The Story of Taxol Production by Plant Cell Culture

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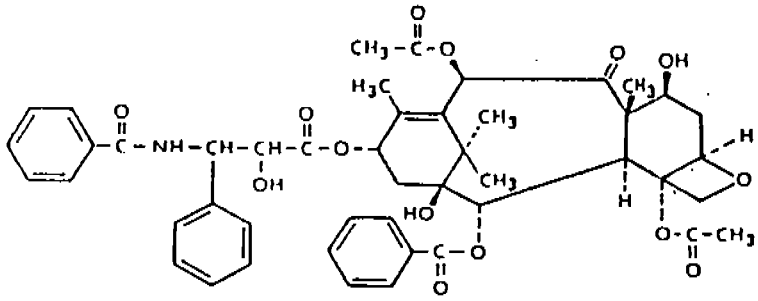
Introduction

Taxol, a promising antineoplastic agent, has been approved by FDA for the treatment of ovarian and breast cancer. It is also under clinical trials for the treatment of other types of cancer. Currently, the taxol is very expensive drug because of short supply. The only commercial source of drug is the bark of yew trees. Slow growth of yew tree and relatively small content of taxol in bark caused short of supply and made a demand of alternative sources.



Plant cell culture of *Taxus* spp. used to be pointed as a potential alternative to the extraction from yew trees. There are some reports for the cell cultures of *Taxus* spp.(1,2,3). These reports, however, don't provide enough information that plant cell culture for the taxol production can be the alternative source.

I have induced callus and cell lines from various explants of *Taxus* spp.. And the following suspension cultures have been developed and used for mass culture. Bioreactors were also applied for the large scale culture. And

other works for the possible commercial application of taxol were made. These several years research experience about taxol production by plant cell culture could possibly review the possibility of these works as a alternative source of that expensive drug.

Characteristics of Cell Culture

Plant Material and Callus Initiation

Various yew trees in Korea and USA were obtained and used as explant sources. *Taxus brevifilia* Nutt and *Taxus baccata* Pendula were from the greenhouse of Rutgers University, New Jersey. Other plants were supplied from The Institute of Forest Genetics, Forestry Administration, Korea.

Table 1. List of plants used for callus initiation

Taxus brevifolia
Taxus baccata Pendula
Taxus cuspidata
Taxus caespitosa
Taxus media Han
Taxus media Hicksii
Taxus media Andersonii
Taxus media Vermuelen
Taxus media Hunnewelliana
Taxus media Robusta
Taxus media Trrandidolia
Taxus media Kelseyi
Taxus media Moon's columnalis

The surface of explants used to be highly contaminated and required intensive surface sterlization. Fig. 1. shows the scheme of callus initiation. For the surface sterlization with sodium hyperchloride, immersion around 20 minutes were recommended.

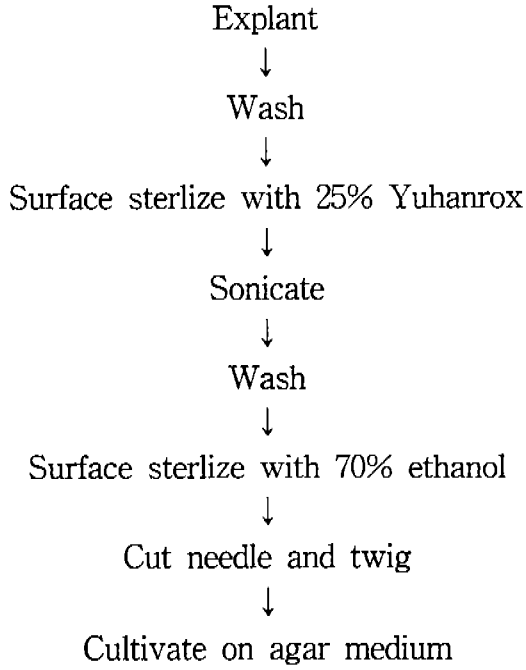


Fig. 1. The scheme of callus initiation

Media Compositions and Supplements

The most frequently used basal media for the culture were B5 and SH. As auxin, either 2,4-D, IBA, or NAA was used. Among them NAA was preferred. Kinetin or BAP was proper cytokinin for cultures.

