Chemosphere 154 (2016) 358–366

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Endophytic bacterium *Sphingomonas* SaMR12 promotes cadmium accumulation by increasing glutathione biosynthesis in *Sedum alfredii* Hance

Fengshan Pan, Qian Meng, Qiong Wang, Sha Luo, Bao Chen, Kiran Yasmin Khan, Xiaoe Yang, Ying Feng*

MOE Key Laboratory of Environment Remediation and Ecological Health, College of Environmental and Resources Sciences, Zhejiang University, Hangzhou 310058, PR China

HIGHLIGHTS

- The antioxidant enzymes of *S. alfredii* root were not sensitive to Cd stress and SaMR12.
- SaMR12 significantly reduced root H_2O_2 concentration under high Cd stress.
- SaMR12 increased root GSH concentration and its biosynthetic genes expression.
- SaMR12 improved Cd accumulation by affecting root GSH and ROS metabolisms.

ARTICLE INFO

Article history:
Received 26 January 2016
Received in revised form
10 March 2016
Accepted 25 March 2016

Handling Editor: Martine Leermakers

Keywords:
Plant growth promoting bacteria
Cd
Reactive oxygen species (ROS)
Glutathione (GSH)
Gene expression

ABSTRACT

A hydroponic experiment was conducted to verify the effects of inoculation with endophytic bacteria *Sphingomonas* SaMR12 on root growth, cadmium (Cd) uptake, reactive oxygen species (ROS), anti-oxidases, glutathione (GSH) and the related gene expression of *Sedum alfredii* Hance under different levels of Cd such as 0, 10, 25, 100 and 400 μM. The results showed that inoculation of SaMR12 improved Cd accumulation and upregulated *glutathione synthase* (*GS*) expression, but slightly reduced malon-dialdehyde (MDA) concentration and alleviated Cd-induced damage in roots. However it didn't alter the activities of antioxidant enzymes. When Cd concentration exceeded 25 μM, SaMR12 increased the concentration of GSH and the expression level of *GSH1*. At high Cd treatment levels (100 and 400 μM), SaMR12 significantly reduced H_2O_2 concentration and enhanced expression level of 1-Cys peroxiredoxin PER1 and ATPS genes. These results indicate that although SaMR12 has no significant effects on anti-oxidases activities, it reduces H_2O_2 concentration by enhancing GSH concentration and relevant genes expression, and subsequently improves Cd tolerance and accumulation.

1. Introduction

Cadmium (Cd) is a non-essential element for plant growth and has a high toxicity to plant (Jia et al., 2013). Excessive Cd results in uncontrolled redox reactions in cells and causes accumulation of reactive oxygen species (ROS) to damage cellular structure and disorder the metabolisms (Singh and Shah, 2014). Plants possess some protective and repair mechanisms to alleviate oxidative damage in the process of evolution. The antioxidative system is composed of two categories. One category comprises antioxidant enzymes that react with oxygen and keep it at a low concentration, such as superoxide dismutase (SOD), catalase (CAT) and guaiacol peroxidase (POD). The other comprises antioxidants or chelates, such as glutathione (GSH) and phytochelatins (PCs) (Gill and Tuteja, 2010; Smirnoff, 1993; Sun et al., 2007). The production of antioxidant enzymes and antioxidants or chelates is closely related to some genes expression, such *PER1*, *peroxidase*, *ATPS*, *GSH1*, *GS* and *PCS*. PER1 represents a subgroup of the peroxiredoxin family of thiol-requiring anti-oxidants with one conserved cysteine residue (1-Cys), and displays in vitro anti-oxidant activity (Stacy et al., 1999). Adenosine triphosphate sulfurylase (ATPS) is the first enzyme in plant sulfur assimilation pathway that catalyzes the

* Corresponding author.
E-mail address: yfeng@zju.edu.cn (Y. Feng).

http://dx.doi.org/10.1016/j.chemosphere.2016.03.120
0045-6535/

formation of adenosine phosphosulfate from ATP and sulfate (Liang et al., 2014). *GSH1* and *GS* are the synthase enzymes genes for GSH (Liang et al., 1999). When the plants are exposed to environmental stress, the expression levels of related genes are altered as adaptive response that minimize the damage from the stress (Clemens, 1999; Na and Salt, 2011; Liang et al., 2014).

With the rapid development of industry, Cd-contamination to soils has become a severe environmental problem and caused extensive concern throughout the world. Compared with physicochemical remediation methods, phytoremediation is an environmentally friendly and cost-effective technique to remedy Cd contaminated soil (Glick, 2010; Gupta et al., 2013). However, phytoremediation efficiency is limited due to phytotoxicity at high heavy metal concentrations and small biomass production by the remediating plants (Mani and Kumar, 2014).

Plant growth promoting bacteria (PGPB) can promote plant growth and improve phytoremediation efficiency. Numerous researches indicate that endophytic bacteria can successfully colonize in the root, promote plant growth, improve plant tolerance to heavy metals and increase heavy metal accumulation in plant (Rajkumar et al., 2009; Leung et al., 2013; Ma et al., 2015). Fourteen endophytic bacteria were isolated from the root of *Sedum alfredii* Hance (*S. alfredii*) and were demonstrated to have properties that promoted plant growth and Zn accumulation in the roots and shoots of *S. alfredii* (Long et al., 2011). The endophytic bacteria *Sphingomonas* SaMR12 was isolated from *S. alfredii*, it could colonize successfully in the root of *S. alfredii* (Zhang et al., 2013). Our previous study indicated that *Sphingomonas* SaMR12 could promote plant growth, protect root of *S. alfredii* from Cd damage, and decrease hydrogen peroxide (H_2O_2) and superoxide anion ($O_2^{\cdot-}$) concentration both in the roots and shoots of *S. alfredii* (Chen et al., 2014a,b). Although there are many reports about the effects of Cd stress on plant antioxidative system, the study of endophytic bacteria affecting the responses of plant antioxidant enzymes and antioxidants, as well as the expression of antioxidant related genes at Cd exposure in heavy metal hyperaccumulators is scanty.

