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Enhanced production of phenylethanoid glycosides by precursor feeding to cell culture of *Cistanche deserticola*

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Abstract

The production of phenylethanoid glycosides (PeG) was enhanced by feeding precursors L-phenylalanine, L-tyrosine, sodium acetate and phenylacetic acid at appropriate concentrations to cell cultures of *Cistanche deserticola*. The optimal precursor was phenylalanine and the suitable feeding time was on the eighth day of the cell culture. Feeding 0.2 mmol l^{-1} phenylalanine on the eighth day resulted in the highest production of phenylethanoid glycosides (1.10 g l^{-1}), which was 75% higher than that obtained in the cell culture without precursors.

Keywords: *Cistanche deserticola*; Cell culture; Phenylethanoid glycosides; Precursors; Phenylalanine

1. Introduction

When considering medicinal activity and safety, more and more attention is being paid to natural products, especially those extracted from plants. *Cistanche deserticola* Y. C. Ma, a traditional Chinese herb, which displays marked medicinal activities in anti-nociception, anti-inflammation [1], sedation [2] and scavenging free radicals [3]. Phenylethanoid glycosides (PeG) are pharmaceutical components isolated from *C. deserticola* and chiefly include echinacoside, acteoside, 2′-acetylacteoside, cistanoside A, etc. [4]. *C. deserticola* is a parasitic plant, like many other species of Chinese traditional herbs; it is now almost extinct due to over-exploiting and being difficult to cultivate. Therefore, cell cultures of *C. deserticola* cells offer great potential for PeG production [5]. In addition, cell cultures provide a convenient system for studying the biosynthesis of secondary metabolites, and offer an easily manipulated source of relevant enzymes [6].

There are many methods for improving the accumulation of secondary metabolites in plant cell cultures and feeding a precursor is an effective one. In the cell culture of *Taxus cuspidata*, the addition of aromatic carboxylic acid and amino acid promoted the biosynthesis and excretion of taxol [7]. Considering the pathway of PeG biosynthesis, phenylalanine and tyrosine are the precursors of PeG, and phenylalanine amino lyase (PAL) is believed to be one of the key enzymes that catalyzes the stereospecific, anti-elimination of ammonia from either phenylalanine or tyrosine to yield cinnamic acid and its derivatives [8]. In the present paper, the effects of phenylalanine, tyrosine, sodium acetate and phenylacetic acid on the cell growth of *C. deserticola* and PeG biosynthesis were investigated.

2. Materials and methods

2.1. Materials

C. deserticola cell lines were maintained in this laboratory for more than 2 years by subculturing every 20 days. PeG standard samples were purified from *C. deserticola* wild plant by the method of Du [9]. L-phenylalanine, L-tyrosine, sodium acetate and phenylacetic acid were of analytic grade.

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2.2. Culture methods

The cell culture medium was B5 medium [10] supplemented with 1 mg l^{-1} 2,4-dichlorophenoxy acetic acid, 800 mg l^{-1} casein hydrolysate and 20 g l^{-1} sucrose. Medium pH was adjusted to 5.50–5.60 with 1 M NaOH and 1 M HCl. *C. deserticola* cells, 0.40 g fresh weight, were inoculated into 40 ml medium held in 100 ml Erlenmeyer flask and cultured at 25 ± 1 °C on a rotary shaker (110 rpm) under 16 h light per day with light intensity of 24 μmol m^{-2} s^{-1} as reported previously [11]. Sterilized precursors were added to the medium in flasks in suitable cell growth phase, and cells were then continuously cultured till the 20th day.

A bubble column bioreactor was constructed of a glass column with 2 l working volume (100 mm i.d × 300 mm h). Before starting the culture, the bioreactor containing 2 l B5 medium was sterilized by autoclaving at 121 °C for 20 min. *C. deserticola* cells were inoculated into the bioreactor and cultured under 16 h light per day with light intensity of 24 μmol m^{-2} s^{-1} at 25 ± 1 °C for 20 days.

