African Journal of Biotechnology Vol. 7 (12), pp. 1924-1926, 17 June, 2008
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2008 Academic Journals

*Short Communication*

# Stimulation of taxane production in suspension cultures of *Taxus yunnanensis* by oligogalacturonides

**Yu Ting Guo and Jian Wen Wang***

School of Pharmaceutical Sciences, Soochow University, Suzhou 215006, P.R. China.

Accepted 3 June, 2008

**When *Taxus yunnanensis* cells of 12-day-old cultures were exposed to oligogalacturonide (OGA) at 20-80 µg ml$^{-1}$ for 3 days, it was found that paclitaxel and 10-deacetyl baccatin III (10-DAB III) content peaked at 23 and 24 µg g$^{-1}$ dry wt, respectively. OGA treatment increased taxane release percentage (extracellular release ratio) from 10 to 90%. The maximum production of paclitaxel and 10-DAB III in cultures elicited with OGA at 40 µg ml$^{-1}$ was demonstrated to be 0.5 and 1.0 mg l$^{-1}$, a 3.6 and 3.4-fold that of the control. This is the first report on the stimulation of taxoid production by OGA.**

**Key words:** 10-deacetyl baccatin III production, oligogalacturonides, paclitaxel producton, *Taxus yunnanensis*.

## INTRODUCTION

Paclitaxel, a diterpenoid secondary metabolite in various *Taxus* (yew) species, is an excellent anticancer drug which has been widely used in the treatment of breast, ovarian and lung cancers as well as AIDS-related Kaposi's sarcoma. Since the supply of paclitaxel from the *Taxus* tree is in great shortage, the semisynthesis from a precursor 10-deacetyl baccatin III (10-DAB III) is viewed as promising alternative ways for antitumor drugs such as Taxol® (paclitaxel) and Taxotere® (*N*-debenzoyl-*N*-*tert*-butoxycarbonyl-10-deacetyl taxol) (Ramesh, 1998). *Taxus yunnanensis* is an evergreen tree indigenous to China and from which many taxoids including paclitaxel and 10-DAB III have been already isolated (Zhang et al., 1995). Cell culture of *T. yunnanensis* is a promising and renewable resource to supply paclitaxel (Zhang et al., 2002).

To improve the paclitaxel yield various kinds of elicitors such as methyl jasmonate, fungal elicitor, chitosan and silver ion have been applied and very effective for cell cultures of *Taxus* spp. (Zhong, 2002). Oligogalacturonides (OGAs), pectic fragments released from the plant cell wall, are among the well-known oligosaccharides triggering a variety of defense responses in plants (Nothnagel et al., 1983; Navazio et al., 2002). OGA elicitor exhibits highly specific activity of inducing secondary metabolite production such as carrot phytoalexin 6-methoxymellen, tropane alkaloids and saponin accumulation in suspension-cultured cells (Zabetakis and O'Hagana, 1999; Kurosaki et al., 2001; Hu et al., 2003). However, there have been no reports up to date regarding OGA elicitation on *Taxus* spp. cells and 10-DAB III production in cell cultures of *T. yunnanensis*. This work aims to characterize the 10-DAB III and paclitaxel production, and to evaluate the effects of OGA on the taxane production in cell suspension cultures of *T. yunnanensis*.

## MATERIALS AND METHODS

### Plant cell cultures

A *T. yunnanensis* cell line induced from the young stems of a *T. yunnanensis* tree was used in this study, which was routinely maintained on solid Murashige and Skoog (1962) medium (MS) with 0.5 mg l$^{-1}$ α-naphthalene acetic acid, 0.08 mg l$^{-1}$ 2, 4-dichlorophenoxyacetic acid and 0.5 mg l$^{-1}$ 6-benzyladenine and subcultured once every 25 days. Cell suspension culture was maintained on liquid MS medium in 150 ml flasks on a rotary shaker at 110 rpm and 25 ± 1°C in dark. Each of the culture flasks was filled with 30 ml medium and inoculated with 3.0 g fresh weight of cells from solid culture.

