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Effect of Lead and γ-Polyglutamic Acid Produced from *Bacillus subtilis* on Growth of *Brassica chinensis* L.

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Abstract

γ-Polyglutamic acid (γ-PGA) has been reported to be an effective biosorbent for metal ions. *In vitro* binding of lead (II) (Pb) by γ-PGA produced from *Bacillus subtilis* NBRC16449 was examined and the effect of Pb and γ-PGA on *Brassica chinensis* L. grown in the laboratory was investigated. The results revealed that the optimal pH for Pb adsorption was 5.0. γ-PGA was stable at 35-50°C and pH 5-7. The maximum removal efficiency was 87.9%. *Brassica chinensis* L. seeds were germinated and grown at 28±1°C on filter paper soaked with Pb solution at 0, 50, 100 and 250 μM and γ-PGA at 0, 100, 500 and 1,000 mg/L for 7 days. The results indicated that Pb markedly inhibited growth of roots by reducing root length (P<0.05). However; the addition of 500 mg/L γ-PGA significantly protected seedlings from the adverse effects of Pb (P<0.05). Thus, γ-PGA has high potential as a biopolymer to be used for alleviation of Pb toxicities in plants.

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Keywords: γ-polyglutamic acid; biosorption; lead; *Brassica chinensis*; *Bacillus subtilis*

1. Introduction

Lead (II) (Pb) is one of toxic heavy metals released by industry and motor vehicles. It is a potential

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pollutant that persists in the soil and is widespread in the urban environment. Although Pb is not an essential nutrient for plants, it is easily taken up by them from the soil and accumulated in edible parts. Excess Pb in plants causes several toxicity symptoms associated with the retardation of growth, inhibition of root elongation, appearance of chlorosis and inhibition of enzyme activities [1]. Vegetables (Brassicaceae) cultivated in land close to traffic roads and highways in Thailand contain Pb levels between 1.0 and 1.6 mg/kg FW [2] while the maximum permissible level of Pb in vegetables set by Codex Alimentarius (2001) is 0.3 mg/kg FW [3]. Thus, the consumption of edible plant parts might cause adverse health effects in humans. Recently, microbial cells and biopolymers derived from microorganisms have been emerging as an alternative treatment for scavenging heavy metals from soil and aqueous systems. γ-Polyglutamic acid (γ-PGA) is a naturally occurring anionic polymer, and is biodegradable, edible and nontoxic to humans and is benign to the environment. PGA consists of D- and L-isomers of glutamic acid linked by amide bonds between the α-amino and carboxyl groups [4]. In the past few years, there has been interest in various applications of γ-PGA in thickeners, humectants, drug carriers, biological adhesives, foods, cosmetics, medicines, water absorbents, bioflocculants and wastewater treatment [4]. Moreover, it has been reported to be an effective biosorbent for cationic dyes [5] and several metal ions: Ni (II), Cu(II), Mn(II), Al(III) and Cr(III) [6], Hg(II) [7] and Cd (II) [8] in water treatment. Thus, it is of interest to consider whether γ-PGA can be used as a Cd biosorbent with plants and to reduce Pb toxicity in plants. This study investigated the optimal conditions for *in vitro* Pb adsorption by γ-PGA and the effects of Pb and γ-PGA on *Brassica chinensis* L. seedlings grown in the laboratory.

2. Experimental Method

2.1. Production and purification of γ-PGA

γ-PGA from *Bacillus subtilis* NBRC16449 was produced in a PGA-production medium and purified by the prescribed method [9].

2.2. In vitro Pb(II) binding experiments

The reaction was carried out by taking 50 mL of 500 μM of lead(II) chloride ($PbCl_2$) (Sigma, USA) solution and adjusting to pH 5.0. Next, 10 ml of 1,000 mg/L γ-PGA was added and the mixture was stirred at 150 rpm for 10 min at 37°C. Then, samples were taken at different time intervals to determine the residual Pb concentration (no further absorption of Pb). Each sample solution was filtered through 0.2-μm membrane filter and the filtrate was analysed for Pb concentration by using a flam atomic absorption spectrophotometer (AAS) (spectrAA 220 F.S. model, Australia) with an oxidizing flame of an air-acetylene mixture at a ratio of 7:1. The optimum pH for Pb adsorption was determined at pH 3-7. For pH stability, γ-PGA was kept at pH 3-9 at 37°C for 2 h. Temperature stability was performed at pH 7.0 at 30-50°C for 2 h. Finally, Pb adsorption was carried out at the optimal conditions and the residual Pb concentration was analysed by AAS.

2.3. Effect of γ-PGA on lead uptake by Brassica chinensis L.

The healthy *B. chinensis* L. seeds were surface sterilised in 10% sodium hypochlorite for 10 min, then rinsed four times with deionised water. The seeds were soaked in deionised water for 30 min. A filter paper was placed on a Petri dish (9 cm in diameter) and moistened with 5 ml of the Pb and γ-PGA mixture in different series of concentrations (Pb; 0, 50, 100, 250 μM and γ-PGA; 0, 50, 100, 500, 1,000 mg/L). Ten seeds were placed on a Petri dish and incubated in a growth chamber with 12 h light and 12 h darkness at 28ºC±1ºC