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ORIGINAL RESEARCH PAPER

# Modeling of tanshinone synthesis and phase distribution under the combined effect of elicitation and in situ adsorption in *Salvia miltiorrhiza* hairy root cultures

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**Abstract** Modeling of tanshinone synthesis and phase distribution under the combined effect of elicitation and adsorption was studied. The simulated results showed that enhancement of tanshinone production was mainly due to the effect of the elicitor and that resin addition resulted in adsorbance of the tanshinones from the root and alteration of tanshinone distribution. Furthermore, parameter sensitivities analysis showed that the rate of transport of tanshinones from the root to the medium was the important factor that influenced tanshinone accumulation in the resin. In conclusion, the modeling of tanshinone synthesis and phase distribution identified the process mechanism under the combined effect of elicitation and adsorption and this modeling can be used in similar plant tissue culture systems.

**Keywords** Modeling · Synthesis · Distribution · Tanshinone · *Salvia miltiorrhiza* hairy root

**Mathematical notations**

| | |
|---|---|
| $X$ | Dry weight of hairy root, g/l |
| $\mu$ | Specific growth rate of hairy root, $d^{-1}$ |
| M | Secondary messenger signal |
| G | DNA concentration |
| E | DXS enzyme concentration |
| $f(\alpha)$ | Generation of secondary messenger signal as a function of elicitor concentration |
| $k_1$ | Degradation rate constant of secondary messenger signal, $d^{-1}$ |
| $\eta$ | Transcription efficiency of the gene |
| $k_2$ | Degradation rate constant of specific mRNA, $d^{-1}$ |
| $k_3$ | Rate constant of enzyme formation, $d^{-1}$ |
| $k_4$ | Rate constant of enzyme degradation, $d^{-1}$ |
| Pi | Tanshinone production in the root, mg/l |
| $k_5$ | Rate constant of tanshinone formation in the root, $d^{-1}$ |
| $n_1$ | Non-linearity influence coefficient |
| $K_p$ | Rate constant of inhibition on tanshinones due to product accumulation |
| $k_6$ | Rate constant of tanshinone degradation in the root, $d^{-1}$ |
| $n_2$ | Non-linearity influence coefficient |

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$k_7$ Rate constant of transport of tanshinones from the root into the medium, $d^{-1}$

$K_e$ Equilibrium constant for tanshinones in and out of the root

Pe Tanshinone production in the medium, mg/l

$k_8$ Rate constant of transport of tanshinones from the medium into the resin, $d^{-1}$

$K_r$ Equilibrium constant for tanshinones in the medium and resin

Pr Tanshinone production in the resin, mg/l

## Introduction

The addition of a solid adsorbent or an extraction solvent to the culture medium combined with other measures can improve the productivity of plant tissue cultures in which secondary products are mainly stored in the cell. The combination of resins and elicitors is an effective technology for promoting the synthesis and recovery of target product in plant tissue cultures (Buitelaar et al. 1993; Asada and Shuler 1989). Our previous study showed that in situ adsorption (using a hydrophobic polymeric resin, X-5) combined with elicitation (using a material derived from yeast extract) enhanced diterpenoid tanshinone production in *Salvia miltiorrhiza* hairy root cultures (Yan et al. 2005). However, the understanding of the mechanism of in situ adsorption and its combination with elicitation is limited and thus requires further investigation. Modeling the culture process for hairy root culture is important because sampling is difficult.

It is therefore crucial to establish and develop a reasonable mathematical model to describe the various phenomena during culture using different product enhancement techniques for further understanding and expanding the process with plant cells or tissues to produce secondary metabolism. There are few mathematical models to describe the process of elicitation or two-phase culture during plant cell or tissue culture (Yuan et al. 2002; Wu et al.2000; Singh et al. 1994). Muccilli (2001) established a model of synthesis and transport of secondary metabolites that considered the contribution of elicitation and in situ extraction as productivity enhancement techniques simultaneously in the California poppy suspension. This model provided a valuable basis for simulation of the combined processes of elicitation and in situ adsorption in other plant tissue culture systems.

In this study, we modeled the dynamic process of tanshinone synthesis and phase distribution under the combination of elicitation and adsorption by revising the model put forward by Muccilli. Under different culture conditions, tanshinone synthesis and phase distribution were simulated and experiments were performed to verify the validity of the model. Finally, we explored the key factors that influence tanshinone accumulation.

