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Isolation of vanadium-resistance endophytic bacterium PRE01 from *Pteris vittata* in stone coal smelting district and characterization for potential use in phytoremediation

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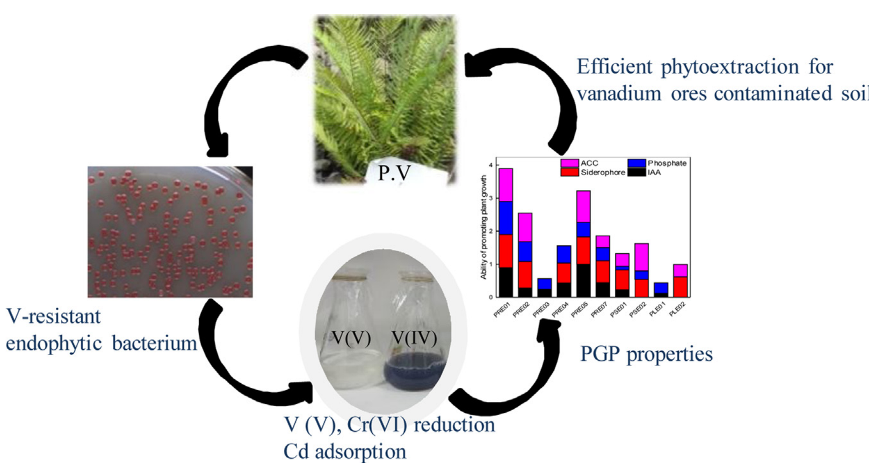
HIGHLIGHTS

- *Pteris vittata* was a better V accumulator and have higher V resistance.
- V-resistant endophyte PRE01 from *P. vittata* was isolated and identified.
- PRE01 had plant growth promoting traits and strong V, Cr and Cd resistance.
- PRE01 possessed ability of V(V) and Cr(VI) reduction and Cd absorption.
- PRE01 was valuable for phytoextraction of vanadium ores contaminated soils.

GRAPHICAL ABSTRACT



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ABSTRACT

This study investigates the V-resistant endophytic bacteria isolated from V-accumulator *Pteris vittata* grown on stone coal smelting district. Among all the ten isolates, the strain PRE01 identified as *Serratia marcescens ss marcescens* by Biolog GEN III MicroPlate^{TM} was screened out by ranking first in terms of heavy metal resistance and plant growth promoting traits. The *S. marcescens* PRE01 had strong V, Cr and Cd resistance especially for V up to 1500 mg/L. In addition, it exhibited ACC deaminase activity, siderophore production and high indoleacetic acid production (60.14 mg/L) and solubilizing P potential (336.41 mg/L). For heavy metal detoxification tests, PRE01 could specifically assimilate 97.6%, 21.7% and 6.6% of Cd(II), Cr(VI) and V(V) within 72 h incubation. Despite the poor absorption of the two anions, most V(V) and Cr(VI) were detoxified and reduced to lower valence states by the strain. Furthermore, the isolate had the potential to facilitate the metals uptake of their hosts by changing heavy metal speciation. Our research may open up further scope of utilizing the endophyte for enhancing phytoextraction of vanadium industry contaminated soils.

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1. Introduction

Vanadium (V) is an important alloying element and widely used in metallurgy, aerospace, chemical, and other industrial sectors in the form of ferrovanadium, V compounds, and metallic V [1]. Vanadium primary resources come from ores, concentrates and vanadiferous slag and are mainly mined in South Africa, China, Russia, and the USA [2]. However, the relevant anthropogenic heavy metals in the environment have increased significantly in recent years due to the high-temperature vanadium industrial activities [3]. Mining and smelting activities especially for vanadiferous magnetite and stone coal have caused serious soil heavy metals pollution worldwide [4–6]. Moreover, as associated minerals, stone coals contain various other metals (e.g. Cr, Cd, As and Pb and so on) besides V [7]. Therefore soil contamination related to mining and smelting of vanadiferous ores might involve multiple heavy metals. Nevertheless more research is biased towards single vanadium contamination and no attention has been paid to other associated metals contamination in vanadium mining area and smelting area [8,9]. Our previous study, the field-investigation around stone coal smelting district, in Hubei Province, China, shows that the soil of the smelter surrounding area has general V, Cr and Cd pollution phenomena; moreover, V and Cr pollution is conspicuous [10]. Additional, in smelting slags contaminated site, the average concentrations of V, Cr, Cd and Cu are 931, 721, 1.4, 279 mg/kg, respectively, which are both several times greater than Soil Environmental Quality Standard set by China [11]. Overall, multiple heavy metals co-pollution of vanadium ores can't be ignored and probably induces potential risks to the ecosystem and human health [12–14].

