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Induction of adventitious roots and extraction of codonoposide from *Codonopsis lanceolata*

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ABSTRACT

Direct rooting from leaf explants of *Codonopsis lanceolata* was achieved on Murashige and Skoog's(MS) medium supplemented with 30g/L sucrose, and different concentrations of growth regulators like Indole acetic acid(IAA), Indole butyric acid(IBA). Basal medium supplemented with 3mg/L indole butyric acid achieved maximum number of roots with 100% response. The roots were cultured on various liquid medium(B5, SH and MS) for the establishment of root-organ culture with the different concentrations of growth regulators and incubated on an orbital shaker at 80rpm at 25±2^{0}C. B5 medium with IBA 0.5mg/L was found to be most effective for the growth of adventitious roots in 250ml flask as well as in bioreactor. The fresh weight of the root is 6.19±0.18g and 0.81±0.02g dry weight was obtained from 250ml flask after one month of sub culture. Similarly 48.56±1.12 g and 8.26±0.19g dry weight was obtained from 2L-bioreactor after one month of sub culture.

KEYWORDS

Induction;
Adventitious roots;
Gambore B5 medium(B5);
Schenk and Hildebrandt a (SH)nd Murashige and Skoog's(MS) medium;
Indole acetic acid(IAA);
Indole butyric acid(IBA);
Codonopsis lanceolata.

INTRODUCTION

Codonopsis lanceolata has long been used as a folk medicine in Korea, Japan and China for the treatment of lung inflammatory diseases. *Codonopsis lanceolata* belongs to the family of Campanulaceae. The roots of this plant have been used as an herbal drug to treat bronchitis, cough, spasm, macrophage-mediated immune responses and inflammation, and as a tonic[1-3]. The roots of these plants mainly contain saponin which has great biological activity. Shin et.al reported[4] the components of the essential oil obtained from the roots of *Codonopsis lanceolata*. Meanwhile, sterols and triterpenes have been isolated from *Codonopsis.pilosula*[5].

Many natural products have been used in traditional and folk medicine for therapeutic purposes. They are generally nontoxic when compared to synthetic chemical compounds. It also provides important sources of promising leads for the development of novel therapeutic drugs. In approximately 66% of the medicinal plants used in traditional medicine, roots are the principal source for drug preparation[6]. The development of a fast growing root culture system would offer unique opportunities for producing root drugs in the laboratory without having to depend on field cultivation[7].

Short Communication

The search for new chemopreventive and anti-tumor agents that are more effective and less toxic has created a great deal of interest in phytochemicals. In particular, saponins are compounds of natural origin and are known to have cytotoxic activities. Lee et al. reported[8] that codonoposide is a natural compound isolated from the root of *Codonopsis. lanceolata*, induces apoptosis of human promyelocytic leukemia cells.

The aim of our study was to establish *in vitro* cultures producing codonoposides of the same quality as those found in native *Codonopsis lanceolata* plants and to scale up its cultivation in a laboratory bioreactor. In the present study, we report the production of codonoposide from *Codonopsis lanceolata* roots cultured in conical flask as well as in bioreactor.

EXPERIMENTAL

Materials

Codonopsis lanceolata plantlets were planted in glass bottles that contained a 70 ml Murashige and Skoog medium with 3%(w/v) sucrose and 0.7% plant agar. The plants were grown in growth room at 25/18^{0}C and a 16-h photo period. General electrical lamps were used delivering irradiance of 8 Wm^{-2}.

General experimental procedures

Melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. Optical rotations were measured on a JASCO DIP-360 digital polarimeter at 25^{0}C. IR spectra were recorded on a Hitachi 260-01 spectrometer in KBr disks. ^{1}H- and spectrum was taken on a Bruker AM-500 spectrometer with TMS as an internal standard.

Induction of Adventitious roots

The leaves were collected from *in vitro* grown plants and cut horizontally into two halves and inoculated on half strength MS[9] medium supplemented with 30g/L sucrose. Various concentrations and combinations of IAA and IBA were added(TABLE 1). The pH of the medium was adjusted to 5.6±0.2 before sterilization. Thirty five explants were taken per treatment and each treatment was replicated thrice.

Mass cultivation of adventitious roots

The induced roots were separated from the explants aseptically and twenty segments per flask(1.0-2.0cm in length and 1g fresh weight) were sub cultured separately into 100ml aliquots of MS, SH and B5 medium supplemented with 0.5mg/L IBA in 250ml Erlenmeyer flasks. The cultures were kept under continuous agitation at 80rpm in an orbital shaker(Orbitek) and incubated at 25±2^{0}C.

Bioreactors were autoclaved for 15min at 121^{0}C each with 1500 ml liquid medium(MS, SH, B5) supplemented with 30g/L sucrose, 0.5mg/L IBA. Before autoclaving, the pH of the medium was adjusted to 5.6 ± 0.2. Five grams mass of root was inoculated into the each bioreactor and culture room temperature was maintained at 25±2^{0}C.