2.3. Assay methods

For dry weight (DW) determination, all cells in flasks were filtered using filter papers, dried at 105 °C to constant weight, and the DW recorded. PeG was extracted from the dried cells with methanol (1:60, w/v) for 24 h; the filtrate was concentrated and diffused with distilled water, then passed through a macroreticular resin column. The absorbance peak of methanol dilution was collected and assayed at 332 nm by ultraviolet-visible spectroscope (Beckman DU-Series 7000) [12]. By comparing sample absorbance with a standard curve, the PeG content could be calculated from the following formula: $OD_{332nm} = 0.0085 + 0.01228\,C$, where C is the concentration of PeG (μg ml^{-1}). The production of PeG was calculated as follows: PeG production (g l^{-1}) = PeG content (%) × DW (g l^{-1}). The activity determination of phenylalanine ammonia lyase was based on the method of Koukol and Conn [13]. All estimations were carried out in triplicate.

3. Results

3.1. Growth curve of C. deserticola cells in suspension culture

The curves of cell growth and PeG accumulation in suspension culture are shown in Fig. 1. The first 8 days were the lag phase when the cells grew slowly. The period from the 8th to the 20th day was the exponential phase, the cells grew fast and the biomass reached the highest of 6.2 g DW l^{-1} on the 20th day. PeG accumulation is closely associated with cell growth. The PeG content in the cells declined slightly in lag phase then increased rapidly in the exponential phase. At the end of the exponential phase, PeG content reached the highest value of 10.2% (based on the dry weight) and PeG production reached 0.63 g l^{-1}.

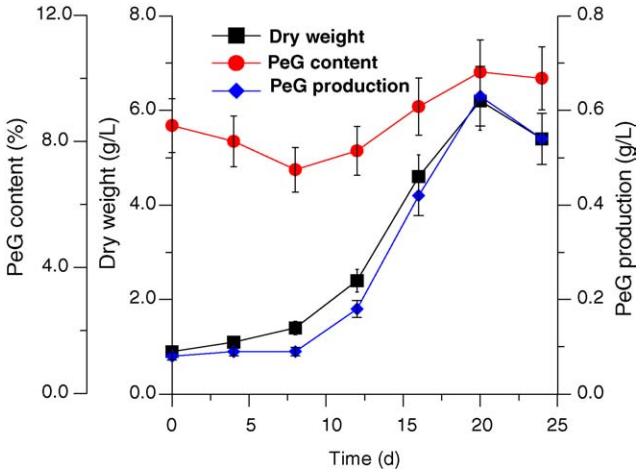

Fig. 1. Curves of *C. deserticola* cell growth and PeG accumulation in suspension cultures. *C. deserticola* cells, 0.4 g fresh weight, were cultured in 40 ml B5 medium held in 100 ml Erlenmeyer flasks for 20 days.

3.2. Effects of precursors on C. deserticola cell growth and PeG biosynthesis

Precursors involved in the biosynthesis of PeG are phenylalanine, tyrosine and phenylacetic acid, etc. In the biosynthesis of secondary metabolites by plant cell cultures, precursor feeding at appropriate concentrations can promote the accumulation of secondary metabolites. But on the other hand, excess precursors may cause feedback inhibition to the metabolite pathway. It is very important therefore to determine the appropriate precursor concentration in the precursor-feeding test.

Secondary metabolites were plentifully synthesized after cell growth entered the exponential phase, so it may be useful to add precursors into the medium in this phase. The effects of L-phenylalanine, tyrosine, sodium acetate and phenylacetic acid added on the 12th day of the culture on *C. deserticola* cell growth and PeG biosynthesis are shown in Fig. 2. Phenylalanine at low concentration (<0.2 mmol l^{-1}) showed a negative effect on the cell growth and a significant positive effect on PeG biosynthesis. After 20 days culture, the cell biomass, PeG content and PeG production in the medium with 0.2 mmol l^{-1} phenylalanine reached 5.9 g DW l^{-1}, 14.6% and 0.86 g l^{-1}, respectively, which were 95, 143 and 137% of those obtained in control. PeG content decreased with the increase of phenylalanine concentration when the precursor concentration was over 0.2 mmol l^{-1}.

Low concentrations (<0.1 mmol l^{-1}) of L-tyrosine had positive effects on both cell growth and PeG biosynthesis. Of the concentrations tested, tyrosine at 0.2 mmol l^{-1} gave the highest biomass (6.7 g DW l^{-1}), at 0.1 mmol l^{-1} gave the highest PeG content (12.7%) and PeG production