### Preparation of OGA

OGA was prepared from polygalacturonic acid (Cat. 81325, Fluka) by the procedure of Roco et al. (1993) with modification. Polygalacturonic acid at 50 µg ml$^{-1}$ with pectinase (Cat. 17389, Fluka) from

*Corresponding author. E-mail: jwwang@suda.edu.cn. Tel: 86-512-67410163. Fax: 86-512-6588-0031.

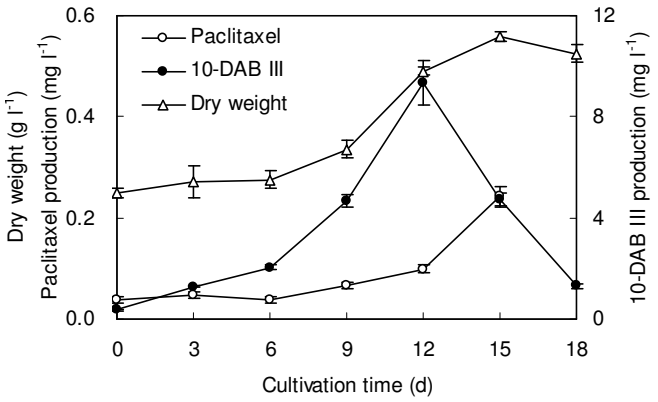


**Figure 1.** Time course for growth and taxane production in cell cultures of *T. yunnanensis*. Values are means of triplicate results and error bars show standard deviations.

*Aspergillus niger* at 30 μg ml$^{-1}$ for 20 min at room temperature. Then the solution was boiled for 5 min and filtered. The filtrate was subsequently dialysed against distilled water using dialysis tubing with a cut-off of approximately 700 Da. The prepared OGA was adjusted to a final concentration of 1 mg ml$^{-1}$ of galacturonic acid equivalents (gal equiv) with distilled water, as determined by the method of Blumenkrantz and Asboe-Hansen (1973).

#### Elicitation experiments

OGA preparations at different concentrations (0 - 80 μg gal equiv ml$^{-1}$) were added to 12-day-old cell cultures. To observe the effect of OGA on the biosynthesis of paclitaxel and 10-deacetyl baccatin III, cell suspension cultures were collected at different times after treated with a specific amount OGA.

#### Analytical procedures

Biomass was quantified in terms of dry weight. The extraction and analysis of paclitaxel and 10-DAB III in the cells and the cell-free medium followed the procedures as our previously described (Wang and Wu, 2004). The content of paclitaxel and 10-DAB III in the extract solution was analyzed by reverse-phase HPLC with UV detection at 227 and 232 nm, using a 250 × 4.6 mm Agilent HC 5 μm C18 HPLC column (Agilent, USA), and a mobile phase consisting of methanol : acetonitrile : water at 25: 35: 45 by volume. The flow rate was kept at 1.0 ml min$^{-1}$. Paclitaxel and 10-DAB III were quantified with genuine standards (Sigma). Taxane production (mg l$^{-1}$) refers to sum of taxane recovered from the cells and the medium. Taxane release ratio was calculated by dividing taxane in the media by total taxane yields.

## RESULTS

### Growth and taxane production

Biomass and the production of paclitaxel and 10-DAB III are presented in Figure 1. After 15 days of cultivation, the biomass was about 11 g l$^{-1}$ dry wt, which was about 2-fold of initial concentration. The production of 10-DAB III and paclitaxel increased linearly with time up to day 12 and day 15 with the highest value of about 0.2 and 0.5 mg l$^{-1}$, respectively.

