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Technical Note

Culturable microbial groups and thallium-tolerant fungi in soils with high thallium contamination

Jialong Sun [a,b], Xiao Zou [c], Zengping Ning [a], Min Sun [a], Jingquan Peng [b], Tangfu Xiao [a,*]

[a] State Key Laboratory of Environmental Geochemistry, Institute of Geochemistry, Chinese Academy of Sciences, Guiyang 550002, China
[b] Guizhou Institute of Environmental Science and Design, Guiyang 550002, China
[c] Institute of Fungal Resources, Guizhou University, Guiyang 550002, China

HIGHLIGHTS

- We reported the culturable microbial groups in Tl-polluted soils.
- The fungal population can grow in the presence of high Tl level up to 1000 mg kg^{-1}.
- We have isolated and identified nine Tl-tolerant fungal strains.
- The isolated Tl-tolerant fungi were potential sources for bioremediation.

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ABSTRACT

Thallium (Tl) contamination in soil exerts a significant threat to the ecosystem health due to its high toxicity. However, little is known about the effect of Tl on the microbial community in soil. The present study aimed at characterizing the culturable microbial groups in soils which experience for a long time high Tl contamination and elevated Hg and As. The contamination originates from As, Hg and Tl sulfide mineralization and the associated mining activities in the Guizhou Province, Southwest China. Our investigation showed the existence of culturable bacteria, filamentous fungi and actinomyces in long-term Tl-contaminated soils. Some fungal groups grow in the presence of high Tl level up to 1000 mg kg^{-1}. We have isolated and identified nine Tl-tolerant fungal strains based on the morphological traits and ITS analysis. The dominant genera identified were *Trichoderma*, *Penicillium* and *Paecilomyces*. Preliminary data obtained in this study suggested that certain microbes were able to face high Tl pollution in soil and maintain their metabolic activities and resistances. The highly Tl-tolerant fungi that we have isolated are potentially useful in the remediation of Tl-contaminated sites.

1. Introduction

The adverse effects of various heavy metals on microbial communities have been well documented (e.g. El-Sharouny et al., 1988; Arriagada et al., 2009; Haller et al., 2011; Margesin et al., 2011). Some studies have also shown that heavy metals can reduce the soil microbial biomass and the size of viable microbial population (e.g. Hu et al., 2007; Zhang et al., 2007, 2010), while certain microbial species can tolerate high metal exposures in soil (Wang et al., 2007a; Margesin et al., 2011; Sousa et al., 2012). Although the focus has been on bacteria (Chen et al., 2009; He et al., 2010; Pepi et al., 2011), some metal-tolerant fungi have been shown to be capable of immobilizing toxic metals by forming insoluble metal oxalates (Norris et al., 1976; Chen et al., 2009). These organisms can therefore successfully serve as efficient adsorbents for removal of heavy metals (Słaba and Długoński, 2011; Yin et al., 2011; Abd-Alla et al., 2012). Since the metal-tolerant fungal isolates can compete with the indigenous bacterial microflora in hostile situations (Lopez Errasquin and Vazquez, 2003; Arriagada et al., 2007), they have an advantage over bacteria for the bioremediation on polluted soils (Peter and Viraraghavan, 2008; Leung et al., 2010; Kalpana et al., 2011; Sharma and Adholeya, 2011).

Thallium (Tl), generally with low concentration in soil, has attracted increasing environmental concerns due to its high toxicity (Zitko, 1975; Tremel et al., 1997; Xiao et al., 2004a, 2007). Besides playing an important role in the dispersion of Tl compounds in the environment (Sklodowska and Matlakowska, 2004), microorganisms can oxidize Tl ion to Tl_2O_3 in their mitochondria (Lindegren and Lindegren, 1973). Studies on Tl uptake kinetics have indicated that Tl is rapidly bound presumably to cell surfaces, and is progressively accumulated by *Saccharomyces cerevisiae* and *Escherichia coli* through energy-dependent transport systems (Norris et al., 1976). However, little information is available about the toxicity of Tl to microbial groups

* Corresponding author at: Guanshui Road 46, Guiyang 550002, Guizhou Province, China. Tel./fax: +86 851 5895318.
E-mail address: xiaotangfu@vip.gyig.ac.cn (T. Xiao).

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in soil. Similarly, little is known about the occurrence of Tl-tolerant fungi in long-term Tl-polluted soils.

Some soils in Guizhou Province, Southwest China are highly contaminated with Tl, derived from the mineralization of Tl-rich sulfide and mining activity. As a result, crops grown in these soils accumulate high amounts of Tl (Xiao et al., 2004a), and Tl has been recognized as a major metal pollutant that causes the chronic poisoning on the local population, evidenced by epidemical symptoms of alopecia and high Tl concentrations in urine (Zhou and Liu, 1985; Xiao et al., 2007). In the present study, we aimed to characterize the culturable microbial groups isolated from the long-term Tl-polluted soils in Guizhou Province, Southwest China. In addition, we aimed to evaluate the in vivo tolerance of fungal isolates to Tl exposure with a view to using the isolates for the remediation of Tl-polluted soils. The results of this study would provide useful information for assessing the microbial susceptibility to Tl pollution, and also provide an insight into Tl remediation potential using such high tolerance fungal isolates.