Isolation of saponin from adventitious roots of *Codonopsis lanceolata*

One month root cultures were collected and dried overnight in an oven at 55°C. The dried roots(8g) were extracted three times with MeOH under reflux. The MeOH extract was filtered and evaporated on a rotary evaporator under reduced pressure to give a viscous mass(1.8g) of MeOH extract. This material was suspended in H_2O and partitioned with $CHCl_3$, EtOAc, and BuOH to give a $CHCl_3$-soluble fraction(0.5g), EtOAc-soluble fraction(0.2g), and a BuOH-soluble fraction(1.1g) after being dried in vacuo.

Isolation of codonoposide

A part of the BuOH-soluble fraction(1.1g) was subjected to column chromatography on silica gel(280g; Merck 7734, Darmstadt, Germany). The column was eluted with $CHCl_3$/MeOH/H_2O(65:35:10, lower phase) and a fraction over the retention volume of 950mL-1420mL. This fraction was filtered using Sephadex LH-20 column chromatography, and the fraction containing a major saponin was dried in *vacuo*. The combined residues were recrystallized in MeOH and resultantly yielded amorphous solid 1(500mg).

Spectral data: Codonoposide(1). Amorphous solid; mp 220^{0}C; $[\alpha]_D^{20}$-29.6^{0}C. IR(KBr): ν_{max}(cm^{-1}) =3432 (OH), 2930(CH), 1735(C=O), 1070, 1045 (glycosidic C-O). ^{1}H NMR(500MHz, pyridine-d_5): triterpene δ0.82(3H, s, H-24), 0.88(3H, s, H-25), 1.03 (3H, s, H-29), 1.05(3H, s, H-26), 1.13(3H, s, H-30), 1.23 (3H, s, H-27), 1.75(3H, s, H-23), 5.60(1H, brs, H-12), sugar moieties δ3.99(1H, m, H-2"), 3.45(1H, m, H-3"), 4.42(1H, m, H-2"), 4.40(1H, m, H-3'), 4.48 (1H, m, H-2'), 4.76(1H, d, J=7.0 Hz, H-1"), 5.07 (1H,

TABLE 1 : Effect of various plant regulators in full length MS medium on root induction from leaf of *Codonopsis lanceolata*

IBA (mg/L)	IAA (mg/L)	Organs	Mean number of roots	Response (%)	Morphology of roots
2.0			7.1±0.4	86.0	R+C
2.0	1.0	Leaf	1.3±0.2	53.0	R+C
3.0			9.2±0.2	100.0	R

R=Roots; C=Callus; ± Standard error of the mean.

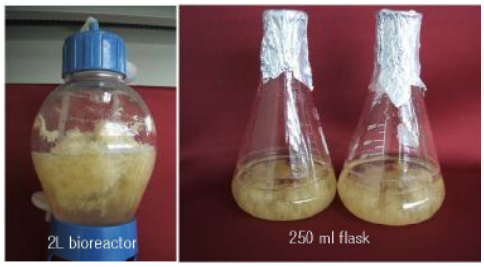

Figure 1 : Roots of *Codonopsis lanceolata* growing in 2L bioreactor and 250ml flask

d, J=6.8 Hz, H-1"), 5.2(1H, d, J=7.4 Hz, H-1"), 5.18 (1H, brs, H-16), 5.6(1H, brs, H-1"), 1.69(3H, d, J=5.7 Hz, H-6") 6.48(1H, brs, H-1").

RESULTS AND DISCUSSION

Growth of the roots was checked on the basis of number of roots per explant. Among the various concentrations and combinations of auxins tested, in a medium(MS) with 3mg/L IBA direct rooting was observed in 100% of the explants within 15-20days of inoculation with a mean number of roots of 9.2±0.2. In other combinations, the response was good, but callus formation occurred after 25days of inoculation(TABLE 1). For further study, the roots were transferred to liquid medium(MS, SH, B5) with 0.5mg/L IBA. The fresh weight of 6.15±0.17g and 0.81±0.02g dry weight was obtained from 250ml flask after one month of sub culture. The roots grown in shake flask were thicker and more sturdy compared with hairy roots[10,11]. The preliminary studies showed that the roots could grow in vigorous aeration. Growing roots(5.0g fresh weight) from the second subculture of root-organ culture were inoculated aseptically into bio reactor containing 1500ml liquid medium(MS, SH, B5) with 0.5mg/L IBA. Profuse root growth was observed in the reactor. The lateral roots were responsible for rapid growth. These roots were thick and brittle. From 2L bio reactor 48.56±1.12 g and 8.26±0.19g dry weight was obtained after one month of sub culture.(Figure 1)

The saponin component of these *in vitro* roots was analyzed by spectral data, which is matching with reported literature[1].

CONCLUSION

We have developed a simple, convenient and effective method for the production of Codonoposides from adventitious roots of *Codonopsis lanceolata* by using various nutrient medium under specific conditions. Codonoposides are important chemical constituents as they act as powerful drug in traditional medicine. Therefore, large-scale cultivation of *Codonopsis lanceolata* roots in bioreactor might be an alternative source to fulfill the global demands for this high value bioactive molecule. To our knowledge, this is the first report on production of Codonoposides from adventitious roots of *Codonopsis lanceolata*.

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