In callus cultures, the darkening of tissues was frequently observed. We call this browning of callus tissue. This is probably the result of oxidation of phenolic compounds by the cell. Cystein, ascorbic acid, or polyvinylpolypyrrolidone was frequently used as browning reducing agent either alone or in combination.

Callus Growth and Suspension Culture.

Besides the browing in callus culture, the slow growth was also frequently observed. Efforts were required to overcome this problem. Frequent subculture, maintenance of high cell density, addition of supplementary nutrients, optimization of the concentration of major media component, maintenance of adequate humidity are part of recommendations. Healthy fast growing callus looked soft and white which was always recommended for good callus culture.

The healthy fast growing callus guaranteed good suspension cultures. The fast growing suspension cells showed almost the same doubling time as other normal plant cell cultures. Fig. 2. shows typical growth kinetics in suspension cultures.

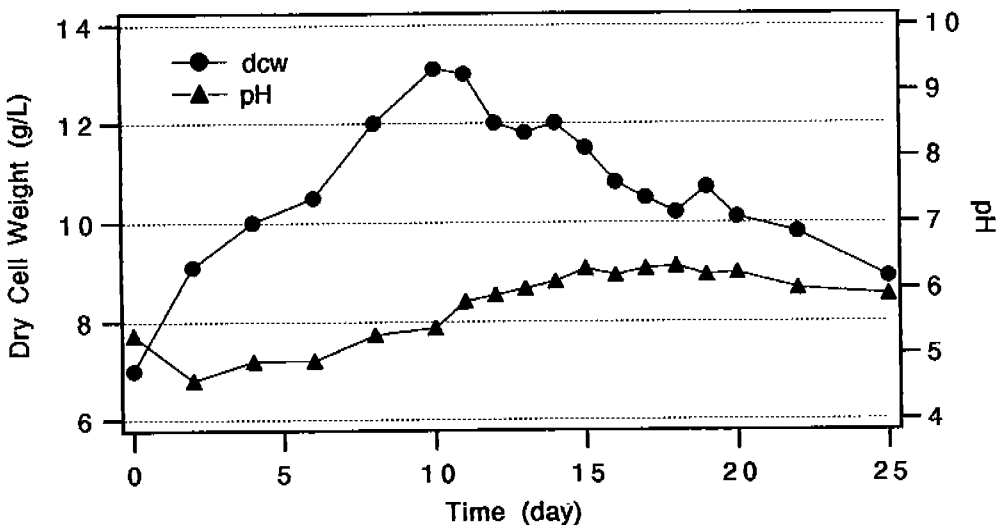


Fig. 2. Time course behavior of cell growth in batch suspension cultures of *Taxus baccata* Pendula

HPLC Analysis of Taxol - A Possible Pitfall

Taxol from cultured cells was extracted with methanol-dichloromethane and analyzed by HPLC. Columns for HPLC analysis could be reversed phase C18, phenyl, pentafluorophenyl(PFP), Curosil™, Taxil™. The proper combination of methanol, water, and acetonitrile was used as mobile phase.

Taxol was detected by UV absorbance at 228 nm.

HPLC analysis of taxol with columns described above showed good results for samples from the bark of yew tree(Fig. 3). The samples extracted from cultured cells, however, should not be analyzed by the simple HPLC. Cultured cells provided unknown chemicals which were not easily separated from taxol with columns described above. Efforts should be made to separate taxol from unknown phytochemicals. Usually the retention time of taxol was more than 40 minutes with adequate mobile phase and separation conditions. Pretreatment of samples from cultured cells to remove unknown phytochemicals was also recommended. Simple UV detector was not adequate to monitor as far as taxol was not separated from other phytochemicals. Photodiode array detector or dual-wavelength detector was strongly recommended to monitor proper taxol peak.