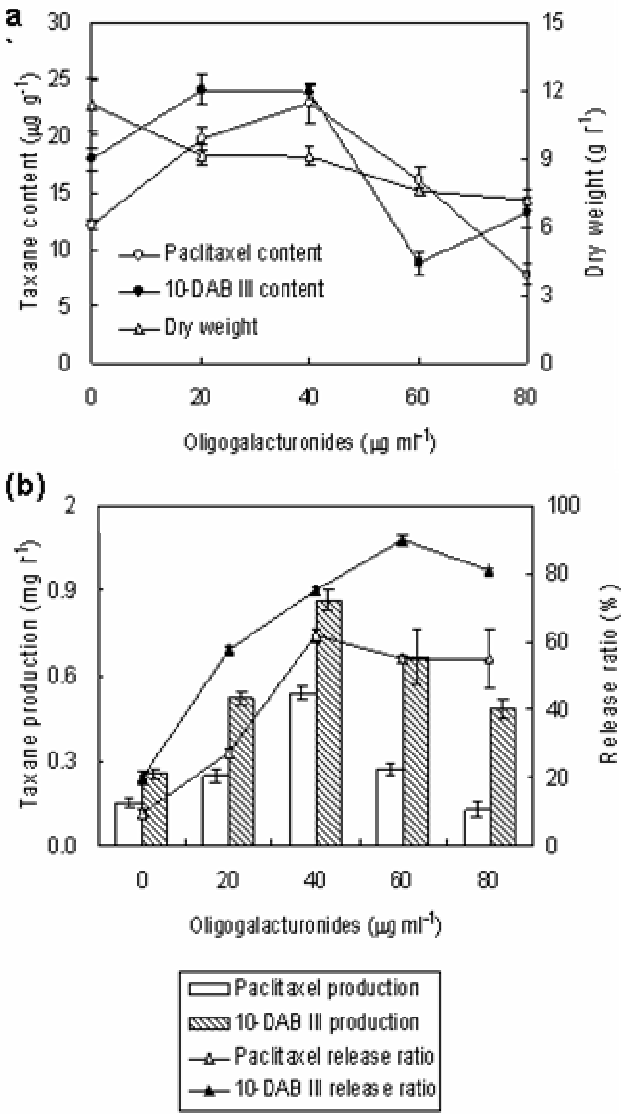


**Figure 2.** Effects of oligogalacturonide elicitor on growth and intracellular taxane content (a), taxane production and extracellular release ratio (b) in cell cultures of *T. yunnanensis*. The elicitor with different concentrations was added to 12 day-old cell cultures for treatment of 3 days. Control received the same volume of water only. Values are means of triplicate results and error bars show standard deviations.

### Effect of OGA dosage

When a preparation of OGA was added to 12-day-old cell cultures, taxane accumulation in cells was dose-dependent (Figure 2a). Furthermore, taxane release percentage (release ratio) to the extracellular medium of the OGA-

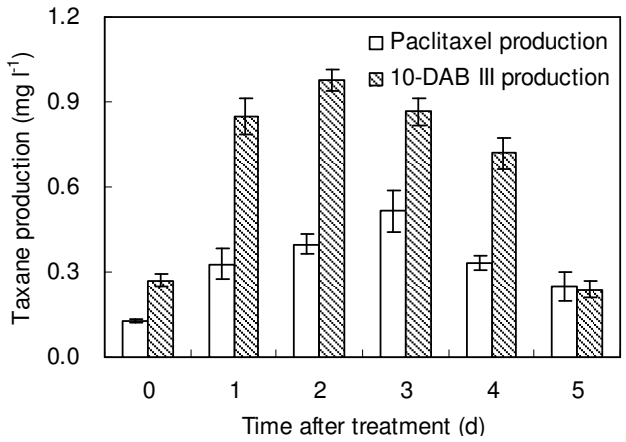


**Figure 3.** Time course of paclitaxel and 10-DAB III production in *T. yunnanensis* cell cultures treated with oligogalacturonide elicitor. Twelve-day-old cell cultures were exposed to oligogalacturonide elicitor (40 µg $ml^{-1}$) for treatment of different time. Controls received the same volume of water only. Values are means of triplicate results and error bars show standard deviations.

treated cells was also significantly higher than that of control (Figure 2b). The highest yield of paclitaxel (0.5 mg $l^{-1}$) and 10-DAB III (0.9 mg $l^{-1}$) was observed after elicitation with OGA at 40 µg $ml^{-1}$ which was then used for subsequent study.