Several physicochemical methods such as soil washing/flushing, vitrification, electroremediation, soil solidifying have been used to remediate these contaminated sites [15–18]. Despite the great progress achieved, these technologies have limiting factors of high cost, application in large-scale areas, generation of secondary pollution and their unsustainable nature. As a low-cost and eco-friendly technology with high public acceptance, phytoremediation refers to using plant roots to take up heavy metals and storing them in harvestable parts and then removing them from contaminated sites [19,20]. However, the efficiency of phytoremediation is restricted by different factors such as low plant yield, low growth rate, multi-metals pollution and excessive metals concentration etc. [21]. Therefore, more researchers focus on the roles of plant-associated bacteria to enhance phytoremediation.

Endophytic bacteria, which have enormous application potentiality in phytoremediation [22,23], can colonize in plant cells or intracellular space without causing harm to the hosts. In polluted soils, endophytes and their host plants have formed a mutual benefit symbiotic partnership during the long-term evolution. The hosts provide a better living environment for endophytes in terms of nutrition and competition pressure etc. As a reward, endophytes can promote plant growth and reduce the damage of heavy metals on the host through their own metal resistance system [24–26]. For example, a multi-metal resistant endophytic bacterium L14 from Solanum nigrum L. could alleviate metal toxicity by removing 75.78%, 80.48%, 21.25% of Cd, Pb and Cu [27]. Inoculated with the Cd-resistant endophyte SaMR12, Sedum alfredii exhibited higher Cd tolerance and accumulation [28].

In recent years, people pay more attention to heavy metal polluted soil by vanadium ores. However, most studies focus on environmental geochemistry and ecological risk of vanadium ores pollution [4,8,29]. There are only a few researches on phytoremediation of these contaminated sites especially for co-pollution of vanadium and its associated metals Cr and Cd [10,30]. The application of plant-associated endophytes to enhance phytoremediation of these multi-metal polluted soils is still blank due to lack of valuable strains having plant growth promotion and metal detoxification capacities. The objectives of this study were to (I) isolate and characterize such valuable endophytic strains from *Pteris vittata* growing on stone coal smelting district, (II) evaluate their ability in plant growth promotion and (III) access their capacity in detoxification of V, Cr and Cd by investigating biosorption or bioreduction. The endophytic bacterium is expected to be a promising bioresource for enhancing phytoremediation in heavy metals pollution of vanadium ores.

2. Materials and methods

2.1. Preparation of medium and metal solution

All reagents in the test were of analytical grade. The certain concentrations of V(V), Cr(VI) and Cd(II) stock solutions were prepared by dissolving exact quantities of $NaVO_3$, $K_2Cr_2O_7$ and $Cd(NO_3)_2$ with deionized water. The working concentrations of V(V), Cr(VI) and Cd(II) solutions were obtained from suitable serial dilution of the stock solutions.

The Luria Bertani (LB) medium containing 10 g/L tryptone, 5 g/L yeast extract, 10 g/L NaCl and sucrose-minimal salts (SMS) medium comprising of 10 g/L sucrose, 1 g/L $(NH_4)_2SO_4$, 2 g/L K_2HPO_4, 0.5 g/L $MgSO_4$; 0.1 g/L NaCl, 0.5 g/L yeast extract, 0.5 g/L $CaCO_3$. The pH of the medium was adjusted to 7.2–7.4.

2.2. Heavy metals analysis

The exact metal concentrations of working solution, filtrate of heavy metal ions and digested samples of plant tissues and rhizosphere soils in the following experiments were determined by inductively coupled-plasma optical emission spectrometer (ICP-OES) (Thermo Scientific ICAP 6000 series, Thermo Fisher Scientific Inc., USA).

2.3. Plant screening and sampling area description

Considering that vanadium hyperaccumulator plants have not yet been found and there have not been many studies on vanadium accumulator plants. To screen a good endophytes carrier for isolating such valuable endophytes, we conducted a related literature review and field investigation. *Pteris vittata* was proved to be the best V accumulator. Healthy *Pteris vittata* plants were collected from the contaminated sites of stone coal smelter in the southeast of Yunxi County (32°57′ N, 110°26′ E), Shiyan City, China.

2.4. Isolation of V, Cr and Cd resistant plant growth promoting endophytes

Plant samples of *P. vittata* were rinsed with tap water extensively followed by three rinses with deionized water and then separated into roots, stems and leaves. For the purpose of surface disinfection, healthy plants tissues were immersed in 75% (v/v) ethanol for 2 min and then 3% sodium hypochlorite solution for 2 min and rinsed three times with sterilized water. After then, the root, stem or leaf tissue was cut and fully grinded in sterile phosphate buffer saline (pH = 7.4). Appropriate dilutions (100 μL) were plated onto LB agar medium amended with 100, 100 and 10 mg/L V, Cr and Cd, respectively. To confirm the surface sterilization is effective, the final rinse water was plated on LB medium. All the plates were incubated at 30 °C for 72 h.