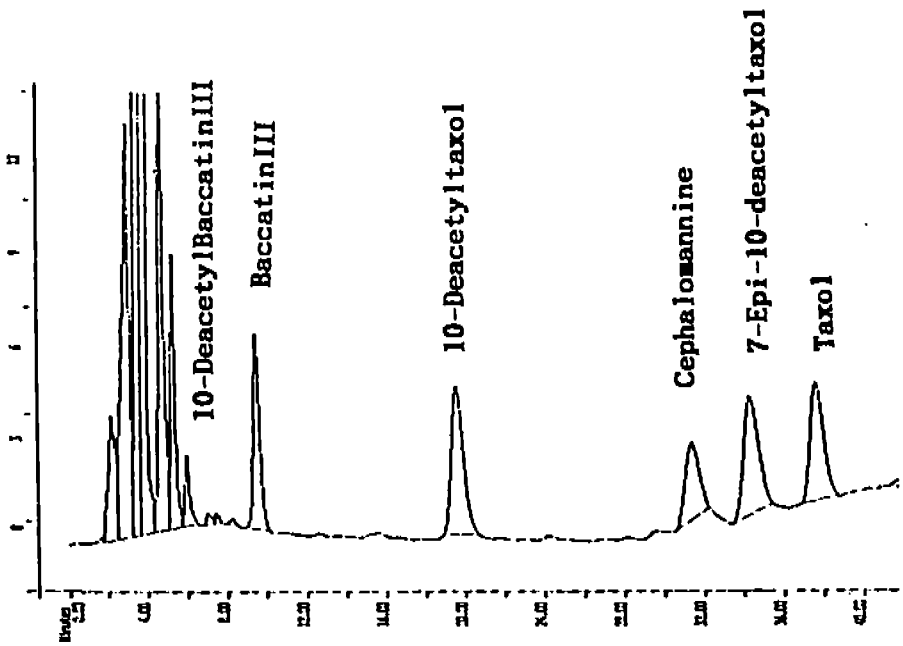


Fig. 3. HPLC chromatogram for taxol and taxanes with reversed phase PFP column.

Production of Taxol in Cultured Cells

In the normal suspension cultures, the kinetics of taxol production didn't show any routine patterns. It is sure that the pattern of taxol production is non-growth associated. After 20 days or later the taxol production starts to increase. The accumulation of taxol, however, was unregularly controlled by several uncertain factors. Fig. 4A. shows one of the time course behaviors of taxol production in suspension cultures. The reproducibility, however, was poor. Fig. 4B. is the same kinetics of taxol production made 1 month after that of Fig. 4A.