### Time course of elicitation

The time course of the effect of OGA on taxane accumulation in 12-day-old cultures is shown in Figure 3. After 2 days, the 10-DAB III yield reached a maximum value (1 mg $l^{-1}$) as compared with that of the non-elicited control (0.3 mg $l^{-1}$). The highest paclitaxel yield of 0.5 mg $l^{-1}$ vs. 0.1 mg $l^{-1}$ in the control culture was obtained after 3 days of OGA treatment.

## DISCUSSION

The stimulation of taxoid production in cell cultures of *Taxus* spp. by the addition of different elicitors is suggested that paclitaxel and other taxanes are phytotoxins (Zhong, 2002). The present results show that accumulations of such phytotoxins including paclitaxel and 10-DAB III can be stimulated by the applied OGA. Moreover, OGA treatment enhanced taxoid release percentage (release ratio) to the extracellular medium. Zhang et al. (2007) reported that the paclitaxel release ratio of *T. chinensis* cells treated by chitosan was increased significantly. It was suggested that paclitaxel released from cells could reduce the probable feedback inhibition and degradation. Thus, the OGA-treated *T. yunnanensis* cell suspension cultures have more efficient taxoid productivity. To our knowledge, this is the first report of the stimulation of the OGA elicitor on toxoid production. With strain improvement and optimisation of elicitation, the greatly enhanced production of both paclitaxel and 10-DAB III in cell suspension cultures of *T. yunnanensis* could be expected.

## ACKNOWLEDGEMENTS

This work was co-financed by grants from NSFC (30772731) and JSNSF (BK2007051).

### RERERENCES

Blumenkrantz N, Asboe-Hansen G (1973). New method for quantitative determination of uronic acids. Anal. Biochem. 54: 484-489.

Hu X, Neill S, Cai W, Tang Z (2003). Hydrogen peroxide and jasmonic acid mediate oligogalacturonic acid-induced saponin accumulation in suspension-cultured cells of *Panax ginseng*. Physiol. Plant. 118: 414-421.

Kurosaki F, Yamashita A, Arisawa M (2001). Involvement of GTP-binding protein in the induction of phytoalexin biosynthesis in cultured carrot cells. Plant Sci. 161: 273-278.

Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15: 473-497.

Navazio L, Moscatiello R, Bellincampi D, Baldan B, Meggio F, Brini M, Bowler C, Mariani P (2002). The role of calcium in oligogalacturonide-activated signalling in soybean cells. Planta 215: 596-605.

Nothnagel EA, McNeil M, Albersheim P, Dell A (1983). Host pathogen interaction XXII: a galacturonic acid oligosaccharide from plant cell wall elicits phytoalexins. Plant Physiol. 71: 916-926.

Ramesh NP (1998). Tour de paclitaxel: biocatalysis for semisynthesis. Annu. Rev. Microbiol. 52: 361-395.

Roco A, Castañeda P, Pérez LM (1993). Oligosaccharides released by pectinase treatment of *Citrus limon* seedlings are elicitors of the plant response. Phytochemistry, 33: 1301-1306.

Wang JW, Wu JY (2004). Involvement of nitric oxide in elicitor-induced defense responses and secondary metabolism of *Taxus chinensis* cells. Nitric Oxide 11: 298-306.

Zabetakis RE, O'Hagana D (1999). Elicitation of tropane alkaloid biosynthesis in transformed root cultures of *Datura stramonium*. Phytochemistry 50: 53-56.

Zhang CH, Fevereiro PS, He G, Chen Z (2007). Enhanced paclitaxel productivity and release capacity of *Taxus chinensis* cell suspension cultures adapted to chitosan. Plant Sci. 172: 158-163.

Zhang CH, Wu JY, He GY (2002). Effects of inoculum size and age on biomass growth and paclitaxel production of elicitor-treated *Taxus yunnanensis* cell cultures. Appl. Microbiol. Biotechnol. 60: 396-402.

Zhang H, Tadeda Y, Sun H (1995). Taxanes from *Taxus yunnanensis*. Phytochemistry 39: 1147-1151.

Zhong JJ (2002). Plant cell culture for production of paclitaxel and other taxanes. J. Biosci. Bioeng. 94: 591-599.