, and then 6 mL of HCl and 2 mL of HNO_3 were added to digest the samples. The closed Teflon vessel was heated under a set program with a microwave digestion instrument. Deionized water was then added to dilute the solution to 50 mL for measurement (Chamba et al., 2017; Q. Li et al., 2014).

2.4. Screening of Hg-resistant strains

2.4.1. Domestication of Hg-tolerant strains

To prepare a bacterial suspension, 10 g of grounded soil S1 sample was placed in a conical flask containing 90 mL of sterile water. Then, the solution was allowed to stand after being shaken for 15 min. The supernatant was extracted and inoculated in beef peptone medium (beef extract 5.0 g/L, peptone 10.0 g/L, and NaCl 5.0 g/L) (Liu et al., 2012). The medium was supplemented with 10 mg/L Hg^{2+}. The samples were then incubated and agitated on a thermostat shaker (140 rpm,