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Dracaena sanderiana endophytic bacteria interactions: Effect of endophyte inoculation on bisphenol A removal

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ABSTRACT

Bisphenol A (BPA) is one of the most abundant endocrine-disrupting compounds which is found in the aquatic environment. However, actual knowledge regarding the effect of plant-bacteria interactions on enhancing BPA removal is still lacking. In the present study, *Dracaena sanderiana* endophytic bacteria interactions were investigated to evaluate the effect of bacterial inoculation on BPA removal under hydroponic conditions. Two plant growth-promoting (PGP) bacterial strains, *Bacillus thuringiensis* and *Pantoea dispersa,* which have high BPA tolerance and can utilize BPA for growth, were used as plant inocula. *P. dispersa*-inoculated plants showed the highest BPA removal efficiency at 92.32 ± 1.23% compared to other inoculated and non-inoculated plants. This was due to a higher population of the endophytic inoculum within the plant tissues which resulted in maintained levels of indole-3-acetic acid (IAA) for the plant's physiological needs and lower levels of reactive oxygen species (ROS). In contrast, *B. thuringiensis*-inoculated plants had a lower BPA removal efficiency. However, individual *B. thuringiensis* possessed a significantly higher BPA removal efficiency compared to *P. dispersa.* This study provides convincing evidence that not all PGP endophytic bacteria-plant interactions could improve the BPA removal efficiency. Different inocula and inoculation times should be investigated before using plant inoculation to enhance phytoremediation.

1. Introduction

BPA has been classified as an endocrine-disrupting compound (EDC) by the US Environmental Protection Agency (USEPA) and World Wide Fund for Nature (WWF) (Badiefar et al., 2015). BPA is widely used for manufacturing polycarbonate products, epoxy resins, phenoplast resins, and other special products (EU, 2003; Staples et al., 1998). BPA can be present in municipal wastewater discharges, leachates from waste landfill and industrial wastewaters (Carlisle et al., 2009; Gavrilescu et al., 2015; Kang et al., 2006; Michalowicz, 2014; Syranidou et al., 2017; Zhang et al., 2013). BPA has been detected at concentrations of approximately 257 $\mu g\,L^{-1}$ in municipal effluent (Pookpoosa et al., 2014) and 1.55 $\mu g\,L^{-1}$ in river waters (Deemoon et al., 2016). However, there are no standardized test guidelines or risk assessment guidance for evaluating BPA effects. Therefore, the EU and Japan have set ecotoxicological hazard values based on conventional effects on mortality and reproduction from standardized studies and concentrations of 1.5 $\mu g\,L^{-1}$ and 1.6 $\mu g\,L^{-1}$, respectively (USEPA, 2010). Exposure to BPA causes adverse health impacts especially to the human reproductive system (Carlisle et al., 2009; EU, 2003; Nakamura et al., 2010; Peng et al., 2015).

Several physico-chemical methods can be used for BPA removal, such as alkaline and acid hydrolysis, Fenton oxidation and peroxidation, ozonation, ultrasonication, membrane filtration, and photocatalysis by TiO_2 and UV (Mohapatra et al., 2010; Wang et al., 2009; Zhang et al., 2006). These cleanup technologies are expensive and involve intensive use of chemicals which can pose significant engineering costs and provide secondary wastes. Sustainable techniques for remediation of BPA contaminated sites therefore need to be developed. Biological treatment of environmental toxic pollutants is an alternative remediation technique which offers a sustainable approach for decontamination. Phytoremediation is considered to be an economical green biotechnology which uses plants and their associated microorganisms to remediate toxic pollutants. Many bacteria can completely degrade many organic pollutants. Phytoremediation efficiency could be improved by using plant combinations with their endophytic bacteria according to three main mechanisms: (1) enhancement of plant growth and biomass production, (2) increasing organic pollutant

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bioavailability, and (3) increasing population size and activity of endophytic bacteria to degrade organic pollutants through gene transfer (Afzal et al., 2014).

There are several plants that have been reported to remove BPA efficiently including *Portulaca oleracea* cv., *Phragmites australis*, *Salvia* spp., *Nasturtium officinale,* forage grasses, and some aquatic plants e.g. *Limnobium laevigatum* and *Spirodela polyrhiza*. Furthermore, the role of microbial degradation has been investigated with rhizosphere bacteria (Imai et al., 2007; Okuhata et al., 2010; Reis et al., 2014; Toyama et al., 2009). In a previous study, the native tropical evergreen plant *Dracaena sanderiana* (ribbon plant) was selected for BPA removal in a hydroponic system. The plant showed a high BPA removal ability depleting up to 85% of the initial concentration of 20 μM and tolerance to BPA up to 80 μM (Saiyood et al., 2010). Although, the previous study showed the important role of rhizosphere bacteria in increasing the degradation rate of BPA, interest in the use of native plant species coupled with their endophytic bacteria towards the treatment of BPA has considerably increased (Khaksar et al., 2016c; Saiyood et al., 2010). Furthermore, knowledge on the effects of endophytic bacterial inoculation for BPA removal is unreported. Traditionally, traits for plant growth promotion by bacteria are assessed through, e.g. nitrogen fixation, phosphate solubilization, 1-amino-cyclopropane-1-carboxylate (ACC) deaminase activity, and the production of siderophores and phytohormones. However, testing plant growth promotion in microbial-assisted phytoremediation through plant hormonal networks, e.g. the indole-3-acetic acid (IAA) biosynthetic pathway and ACC deaminase activity seems lacking (Khaksar et al., 2017). Endophytic bacteria could not only enhance the degradation of organic pollutants in plant tissues but also alleviate stress-mediated impacts of pollutants and enhance phytohormone production, e.g. IAA, to protect host plants by controlling intracellular reactive oxygen species (ROS) (Afzal et al., 2014; Khaksar et al., 2017). In order to assess the contribution of bacteria to BPA removal, each bacterial species was inoculated into the plants to determine the effects on BPA removal ability.