## Materials and methods

### Hairy root culture

*Salvia miltiorrhiza* hairy roots were cultured as described by Yan et al. (2005).

### Elicitor preparation

The yeast elicitor (YE) was the carbohydrate (polysaccharide) fraction of yeast extract (Sigma) prepared by ethanol precipitation as described elsewhere (Ge and Wu 2004).

### Adsorbent preparation

X-5 macroporous polystyrene resin (Nankai University Chemical Plant, Tianjin, China) was used as adsorbent and pre-treated as previously described (Yan et al. 2005).

### Assay of 1-deoxy-D-xylulose-5-phosphate synthase (DXS)

The extraction and analysis of DXS was performed as described by Ge and Wu (2004). The formation and degradation of the enzyme were represented by the terms $k_3[mRNA]$ and $k_4[E]$ in Eq. (4), respectively, and were predicted from calculations.

### Extraction and analysis of tanshinones

The method and instruments for extracting tanshinones from the roots, the resin, and the culture medium were described previously (Yan et al. 2005).

Dynamic experiments

X-5 resin (1 g) in a nylon bag or YE (100 mg carbohydrate/l) was added separately or simultaneously to each culture flask on day 22 for the experimental group whereas nothing was added to the control group. The samples were analyzed continuously for 7 days. All treatments were performed in triplicates and the results are represented as mean and standard error (S.E.).

## The mathematical model

Hypothesis of the model

According to the inherent character of hairy root culture, the elicitation process, product synthesis, and hypothesis of the model were performed as follows:

(1) Growth of hairy root: the hairy root was considered the homogeneous growth whole and the specific growth rate was the basic variable in the model.
(2) The process of elicitation on enzyme activity: assuming that YE first induced the generation and transfer of a secondary messenger signal, then changes in the specific mRNA concentration codified by the *dxs* gene followed, which was a function of messenger signal (symbol "M" in the mathematical model) and DNA concentration (symbol "G" in the mathematical model). Finally, specific mRNA was translated into the corresponding intracellular enzyme (DXS enzyme, symbol "E" in the mathematical model).
(3) Synthesis and distribution of tanshinones: the supposed mode of tanshinone synthesis and distribution was established according to the mode put forward by Muccilli (2001) and is shown in Fig. 1. The main point is that tanshinone concentrations in different phases are in equilibrium. The resins in the bag were considered as a whole ignoring the asymmetry of tanshinone distribution in the resins.

The mathematical model

According to the above hypothesis, the mathematical model put forward by Muccilli (2001) was used with suitable revisions:

(1) The growth rate of hairy root:

$$\frac{d[X]}{dt} = \mu[X] \tag{1}$$

(2) The effect of elicitation on the enzyme activity:

$$\frac{d[M]}{dt} = f(\alpha) - k_1[M] - \mu[M] \tag{2}$$

$$\frac{d[mRNA]}{dt} = \eta[M][G] - k_2[mRNA] - \mu[mRNA] \tag{3}$$

$$\frac{d[E]}{dt} = k_3[mRNA] - k_4[E] - \mu[E] \tag{4}$$

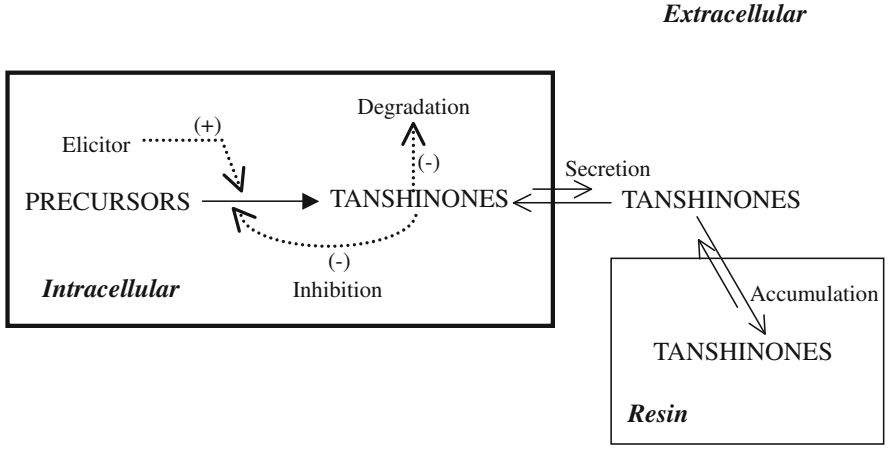


**Fig. 1** The potential modes of tanshinone synthesis and distribution under elicitation and in situ adsorption

(3) Synthesis and distribution of tanshinones:

$$\frac{d[P_i]}{dt} = \frac{k_5[E]^{n_1}}{1+\frac{[P_i]}{K_p}} - k_6[P_i] - \mu^{n_2}[P_i] - k_7\left([P_i] - \frac{[P_e]}{K_e}\right) \quad (5)$$

$$\frac{d[P_e]}{dt} = k_7\left([P_i] - \frac{[P_e]}{K_e}\right) - k_8\left([P_e] - \frac{[P_r]}{K_r}\right) \quad (6)$$

$$\frac{d[P_r]}{dt} = k_8\left([P_e] - \frac{[P_r]}{K_r}\right) \quad (7)$$

where $n_1$ and $n_2$, the non-linearity influence coefficient in the Eq. (5), were added to the original equation. Equation (7) left out 1 coefficient compared with the original equation. The revision of these non-linearity coefficients should closely reflect the real experimental process.

### Determination of the model parameters

The method of least squares was used to fit the model to the experimental data from the dynamic experiments combining YE elicitation and resin X-5 adsorption. The parameters' values are shown in Table 1.

## Results and discussion

### Fitting experimental data

Figures 2 and 3 show that the established model fits the experimental results. Changes in DXS activity followed changes in mRNA, and there was a similar tendency to change between the mRNA and DXS enzyme activity in Fig. 2. These results support the above hypothesis. The tanshinones produced by the root could be absorbed by the resin and thereby did not accumulate in the root (Fig. 3). The total tanshinone production at day 7 after treatment (the combination of elicitation and adsorption) was 2.8 times higher that of the beginning production.

**Table 1** Model parameters determined by mathematically fitting the model to experimental dada

| Parameter | Value |
|---|---|
| $k_1$ | 1.425 $d^{-1}$ |
| $\eta[G]$ | 180.864 $d^{-1}$ |
| $k_2$ | 0.0536 $d^{-1}$ |
| $k_3$ | 60.762 $d^{-1}$ |
| $k_4$ | 15.568 $d^{-1}$ |
| $k_5$ | 1.012 $d^{-1}$ |
| $n_1$ | 0.152 |
| $K_p$ | 10.106 |
| $k_6$ | 0.115 $d^{-1}$ |
| $n_2$ | 0.124 |
| $k_7$ | 0.4945 $d^{-1}$ |
| $K_e$ | 0.1112 |
| $k_8$ | 15.238 $d^{-1}$ |
| $K_r$ | 140.369 |

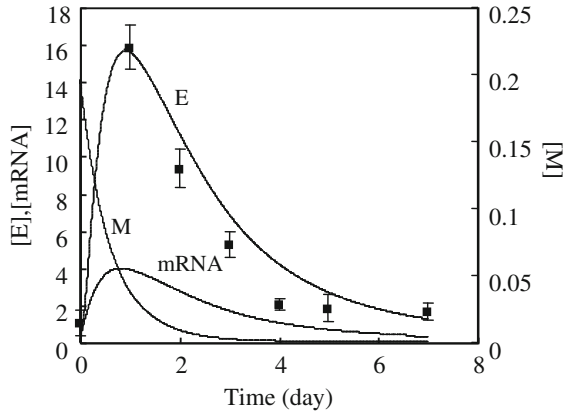


**Fig. 2** The fit curve of experimental enzyme activity results (*E* the DXS enzyme concentration; and *M* the secondary messenger signal)

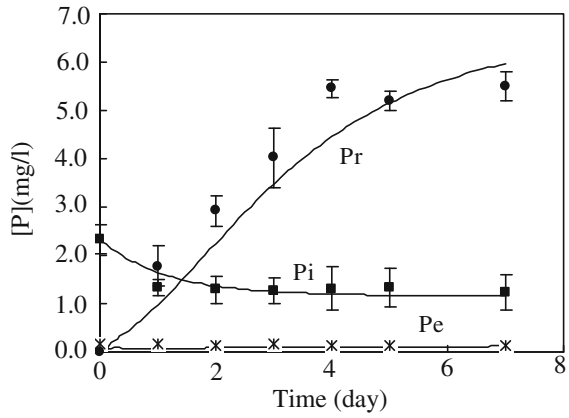


**Fig. 3** The fit curve of experimental results of tanshinones with a combination of elicitation and in situ adsorption (*Pr* the tanshinone production in the resin; *Pi* the tanshinone production in the root; and *Pe* the tanshinone production in the medium)

