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Screening, characteristics and mechanism of Cd-tolerance *Cunninghamella bertholletiae*

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ABSTRACT

An isolate, designated as ZZY, was obtained from a long-term heavy metals contaminated soil with the potential to remove Cd^{2+}. The strain's screening, identification and biosorption characteristics including the effect of initial concentration of metal ions, pH value, biosorption temperature, biosorption time, inoculum size, mineral nutrients, and competing ions on biosorption were studied. Based on morphological observations and 18 S rRNA gene sequence identification, ZZY had affinity to *Cunninghamella bertholletiae*. The optimum parameters for Cd^{2+} biosorption by the strain were: Cd^{2+} tolerance of 4 mg/L, pH value of 4, biosorption time of 84 h, biosorption temperature of 35 °C, quantity of added thallus of 6% (v/v), the mineral collaborative concentration of 0.05 g/L (mass of mineral nutrition per liter of medium). In addition, the determination of biosorption sites distribution and the results of Fourier transform infrared spectroscopy (FT-IR) showed that biosorption occurred at the thallus surface, the chief active biosorption sites being O–H, C=O, and N–H.

1. Introduction

An important class of pollutants that is of significant concern, because of its presence in agricultural soil and in water, recalcitrance towards biodegradation, potential to bio-accumulate, is heavy metal contamination. Heavy metals may be introduced into soil by waste water irrigations and by application of solid waste, metal mine tailings, pesticides, and fertilizer. They can damage organizational structure and function of some plants, thus lead to the decline of yield and quality in crops, for example excessive cadmium content would destroy the structure of chlorophyll, cause wilting and even death of plants. In addition, the enrichment of heavy metals in plants and human bodies via food chain (Li and Li, 2010) would definitely do harm to human health. Due to the lack of appropriate cost-effective technologies, remediation of such contamination mainly including physical-chemical remediation, agricultural-ecological remediation and bioremediation (Li et al., 2014) has been challenging.

Under normal conditions, soil microbes are vulnerable to heavy metal pollutants, showing a sharp decrease in the density of the microbial population or even its extinction. However, over time, the microbes may adapt to the heavy metals, surviving and gradually becoming the dominant population in the soil (Åkerblom et al., 2007). In the presence of high concentration heavy metals, certain microbes tolerant to heavy metals to some extent might survive and some could reduce the toxicity by biological transformation or metabolic activity. The mechanisms of microbial resistance to metals mainly includes biosorption, extracellular precipitation, biological accumulation and excretion (Wang et al., 2015). In recent years, many researches showed that cadmium contamination had become an increasingly serious problem in agricultural soil in China (Huang et al., 2005). Hence, novel strains isolated from mixed contaminated soils and displaying high tolerance to heavy metals could be used as a potential cost effective remediation strategy. With this view, the present study concentrated on isolating and characterizing a strain capable of extensive Cd^{2+} biosorption, and on further exploring the influence of initial metal concentrations, pH value, biosorption temperature, biosorption time, inoculum size, mineral nutrient concentration, and effect of competing ions on biosorption, which was followed by a preliminary study of the biosorption mechanism.

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2. Materials and methods

2.1. Soil sampling

The strain ZZY (*Cunninghamella bertholletiae*) was isolated from contaminated soils from Huaihua city in Hunan province (heavy metal polluted area) and entered into the China General Microbiological Culture Collection Center (number CGMCC 7.259). The GenBank accession number for the 18 S rRNA gene sequence of Cunninghamella bertholletiae was KU375550.1. The mineral nutrient used in the study was provided by Tianjin Aolvshennong Science and Technology Company Limited and the product contained 16 kinds of nutrient elements including Mn, B, Cu, Fe, Zn, Mo and so on.

2.2. Screening, isolation and purification of strains

The flowchart of the experiment procedure was showed in Fig. 1. Firstly, soil samples (10 g) were mixed with 90 mL sterile distilled water in flasks at a constant shaking rate of 150 rpm. After centrifugation and serial dilution, supernatants were daubed in sequence to Luria-Bertani broth medium, Gause I medium and Martin solid medium containing 100 mg Cd^{2+} per liter. Bacteria were cultured at 37 °C for 1–2 d; fungi were cultured at 28–30 °C for 3–5 d. After preliminary culturing, strains were re-inoculated on medium containing successively higher concentrations of Cd^{2+} in order to obtain strains with increased Cd-tolerance and the highest Cd^{2+} concentrations in medium was 1200 mg/L.

