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Production of tropane alkaloids by small-scale bubble column bioreactor cultures of *Scopolia parviflora* adventitious roots

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Abstract

The mass production of tropane alkaloids from adventitious root cultures of *Scopolia parviflora*, in small-scale bubble column bioreactor (BCB) was attempted. Adventitious roots of *S. parviflora* produced relatively enhanced levels of scopolamine and hyoscyamine in bioreactor compared to flask type cultures, and rapidly produced root clumps, with continuously increasing biomass throughout the culture period. The production of scopolamine and hyoscyamine in the top and bottom regions of root clumps were higher than in the core region. The adventitious root cultures of *S. parviflora* in the BCB required a relatively high level of aeration. The optimized conditions for the bioreactor culture growth and alkaloid production were found to be 3 g of inoculum, on a fresh weight basis, a 15-day culture period and 0.4 vvm of airflow. The elicitation by *Staphylococus aureus* increased the specific compound of scopolamine, while the production of hyoscyamine was slightly inhibited in BCB cultures.

Keywords: Aeration; Bubble column bioreactor; Inoculum density; *Scopolia parviflora*; Tropane alkaloids

1. Introduction

Higher plants produce diverse ranges of pharmaceutically important secondary metabolites (Cho et al., 2003). *Scopolia parviflora*, a solanaceous perennial plant, is endemic to Korea and recently classified as rare endangered species. *S. parviflora* produces considerable levels of tropane alkaloids with anticholinergic activity (Baiza et al., 1998). Bioreactor culture studies have been initiated in solanaceous crops, such as *Atropa, Datura, Duboisia* and *Hyoscyamus* species for the production of secondary metabolites. However studies on use bioreactors for the commercial production of scopolamine and hyoscyamine from *S. parviflora* are limited.

Cultures of adventitious or hairy roots are potential source for the production of valuable plant secondary metabolites on commercial scale. Biosynthesis of scopolamine and hyoscyamine is correlated with root differentiation (Endo and Yamada, 1985). Dedifferentiated cell culture studies for the production of scopolamine and hyoscyamine were found unsuccessful, as they are predominantly synthesized in roots. This suggests that development of suitable methods for large-scale culturing of adventitious and/or hairy roots would be a viable approach for the production of tropane alkaloids. The ability of hairy roots to grow to high density and to produce significant amount of secondary metabolites makes them a suitable system for large-scale culture in reactor (Wilson, 1997). Keeping this in view, we devised bubble column bioreactor (BCB) to scale-up of culturing of adventitious roots. Many factors are severely affected during scale up

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of any kind of cell culture. These include oxygen transfer rate, heat transfer, mixing and the associated shear stress, superficial air velocity and culture age and stability (Humphery, 1998; Kim et al., 2002). Scale up of hairy root cultures remains very challenging, and despite recent advances, the objectives of optimal growth and production in bioreactors are far from being reached (Kim et al., 2002; Ogbonna et al., 2001; Kaewpingtong et al., submitted). In the present study, the optimal conditions, such as culture period, initial inoculum density and aeration, required for culturing adventitious or hairy root cultures of *S. parviflora* in a BCB for the production of scopolamine and hyoscyamine were evaluated.

2. Methods

2.1. Plant material and adventitious root culture

The mother plant of *S. parviflora* was provided by the National Arboretum of Korea. The adventitious roots were induced from the rhizome of *S. parviflora* (Jung et al., 2002) and maintained by sub culturing in B5 medium supplemented 50 g l^{-1} sucrose and 0.1 mg l^{-1} IBA from last three years. The media used in flask cultures, consisted of B5 medium supplemented with 50 g l^{-1} sucrose and 0.1 mg l^{-1} IBA. The liquid culture was established by the inoculation of 0.5 g fresh weight (F.W.) of roots into a 100 ml conical flask containing 30 ml medium. The flasks were maintained in the dark at 25 ± 1 °C, 100 rpm on a rotary shaking incubator.

