Biotechnology Letters, Vol 19, No 4, April 1997, pp. 353–355

Enhanced production of taxol in suspension cultures of Taxus chinensis by controlling inoculum size

Hong-Qiang Wang, Jian-Jiang Zhong* and Jun-Tang Yu

State Key Laboratory of Bioreactor Engineering, East China University of Science & Technology, Shanghai 200237, China

Inoculum size (1.5–6.0 g dry weight/l) significantly affected cell growth and accumulation of intracellular and extracellular taxol in Taxus chinensis. A shorter cultivation time and a higher biomass productivity were achieved using inoculum size of 6.0 g DW/l. Both the intracellular content and total production of taxol were increased almost 30% with an increase of inoculum size from 1.5 to 3.0 g DW/l, while an even higher inoculum size decreased taxol formation. The extracellular taxol concentration was relatively higher (0.091 mg/l) at low inoculum sizes of 1.5 and 2.0 g DW/l; and in various cases it was less than 25% of the total amount of taxol produced.

Introduction

Taxol is an effective anticancer drug. Its supply from bark extract of yew trees is limited because they grow very slowly and contain only a very small amount of it. The production of taxol by cell cultures of *Taxus* spp. has therefore received great attention as a promising alternative supply source (Wickremesinhe & Arteca, 1993; Fett-Neto *et al.*, 1994; Kim *et al.*, 1995; Mirjalili & Linden, 1995; Seki *et al.*, 1995; Srinivasan *et al.*, 1996; Vanek *et al.*, 1996; Yukimune *et al.*, 1996).

Inoculum size may affect metabolite formation in plant cell cultures. Its effect is well demonstrated in cell cultures of *Perilla frutescens* and *Panax notoginseng* for production of anthocyanin and ginseng saponin and polysaccharide, respectively (Zhong & Yoshida, 1995; Zhang & Zhong, 1997). However, until now there is no information regarding the effect of inoculum size on taxol production. In this work, we show that both the cell growth and accumulation of taxol (both intracellular and extracellular) were greatly affected by inoculum size in suspension cultures of *Taxus chinensis*.

Materials and methods

Cell cultures

Suspension cells of *Taxus chinensis* were cultured in Murashige and Skoog medium supplemented with 0.5 mg 6-benzyladenine/*l*, 0.2 mg 2,4-dichlorophenoxyacetic acid/*l*, 0.5 mg naphthaleneacetic acid/*l*, 100 mg ascorbic acid/*l*, and 30 g sucrose/*l* (Wang *et al.*, 1996). A 250 ml Erlenmeyer flask containing 50 ml medium was incubated on a rotary shaker at 100 rpm and 25°C in the dark. The subculture period and inoculum size (normal) were 14 days and 4 g DW/*l*, respectively.

Taxol extraction

From harvested cells: The dried cells (about 200 mg) were powdered and ultrasonicated twice for 40 min in 3 ml methanol. The extracts were dried at 25°C. The residue was dissolved by adding 2 ml dichloromethane and 2 ml distilled water. After sufficient mixing, the mixture was centrifuged at 4000 rpm for 5 min. The dichloromethane fraction was collected and dried at 25°C. The residue was dissolved in 0.5 ml n-hexane/acetone (1:1, v/v), and applied to SEP-PAK Silica cartridge (Waters, USA). The cartridge was washed with 6 ml n-hexane/acetone (1:1, v/v). The elution was collected and dried at 25°C, and dissolved in 0.5 ml methanol for HPLC analysis.

From culture medium: Two ml of cell-free medium was extracted with 2 ml dichloromethane for twice. The bottom layer (dichloromethane phase) was collected and evaporated to dryness at 25°C, and dissolved in 0.5 ml methanol for HPLC analysis.

HPLC analysis

The above methanol solution (20 μl) was injected for taxol analyses on a reverse-phase column (Whatman Pentafluorophenyl, 5 μm, 250 × 4.6 mm). The mobile phase consisted of acetonitrile and water (37:63, v/v), and the flow rate was 1 ml/min. Taxol was quantitated at 227 nm. The authentic taxol was purchased from Sigma and internal standard samples were used to confirm the product.

Table 1 Effect of inoculum size on the growth (A) and taxol production (B) in cell cultures of T. chinensis

(A)

Inoculum size (g/l)	Culture time[a] (d)	Maximum biomass (g/l)	Average growth rate[b] (1/d)	Biomass productivity (g/l d)
1.5	26	10.8	0.24	0.36
2.0	24	12.9	0.23	0.45
3.0	21	13.1	0.16	0.48
4.0	19	13.4	0.12	0.49
5.0	16	14.8	0.12	0.61
6.0	14	16.0	0.12	0.71

[a]Culture time when the maximum cell mass was reached. [b]Average growth rate = (Maximum DW – Initial DW)/Initial DW/Cultivation time.

(B)

Inoculum size (g/l)	Culture time[a] (d)	Intracellular taxol content (μg/g DW)	Intracellular taxol production (mg/l)	Extracellular taxol amount (mg/l	Total taxol (mg/l)	Taxol productivity (μg/l d)
1.5	26	25.6	0.276	0.091	0.37	14.1
2.0	26	31.6	0.360	0.091	0.45	17.3
3.0	23	32.7	0.396	0.073	0.47	20.4
4.0	19	20.5	0.276	0.077	0.35	18.6
5.0	16	22.4	0.332	0.035	0.37	22.9
6.0	16	13.0	0.192	0.045	0.24	14.8

[a]Culture time when the maximum taxol production was obtained.

Results and discussion

The effects of inoculum size within the range of 1.5 to 6.0 g DW/*l* on the cell cultures of *T. chinensis* were investigated on a shaker flask scale. Table 1A shows that an increase of inoculum size greatly shortened the cultivation period, but the net biomass increase was almost the same in the various cases. For example, at an inoculum size of 6.0 g DW/*l*, the cell growth reached its peak after 14 days cultivation, while 26 days was required for the cells under an inoculum size of 1.5 g DW/*l*. In addition, the cell cultures with a relatively higher inoculum size had a higher biomass productivity although their average growth rate was lower.

Table 1B indicates that the maximum taxol production was reached on the same day as the cell peak or a few days later, which coincided with our previous work (Wang *et al.*, 1996). The taxol (both intracellular and total) production was increased with an increase of inoculum size from 1.5 to 3.0 g DW/*l*, while a further increase of inoculum size decreased its accumulation. The taxol content, total intracellular taxol and total taxol production were reached the highest in the cell cultures with 3.0 g DW/*l* of inoculum size on day 23. The taxol productivity was relatively higher (ca. 20 μg/*l* d) in the cultures with an inoculum size of 2.0–5.0 g DW/*l*. In several other plant cell cultures, it is also reported that an intracellular metabolite accumulation was improved under a moderately higher inoculum size (Zhong & Yoshida, 1995; Zhang & Zhong, 1997). The mechanism of inoculum size effect on product synthesis may be related with enhanced activities of enzymes in the metabolic pathways (Moreno *et al.*, 1993).

The results also show that the extracellular taxol accumulation was relatively higher at low inoculum sizes of 1.5 and 2.0 g DW/*l*; and in all the cases the amount of taxol excretion was less than 25% of the total metabolite produced by the cells. We note that until now there have been no reports about the effect of inoculum size on metabolite excretion in cell cultures. The above finding is interesting, but the reason why more taxol was excreted under a relatively lower inoculum size is still unknown, and it requires further studies.

Acknowledgements

This work was supported by National Natural Science Foundation of China (No. 29606003). We thank X.F. Chen for his help during the project initiation.

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Received as Revised 25 February 1997