To the best of our knowledge, there have been few publications on the feasibility of employing plants inoculated with endophytic bacteria to enhance BPA removal. This research addresses this by investigating the isolation, screening, characterization and identification of endophytic BPA-degrading bacteria in the rhizo- and endosphere of *D. sanderiana* roots. Growth of the bacterial isolates using BPA was studied in order to select those that grew well on BPA for plant inoculation. Interactions between the plant and the selected endophytic bacteria were assessed regarding BPA removal, phytotoxicity tolerance, phytohormone induction and the degree of bacterial colonization of the plant tissues.

2. Materials and methods

2.1. Plant preparation for BPA treatment

Two-month old *D. sanderiana* plants were obtained from a plant nursery in Bangkok, Thailand. Each plant was thoroughly cleaned with distilled water to remove soil and dirt. Plants were then cultured in a hydroponic system using a 2-L glass jar containing 400 mL half-strength Hoagland's nutrient solution for 4 weeks until their roots were fully grown (Saiyood et al., 2010). Afterwards, freshly harvested plants were transferred to a 0.8-L glass jar containing 400 mL of 20 μM BPA in distilled water (one plant per pot). In our previous study, at an initial BPA concentration of 20 μM, *D. sanderiana* could completely remove the BPA within 16 days. Thus, this initial concentration was used in this study. The plants were placed 30 cm under natural luminescence using two cool-white fluorescence lamps (1200–1300 lx) and a 12 h photoperiod at room temperature (28–30 °C).

2.2. Isolation of epi- and endophytic bacteria from D. sanderiana roots

To isolate epiphytic bacteria, plant roots were washed three times with sterile distilled water and placed on the surface of a mineral medium (MM) agar plate containing 20 μM BPA as a sole carbon source. Isolates which grew on MM media were picked off and re-inoculated onto fresh BPA agar plates to obtain pure cultures.

To isolate endophytic bacteria, a process modified from Khaksar et al. (2016b) and Luo et al. (2011) was used. Briefly, root samples were incubated in sterile phosphate-buffered saline (pH 7.0) for 15 min. Then, plant roots were surfaced sterilized using 2.5% NaOCl for 30 min, washed three times with sterile distilled water. A 100-μL of the final wash was inoculated onto a MM agar plate for sterility checking. The root samples were ground in a pre-sterilized mortar and transferred to a MM liquid medium containing 20 μM BPA and incubated overnight at room temperature on a rotary shaker at 150 rpm. The overnight grown culture was spread on MM agar plates and incubated at 30 °C for 48 h. Bacterial colonies were selected and sub-cultured three times to ensure purity and stability.

Pure bacterial isolates were tested for BPA tolerance using BPA concentrations from 20 to 200 μM in MM agar plates (Silambarasan and Vangnai, 2016). The fastest growing isolates with the highest BPA tolerance were selected for further experiments.

2.3. Screening bacterial isolates with high BPA removal efficiency

One loop of a bacterial colony was inoculated into a 5-mL Luria-Bertani (LB) liquid medium starter culture. This was inoculated into mineral medium containing 0.1% (w/v) yeast extract at 3% (v/v), and inoculated at 200 rpm at 30 ± 2 °C for 8 h. The bacterial cells were centrifuged (4,180 × *g*, 10 min) and washed twice with 0.85% sterile sodium chloride. The cell pellet was re-suspended in 50 mL of mineral medium containing 0.1% (w/v) yeast extract and 100 μL of 20 μM BPA. Samples were taken at 12, 24, 36 and 48 h and centrifuged (4,180 × *g*, 10 min) to obtain cell-free medium. This was used for BPA analysis by high performance liquid chromatography (HPLC Model 1200 series, Agilent Technologies, U.S.A.). All experiments were performed in triplicate.

2.4. Identification and characterization of the selected bacteria

The selected bacteria were identified and characterized by colony morphology, Gram-staining and analysis of 16S ribosomal RNA (rRNA) gene sequences. This analysis was amplified with 785F and 907R primers supplied by Macrogen (Seoul, Korea). The obtained sequences were checked for sequence similarity using the NCBI database (Nucleotide-nucleotide BLAST).

2.5. Growth of endophytic bacteria

Overnight-grown cells were pre-cultured in LB medium. The culture was inoculated into mineral medium containing 0.1% (w/v) yeast extract and with and without BPA at a concentration of 20 μM. At time intervals, cell growth was measured by OD_{600}.

2.6. Plant growth-promoting (PGP) traits of endophytic bacteria

IAA production and ACC (1-aminocyclopropane-1-carboxylic acid) deaminase activity of the selected endophytic bacteria were examined following the methods of Khaksar et al. (2016b). More details are provided in the supplementary data.

2.7. Bacterial preparation and root inoculation

Each bacterial isolate was grown in LB medium at 30 °C on a rotary shaker at 150 rpm to an approximate cell concentration of 10^9 CFU