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Optimizing co-culture conditions of adventitious roots of *Echinacea pallida* and *Echinacea purpurea* in air-lift bioreactor systems

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ABSTRACT

Co-culture is a new strategy to enhance metabolite biosynthesis of plants. In the present study, effects of Murashige and Skoog (MS) medium strengths and sucrose concentrations on adventitious root (AR) biomass, bioactive compound accumulation, and antioxidant activity were investigated to optimize the bioreactor co-culture conditions of the ARs of *Echinacea pallida* and *Echinacea purpurea*. A kinetic study was also conducted to confirm the suitable culture period. MS medium strengths and sucrose concentrations critically affected the efficiency of the AR co-culture. The maximum AR biomass and accumulation of total polysaccharide, total phenolics, total caffeic acid derivatives, and their monomers (caftaric acid, chlorogenic acid, caffeic acid, cynarine, echinacoside, and cichoric acid) were determined at 0.75× MS medium supplemented with 50 g L^{-1} sucrose, resulting in the occurrence of the highest antioxidant activities, namely, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity and reducing power. In the kinetic study, the bioactive compound production peaked on day 35, and 502.1 mg L^{-1} polysaccharides, 564.3 mg L^{-1} phenolics, 457.4 mg L^{-1} flavonoids, and 416.1 mg L^{-1} caffeic acid derivatives were produced. The DPPH radical scavenging activity and reducing power also reached the maximum on day 35 of culturing. Therefore, the optimized conditions of the AR co-culture of *E. pallida* and *E. purpurea* can be used for the mass production of bioactive compounds during the industrial production of *Echinacea* products.

1. Introduction

Echinacea pallida (Nutt.) Nutt. and *E. purpurea* (L.) Moench are perennial herbs that belong to the Asteraceae family. These *Echinacea* species are mainly used in drug production. They have also been widely explored because of their increasing economic value and valuable medicinal functions [1]. The whole plants of *E. pallida* and *E. purpurea* contain numerous bioactive compounds, including caffeic acid derivatives, phenolics, flavonoids, and polysaccharides [2]. However, both species contain diverse types and amounts of metabolites. For instance, 0.3% to 0.7% echinacoside, a caffeic acid derivative, is detected in *E. pallida* roots but not in *E. purpurea* roots [3]. The plant extracts of both *Echinacea* species possess antioxidative, antibacterial, antiviral, and antifungal properties, and they are often used to treat common cold and respiratory and urinary diseases [4]. In the production of *Echinacea*-containing goods, artificially cultivated plants have been mainly used as raw materials, but the limited cultivation yield of *Echinacea* species cannot satisfy market demands [5].

Plant cell, tissue, and organ cultures have emerged as a valuable route for phytochemical biosynthesis, and bioreactor systems have been used for the mass production of useful plant metabolites [6]. Among these cultures, in vitro-induced adventitious roots (ARs) are considered good biological materials because of their stable commercial production of high metabolites [1]. Thus, AR culture has been extensively utilized for various plant species to produce bioactive compounds, such as ginsenosides [7], glycyrrhetinic acid [8], polysaccharides, phenolics [9], and caffeic acid derivatives [1]. Studies on the AR bioreactor culture of *Echinacea* species have also been performed. Jeong et al. [6] evaluated the feasibility of the mass culture of *E. purpurea* ARs in bioreactors. Wu et al. [10] developed a bioreactor AR culture of *E. pallida* to produce caffeic acid derivatives. Cui et al. [1] established large-scale (20 L) and pilot-scale (500 L) culture systems of *E. angustifolia* ARs. Their findings indicated that the AR cultures of *Echinacea* are novel plant materials that can be efficiently used in the industrial production of *Echinacea* products.