### Simulation of tanshinone synthesis and distribution under different culture conditions

The dynamic process was simulated under different culture conditions: the normal culture (without any

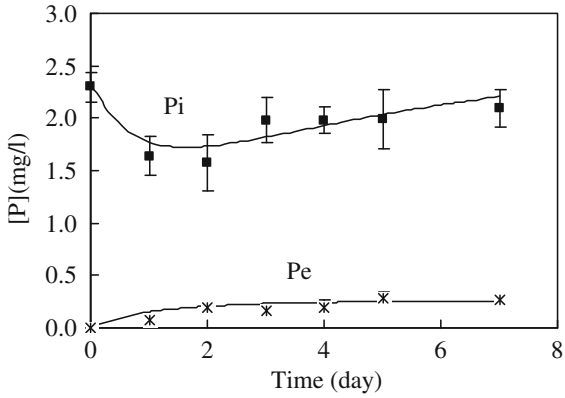


**Fig. 4** The stimulation model of products of the dynamic process of the normal condition without elicitation and in situ adsorption (*Pi* the tanshinones production in the root; and *Pe* the tanshinones production in the medium)

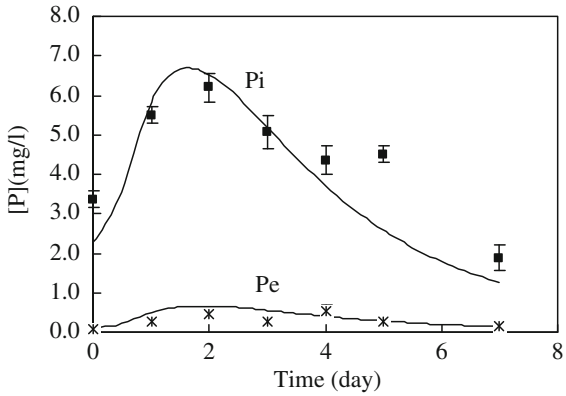


**Fig. 5** The stimulation model of products of the dynamic process of elicitation (*Pi* the tanshinone production in the root; and *Pe* the tanshinone production in the medium)

addition), elicitor YE added alone, or resin X-5 added alone. In the normal culture, tanshinone production in the hairy roots culture reached 2.3 mg/l (Fig. 4). The addition of the elicitor YE increased tanshinone production by 2.9-fold in the root reaching a peak 2 days after treatment (Fig. 5). Addition of the resin X-5 alone decreased tanshinone content in the root 2 days after treatment, and this change is the same as that of tanshinone productions in the root observed when using a combination of the elicitor YE and the resin X-5 (Figs. 3, 6).

Tanshinone production in the medium remained at low during the entire growth period for all four culture conditions (Figs. 3, 4, 5, and 6).

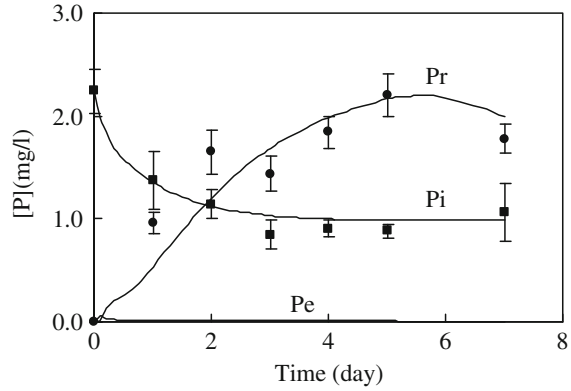


**Fig. 6** The stimulation model of products of the dynamic process of in situ adsorption (*Pr* the tanshinone production in the resin; *Pi* the tanshinone production in the root; and *Pe* the tanshinone production in the medium)

Accumulation of tanshinones in the resin X-5 can be seen in Figs. 3 and 6. Tanshinones accumulated more in the resin and the speed of accumulation was quicker when the resin was added at the same time as the elicitor compared to resin alone. However, the presence of the elicitor did not influence the adsorption ratio of the resin X-5 on tanshinones and the adsorption ratio in the resin alone and resin and elicitor together culture conditions was almost identical. Furthermore, the maximal tanshinone production under the combined application of elicitation and adsorption reached 6.9 mg/l but the maximal tanshinone production under the individual adsorption reached only 3.1 mg/l.