2. Materials and methods

2.1. Study area

The study area is located on Lanmuchang (105°30′23″E, 25°31′28″N), a small town with approximately 1000 inhabitants, in the southwest Guizhou Province of Southwest China. The local residents suffer from chronic Tl poisoning and display some symptoms, such as weakness, muscle and joint pain, disturbance of vision, hair loss, and high Tl levels in urine, and these symptoms are all induced by high Tl contaminations in local soils, water and crops (Zhou and Liu, 1985; Xiao et al., 2007). The Tl source is the local sulfide mineralization of Tl, arsenic (As) and Hg, and mining activities. For example, Tl concentration is 100–35,000 mg kg^{-1} in sulfide minerals, 40–124 mg kg^{-1} in soils, 0.8–495 mg kg^{-1} in crops, 13–1966 μg L^{-1} in groundwater and 1.9–8.1 μg L^{-1} in stream water (Xiao et al., 2003; Xiao et al., 2004a,b,c). The elevated Tl contents in the local environment are prone to Tl pollution, thereby presenting a severe threat to public health of the local population (Xiao et al., 2007, 2012).

This area presents a karst topography, exhibiting a higher elevation in the northwest and a lower elevation in the southeast. The average altitude is 1400 m, and the relative relief is 100–200 m. The local outcropping rocks are composed of limestone, argillite and siltstone from Permian to Triassic in age. Previous studies have described the local geology and sulfide mineralization in details (Xiao et al., 2003, 2004c). The Lanmuchang area has been widely developed for agricultural and residential purposes.

2.2. Sampling and analysis

A total of 10 soil samples were collected from the Tl mineralized area within Lanmuchang and a background area without major Tl pollution (Fig. 1). The sampling patterns, regardless of sequential or random, corresponded to the characteristics of soils associated with pedogenesis, mining-related disturbance, and topographical characteristics (e.g. hill top, hill slopes and lowland areas) related to the Tl mineralization. These patterns served to delineate the variations of Tl in this study area, and were categorized into groups as follows: soils in the mining area (soils derived from mine wastes and arable soils in the mining areas), slope wash materials, undisturbed natural soils and background soils.

All the soil samples were kept in polyethylene bags and air-dried in the laboratory prior to final processing. The soils were passed through a 2-mm sieve for geochemical analysis. The sieved fractions were then ground in a Bico ceramic disk grinder, and they were further ground to 80-mesh (−180 μm) powder in a ceramic ball mill. A portion of each soil sample for microbiological analyses was immediately kept in sterile sacks at site and stored in coolers at 4 °C and then shipped to the laboratory where they were kept in coolers in the dark at the same temperature.

Soil samples for geochemical analysis were digested in a mixture of concentrated acids ($HF/HNO_3/HClO_4$), and the initial contents of Tl, Hg and As were determined using an inductively coupled plasma mass spectrometry (ICP-MS, ELAN DRC-e, Perkin-Elmer, USA). The soil pH was determined by a pH meter (AISI pHB9901, Taiwan; solid:de-ionized water = 1:5). Moreover, total carbon (TC) and total nitrogen (TN) were analyzed by dry combustion using an element analyzer (PE2400II, Perkin-Elmer, USA).

Standard references of soils GBW07403 and GBW07408 (National Institute of Standard Materials, China) were used to control the analysis quality. The analytical precision was determined by quality assurance/quality control procedures using duplicates, blanks, internal standards (Rh at 500 μg L^{-1}) and reference samples, and the result was better than ±10%.

2.3. Microbial assays in soil samples

The culturable microbial groups were determined from the fresh soil samples collected at the site. The culturable microbes in soils were enumerated for viable cells by the plate-count method. In order to obtain a soil suspension, 5 g fresh soil was added into 45 mL of sterile Milli-Q water, and the mixture was shaken at 180 rpm for 15 min. The serial dilutions of soil suspensions were arranged in Milli-Q water for the enumeration and isolation of the culturable microorganisms, and were prepared 3 min before use. Then, the dilutions of soil suspensions were surface spread onto agar plates. Colony-forming units (CFU g^{-1} dry soil) of culturable bacteria, actinomyces and filamentous fungi were determined on meat-peptone agar, Gause's starch agar and Martin agar, respectively (Huang, 1999). Bacterial colonies were counted at 28 °C after 48 h. Both actinomyces and fungal colonies were counted at 25 °C after 72 h, respectively.

2.4. Tolerance assay and isolation of Tl-tolerant strains

Tolerance assay was conducted in a 60-mm Petri dish test unit with agar medium containing Tl. To explore the tolerance of the isolated fungal strains, optimal culture conditions were employed with various initial Tl concentrations. Each test unit was supplemented with $TlNO_3$ (Merck, Germany) at concentrations of 200, 400, 600, 800 and 1000 mg kg^{-1}, respectively. After an incubation period of 24–48 h, CFUs were determined in each test unit. Agar medium without Tl addition was used as a control. All tests were performed in triplicate.

At a Tl level of 1000 mg kg^{-1}, all the fungal colonies were microscopically analyzed and transferred to Gause's starch agar. The fungal isolates were then purified and stored on potato dextrose agar slants at 4 °C until further analysis.

2.5. Identification of Tl-tolerant fungal isolates

The fungal isolates were identified by morphological traits and fungal ITS1-5.8S-ITS2 region sequence analysis. DNA was extracted from fungal isolates using a cetyltrimethylammonium bromide (CTAB) method (Stewart and Laura, 1993). The ITS1-5.8S-ITS2 region was amplified and sequenced using fungal-specific primers ITS1F (5′-CTTG GTCATTTAGAGGAAGTAA-3′) and ITS4 (5′-TCCTCCGCTTATT GATATGC-3′) as previously described (Martin and Rygiewicz, 2005). The sequences were searched against those already known at NCBI GenBank (http://www.ncbi.nih.gov/index.html) using BLAST search option. The sequences of ITS region were aligned with the sequences of similar fungi retrieved from databases using CLUSTAL X, and a phylogenetic tree was constructed using the neighbor-joining algorithm (MEGA version 4.0) with the bootstrap analysis of 1000 replicates (Kumar et al., 2004).