2.2. Preparation of the bubble column bioreactor (BCB) culture

The bioreactor consisted of a glass vessel culture chamber (7.5 × 7.5 × 25 cm), air inlet and outlet, sampling ports, accessory connector and an air filter (0.20 μM pore size, Midisart® 2000, Sartorius, Germany), connected with silicon tubes. The sampling ports and accessory connector were locked for efficient operation. The internal diameter of the bioreactor was 6.5 cm, with a working volume of 300 ml (Fig. 1). The pH of medium used in the bioreactor was adjusted to 5.8 with 0.1 N NaOH or HCl by pH controller before autoclaving at 121 °C for 15 min. Filter-sterilized air was supplied through a sparger from the bottom of the bioreactor. Five grams F.W. of 14-day-old randomly cut 1.5–2 cm segments of adventitious roots was used as inoculum. The entire operation was carried out at 25 ± 1 °C in the dark.

2.3. Optimization of culture condition

A time-course test for the bioreactor was accomplished with 5 g of inoculum density fresh roots, operating with a 0.3 vvm air flow, for 25 days, with samples taken at 5 day intervals. To determine the optimal inoculum density, different levels of inoculants (3, 5, 10, 15 and 20 g F.W.) were used separate experiments. Efficient aeration is an important factor in bioreactor culture studies. Therefore, air flows of 0.1, 0.2, 0.3, 0.4 and 0.5 vvm were evaluated with the 5 g (F.W.) inoculum density.

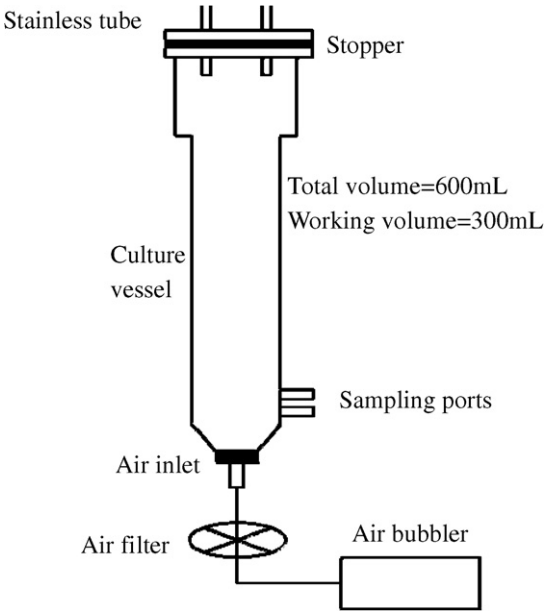

Fig. 1. Design of the bubble column bioreactor (BCB) used in this study. The vessel volume was 600 ml (7.5 × 7.5 × 25 cm) and the working volume was 300 ml. The glass growth chamber was connected to an air bubble to provide oxygen. Supplied oxygen was sterilized by passing an air filter and bubble were created through a sparger.

2.4. Measurement of root growth, conductivity, and dissolved oxygen

The growth of adventitious roots was measured as described in Jung et al. (2002). The adventitious roots were separated from the medium, blotted and weighed. The growth index (G.I.) was equal to the fresh weight of harvested roots minus fresh weight inoculated roots divided by the fresh weight inoculated roots. The nutrient, as a measure of conductivity, and dissolved oxygen (DO) utilization by the growing roots was recorded for a period of 25 days. The electrical conductivity and DO of the medium were measured using an electrical conductivity electrode (MultiLine P4, Tetracon® 325) and DO electrode (MultiLine P4, CellO × 325), respectively.

2.5. Extraction and quantification of scopolamine and hyoscyamine

Tropane alkaloids from harvested roots and media were prepared for HPLC analysis according to the method of Kang et al. (2004). The HPLC separated scopolamine and hyoscyamine were analyzed using EI mass spectrometer (JMS-AX505 WA).