These results showed that the individual addition of the resin X-5 can promote the release of tanshinones from the root but cannot effectively enhance tanshinone production. Tanshinone production that increased under the combined application of elicitation and adsorption was mostly because of the effect of the elicitation whereas the addition of resin only adsorbed the tanshinones and changed the distribution of tanshinones in the culture system.

## Parametric sensitivity of tanshinones in resin to parameters of the model

The parameters were divided into three groups to examine their sensitivities to tanshinones in resin (Figs. 7, 8, and 9). Throughout the overall analysis, the final accumulation of tanshinones in the resin was consistently most sensitive to variations in $k_3$, $\eta(G)$, $k_4$,

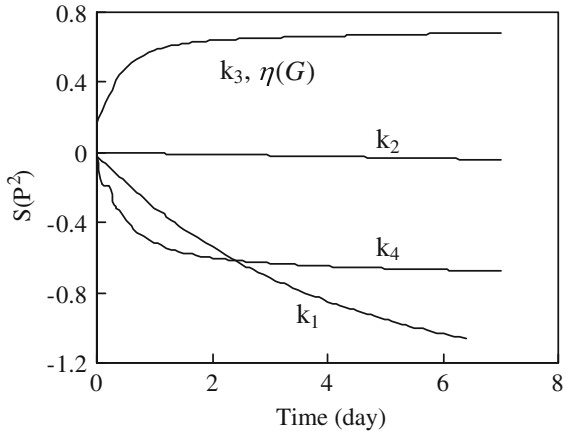


**Fig. 7** Sensitivities of tanshinone production level in the resin to parameters describing messenger signal, transcription, and translation ($k_1$ the degradation rate constant of secondary messenger signal; $k_2$ the degradation rate constant of specific mRNA; $k_3$ the rate constant of enzyme formation; $k_4$ the rate constant of enzyme degradation; and $\eta[G]$ the grouped parameter with the transcription efficiency of gene and DNA concentration)

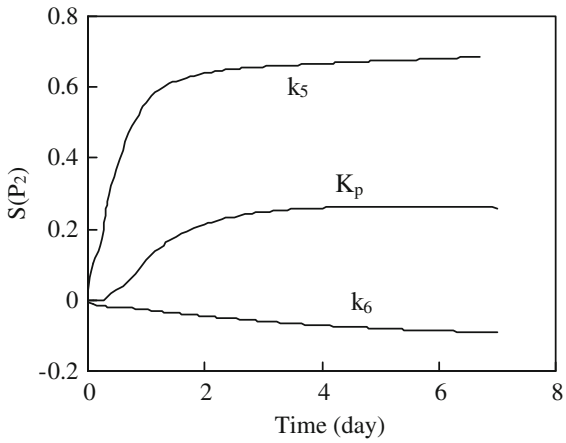


**Fig. 8** Sensitivities of tanshinone production level in the resin to parameters describing intracellular product formation, degradation, and inhibition ($k_5$ the rate constant of tanshinones formation in the root; $k_6$ the rate constant of tanshinones degradation in the root; and $K_p$ the rate constant of inhibition on tanshinones due to product accumulation)

and $k_5$, and was slightly less sensitive to variation in $K_p$. This reflected the importance of intracellular tanshinone formation and enzyme formation, degradation, and inhibition on ultimate tanshinone accumulation in the resin. In addition, the sensitivity of [Pr] to parameters $k_7$ and $k_8$ gradually decreased to half and zero, respectively, over the time course but the accumulation of tanshinones in the resin increased at all times. This