The aim of this study was to test, at different Cd levels, the effects of SaMR12 inoculation on: 1) Cd concentration and uptake in plant; 2) the responses of ROS, antioxidant enzymes and related gene expression; and 3) GSH concentration and related gene expression in the root of *S. alfredii*.

2. Materials and methods

2.1. Plant collection and culture

Cd hyperaccumulating ecotype (HE) *S. alfredii* was collected from an old Pb/Zn mined site in Zhejiang province of China. Healthy and uniform shoots of *S. alfredii* were selected and grown 2 weeks with distilled water, and then precultured 4 weeks with 1/4, 1/2 and total modified Hoagland medium in turn (Tian et al., 2011). The pH of nutrient medium was adjusted to 5.5–5.8 using 0.1 M NaOH or 0.1 M HCl and was continuously aerated and renewed every 3 d. *S. alfredii* were randomly placed in a controlled environment chamber (made by http://www.zjuee.cn) with a 14 h photoperiod and day/night temperatures of 26/23 °C. The relative humidity was maintained at 70% (day) and 85% (night).

2.2. Cd treatment, inoculation and sample preparation

After 4 weeks of preculture, the uniform young plants were exposed to a series of Cd levels (0, 10, 25, 100 and 400 μM) as $CdCl_2$. Another group of uniform young plants were inoculated with SaMR12. Bacterial strain SaMR12 was isolated from surface-sterilized roots of healthy *S. alfredii* as described in our previous study (Zhang et al., 2013). The strain was stored in 30% glycerine at −70 °C. Briefly, SaMR12 bacteria were cultured in lysogeny broth (LB) medium overnight, and then centrifuged, washed three times with distilled water, and resuspended. The roots of plant were immersed in the bacterial suspensions for 2 h. After inoculation, the plants were treated with different Cd concentrations as above.

After 6 d of treatment, roots and leaves were collected separately. Roots were soaked in 20 mM Na_2-EDTA for 15 min to remove Cd ions adhering to the root surface. Fresh materials of leaves and roots were immediately frozen in liquid nitrogen and stored at −80 °C prior to analysis.

2.3. Determination of Cd content and uptake

The whole plants were carefully separated into shoots and roots, and washed with 20 mM Na_2-EDTA and deionized water. Then samples were oven dried at 65 °C, weighed, ground and passed through 0.5 mm sieve, and then digested in HNO_3 and $HClO_4$ (5: 1, v/v) until clear. Digested samples were diluted with deionized water and Cd concentration was determined using an Inductively Coupled Plasma Mass Spectrometer (ICP-MS, Agilent 7500a, USA).

2.4. Loss of plasma membrane integrity and malondialdehyde (MDA) concentration

Histochemical detection of loss of plasma membrane integrity in root apices was performed as described by Yamamoto (2001). After different treatments, intact roots of plant were rinsed several times with 0.5 mM $CaCl_2$ (pH 4.5), dried with filter papers. Then roots were incubated in 4 ml of Evans blue solution (0.025% [w/v]) prepared in 0.1 mM $CaCl_2$ (pH 5.6) solution for 30 min. All of the stained roots were washed three times with deionized water and observed under a light microscope (model SZH-ILLD; Nikon, Japan). The tips of root were weighed and ground in liquid nitrogen. MDA concentration was determined using a MDA assay kit (thiobarbituric acid method) (Nanjing Jiancheng Bioengineering Institute, China).

2.5. ROS (H_2O_2 and ($O_2^{\cdot-}$)) content assay

The H_2O_2 concentration was determined according to the method of Jin et al. (2008a). The concentration of ($O_2^{\cdot-}$) was determined by method with 2-naphthylamine-4-aminobenzenesulfonic acid (Tang et al., 2004).

2.6. Antioxidant enzyme assays

Antioxidant enzymes activities were determined according to the method described by Jin et al. (2008b). SOD (EC 1.15.1.1) activity was determined by the photochemical method (Giannopolitis and Ries, 1977). One unit of SOD activity was defined as the amount of enzyme required to cause a 50% inhibition of the nitroblue tetrazolium reduction rate at 560 nm. CAT (EC 1.11.1.6) activity was assayed in a reaction mixture containing 25 mM potassium phosphate buffer (pH 7.0, containing 0.1 mM EDTA), 10 mM H_2O_2, and the enzyme. The decrease in absorbance of H_2O_2 within 1 min at 240 nm ($E = 39.4\ mM^{-1}\ cm^{-1}$) was recorded (Cakmak et al., 1993). Analysis of POD (EC 1.11.1.7) activity was based on the oxidation of guaiacol using H_2O_2. The increase in absorbance at 420 nm was recorded (Liu et al., 2008).

2.7. Determination of GSH

The tips of root were weighed and ground in liquid nitrogen. GSH content was determined using an assay kit (Nanjing Jiancheng