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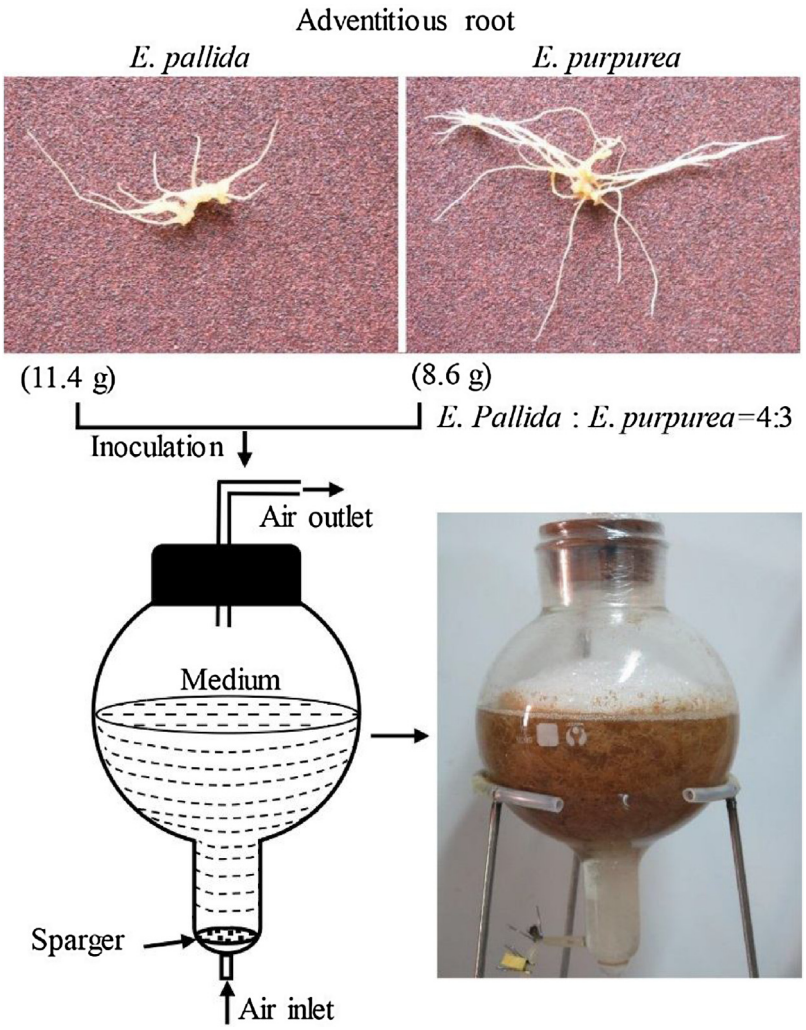

Fig. 1. The procedure of AR co-culture of *Echinacea pallida* and *Echinacea purpurea* in a balloon-type airlift bioreactor.

In bioreactor culture, the production of a culture's biomass and metabolites has been increased by using different strategies, including culture medium manipulation, environmental conditions, precursor addition, and elicitation [11]. Furthermore, co-culture is a new strategy [12] involving different plant species or various explants that can enhance metabolite biosynthesis and produce new metabolites [13,14]. In our previous study, ARs of different *Echinacea* species are co-cultured in one bioreactor, and our results revealed that different combinations and proportions of species have a diverse effect on AR biomass and metabolite accumulation; the optimal proportion of *E. pallida* and *E. purpurea* is 4:3 [12]. In the present study, to improve the AR co-culture conditions of *E. pallida* and *E. purpurea* (4:3 proportion), we investigated the effects of medium conditions, including Murashige and Skoog (MS) medium strength and sucrose concentration, on the accumulation of AR biomass, bioactive compounds (polysaccharides, phenolics, flavonoids, and caffeic acid derivatives), and antioxidant activity. We also conducted a kinetic study to select a suitable co-culture period.

2. Materials and methods

2.1. AR maintenance

The ARs of *E. pallida* and *E. purpurea* were induced from natural roots according to the method described by Wu et al. [15]. The induced ARs of both *Echinacea* species were separately cultured in 5 L balloon-type airlift bioreactors containing 4 L of half-strength MS medium [16] supplemented with 1 mg L^{-1} indole-3-butyric acid (IBA) (Beijing Solarbio Science and Technology Co., Ltd., Beijing, China) and 30 g L^{-1} sucrose (Tianjin Kemiou Chemical Reagent Co., Ltd., Tianjin, China). The pH of the medium was adjusted to 5.8 before sterilization at 121 °C and 1.2 kg cm^{-2} for 20 min. The bioreactors were maintained at 25 °C ± 2 °C in the dark, aerated with sterile air at 100 mL min^{-1}, and sub-cultured once in 4 weeks.

2.2. Effects of medium condition and culture period on biomass, bioactive compound accumulation, and antioxidant activity of co-cultured ARs

Three sets of experiments were employed. In all of the experiments, 5 L balloon-type airlift bioreactors containing 4 L of culture medium were used. The pH of the medium was adjusted to 5.7. Each bioreactor was inoculated with 20 g of fresh 4-week-old ARs of *E. pallida* (11.4 g) and *E. purpurea* (8.6 g) (*E. pallida*: *E. purpurea* = 4:3) (Fig. 1). In the first experiment, the effect of the MS medium strength was investigated. The basic MS media with different strengths (0.5×, 0.75×, and 1×) were supplemented with 1 mg L^{-1} IBA and 50 g L^{-1} sucrose. In the second experiment, various concentrations of sucrose (30, 50, 70 and 90 g L^{-1}) were added to 0.75× MS medium supplemented with 1 mg L^{-1} IBA. After 30 days of co-culture, the AR biomass and content of total polysac-