Strains with higher Cd-tolerance were selected for further experiment. For purification, the edge of a single bacterial colony was streaked on fresh agar medium containing no added heavy metal under aseptic conditions. After incubation several days, the streaking was repeated three times and the morphology of the colonies was observed. Besides, fungi were purified by picking slight strains on fresh medium using inoculating loop under aseptic conditions. The operation was repeated three times and the colony morphology were also observed. The other procedure was similar with that of bacteria.

2.3. Morphology and biological characteristic of strains

The fungus strain ZZY with the highest Cd-tolerance was inoculated on Martin and PDA medium and cultured at 29 °C for 5 days. Colonial morphology, surface texture, colony exudate, and soluble pigment were observed with a low-power lens of an optical microscope. Hyphae and meristematic brussel of colonies were dyed with lactophenol cotton blue solution and observed by a high-power optical microscope. The detailed process was summarized as follows. Firstly, a drop of lactophenol cotton blue solution was added on the slide. Then, a small amount of hyphae with meristematic brussel was picked by a anatomical needle and placed in the solution. When the hyphae was dispersed evenly in the aid of the anatomical needle, the coverslip was placed on it and hyphae as well as meristematic brussel was observed.

2.4. Molecular identification of strains

For molecular identification, strain ZZY was cultured in conical flasks in liquid medium at 35 °C, 150 rpm, for 132 h. The composition of improved liquid medium was listed in Table 1. The culture was harvested by centrifugation at 10,000 rpm for 10 min. The sedimented mycelium was freeze-dried with liquid nitrogen and pulverized. Fungal genomic DNA was extracted according to CTAB method (Attitalla, 2011). The 18 S rRNA gene was amplified and sequenced by universal fungus primers: ITS1 and ITS4 (Hunt et al., 2013). The PCR conditions were as follows: initial denaturation at 94 °C for 4 min, followed by 40 cycles comprising denaturation at 94 °C for 40s, annealing at 56 °C for 1 min, extension at 72 °C for 2 min. Final extension was done at 72 °C for 10 min. The sequence was submitted in NCBI nucleotide database and compared with

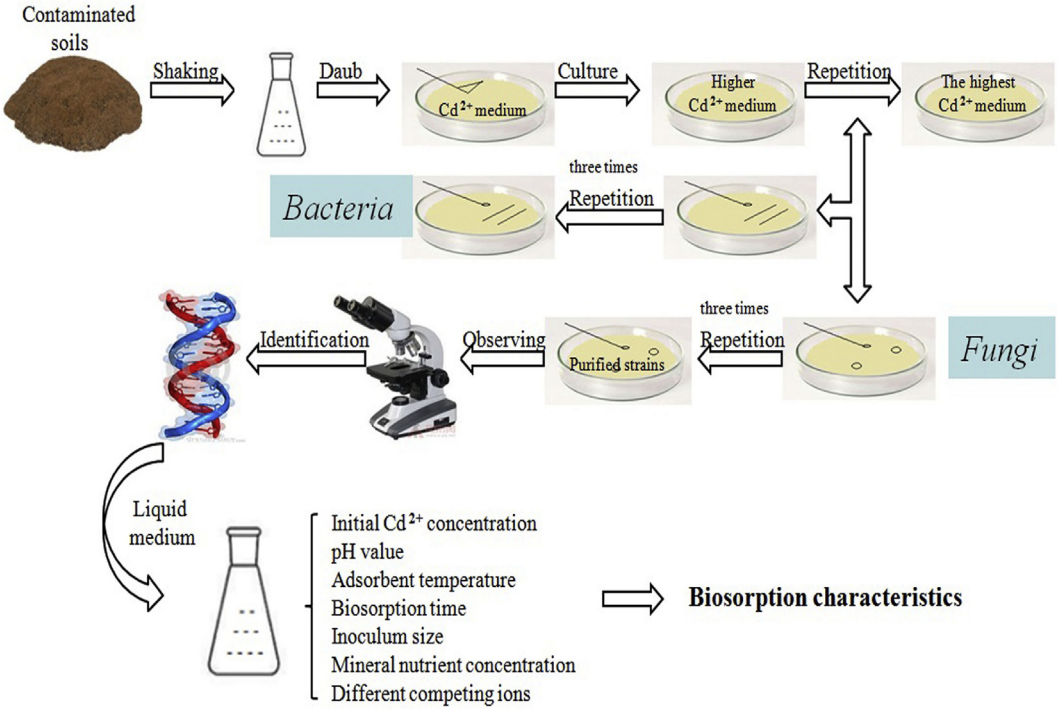

Fig. 1. The flowchart of the experiment procedure.