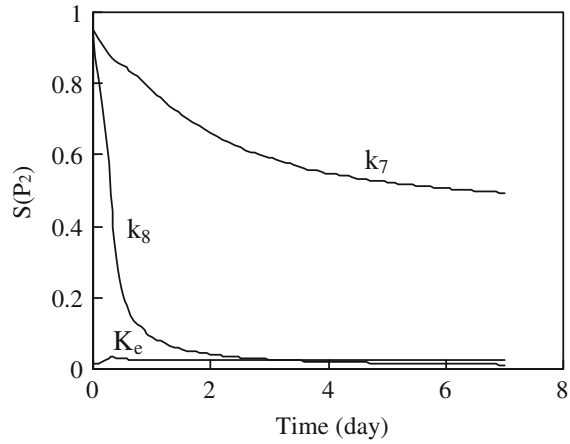


**Fig. 9** Sensitivities of tanshinone production level in the resin to parameters describing transport of product ($k_7$ the rate constant of transport of tanshinones from the root into the medium; $k_8$ the rate constant of transport of tanshinones from the medium into the resin; and $K_e$ the equilibrium constant for tanshinones in and out of the root)

indicated that the model had a higher sensitivity to the parameter describing the rate of transport of tanshinones from the root to the medium than to the parameter describing rate of transport of tanshinones from the medium to the resin.

Our simulated results produced different conclusions from those of Muccilli (2001) although our simulations are based on his revised model. Muccilli (2001) concluded that the combined application of elicitation and in situ extraction results in approx. 7-fold increase of alkaloid products and this is due to the synergistic improvement obtained by the two treatments together. As mentioned above, we concluded that the increase in tanshinone production was mostly because of the effect of the elicitation, whereas the addition of the resin only adsorbed the tanshinones and changed the distribution of tanshinones in the culture system under the combined application of elicitation and adsorption. The reason that a similar model gives different simulated results is not clear, but the significance of transport in the accumulation of the products in the second phase or a solid absorbent is same in different simulations.

## Conclusions

In this work, the dynamic process of tanshinone synthesis and distribution by integrating the

productivity enhancement techniques of elicitation and adsorption was modeled using a revised model proposed by Muccilli (2001). The process simulations identified the mechanism of the synergic effects well at a certain extent. The analysis of simulated results showed that the elicitor mainly enhanced tanshinone production, and the addition of the resin mostly affected adsorbance of the tanshinones from the root and altered tanshinone distribution. Furthermore, the parameter sensitivities analysis indicated that the rate of tanshinone transport from the root to the medium was the important factor influencing tanshinone accumulation in the resin. These results confirmed that the model proposed by Muccilli is feasible and can be used to analyze the process of two-phase plant cell or tissue culture after appropriate revision.

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## References

Asada M, Shuler M (1989) Stimulation of ajmalicine production and excretion from *atharanthus roseus*: effects of adsorption in situ, elicitors and alginate immobilization. Appl Microbiol Biotechnol 30:475–481

Buitelaar RM, Leenen EJTM, Geurtsen G, Groot Æde, Tramper J (1993) Effects of the addition of XAD-7 and of elicitor treatment on growth, thiophene production, and excretion by hairy roots of *Tagetes patula*. Enzyme Microb Technol 15:670–676

Chen H, Chen F, Zhang YL, Song JY (1999) Production of lithospermic acid B and rosmarinic acid in hairy root cultures of Salvia miltiorrhiza. J Ind Microbiol Biotechnol 22:133–138

Ge XC, Wu JY (2004) Tanshinone production and isoprenoid pathways in *Salvia miltiorrhiza* hairy roots induced by Ag + and yeast elicitor. Plant Sci 168:487–491

Muccilli TA (2001) In situ extraction of benzophenanthridine alkaloids from calfornia poppy suspension cultures. PhD thesis, Rutgers University, USA

Singh G, Reddy GR, Curtis WR (1994) Use of binding measurements to predict elicitor dosage requirements for secondary metabolite production from root cultures. Biotechnol Prog 10:365–371

Wu ZL, Hu B, Yuan YJ, Hu ZD (2000) Kinetic model on two-liquid-phase culture *Taxus chinesis var.mairei* cells for production of taxol. J Chem Ind Eng (China) 51:637–642

Yan Q, Hu ZD, Tan RX, Wu JY (2005) Efficient production and recovery of diterpenoid tanshinones in *Salvia miltiorrhiza* hairy root cultures with in situ adsorption, elicitation and semi-continuous operation. J Biotechnol 119:416–424

Yuan YJ, Li C, Wu JC, Hu ZD (2002) A model for signal transduction in suspension cultures of *Taxus chinensis* var. *mairei* induced by an oligosaccharide from *Fusarium oxysporum*. Biotechnol Lett 24:407–412