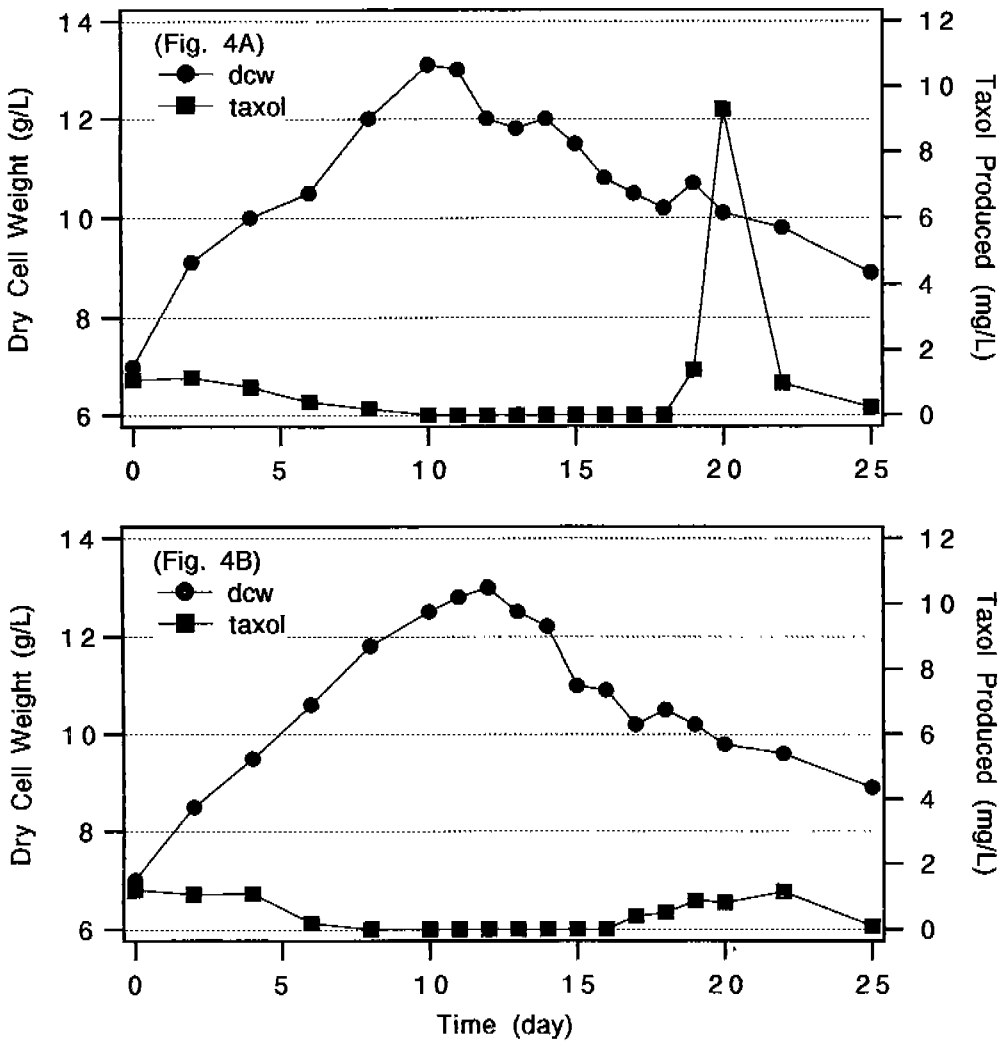


Fig. 4. Time course behavior of taxol production in suspension cultures of *Taxus baccata* Pendula

Special Approaches for the Enhanced Production of Taxol

The major reason that the taxol production by plant cell culture is not optimistic is that the matabolic pathway of taxol synthesis is not completely known yet. Hardly the taxol production can be expected or controlled without information about metabolic pathway. Only a small part of total pathway is known.

Several special approaches to increase taxol production in suspension culture could be applied even if the matabolic pathway was not completely known. Among them optimization of media component, elicitation, precursor feeding, two-phase culture, production medium techniques were applied and their results are shown in Fig. 5.-7. Application of bioreactor was also made for the large scale operation. One of the results which was operated with low shear stirred tank bioreactor is shown in Fig. 8.

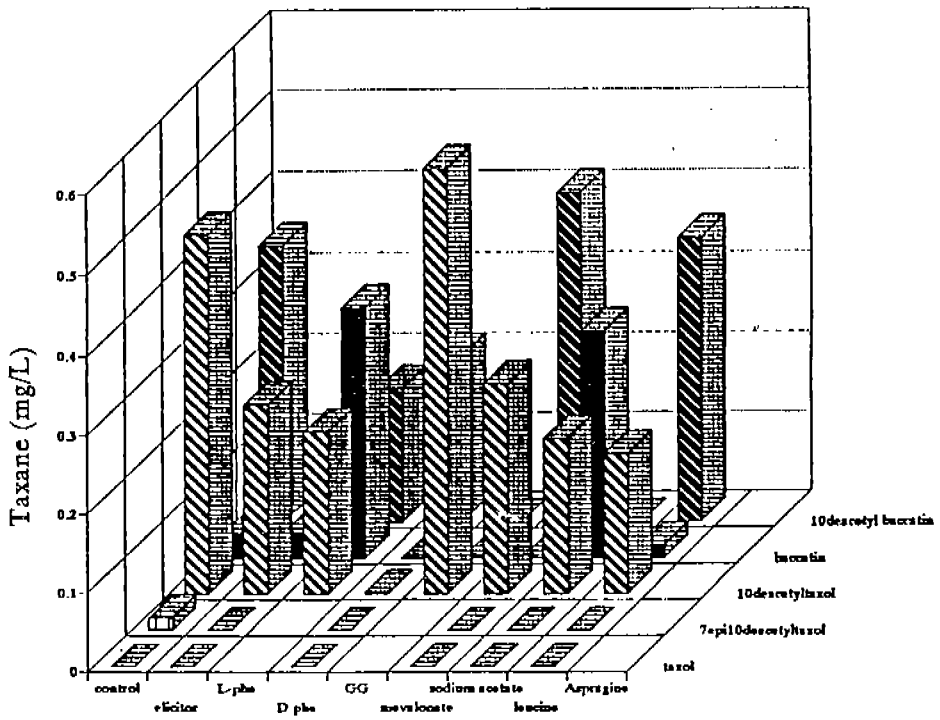


Fig. 5. Precursor feeding effects on taxane production

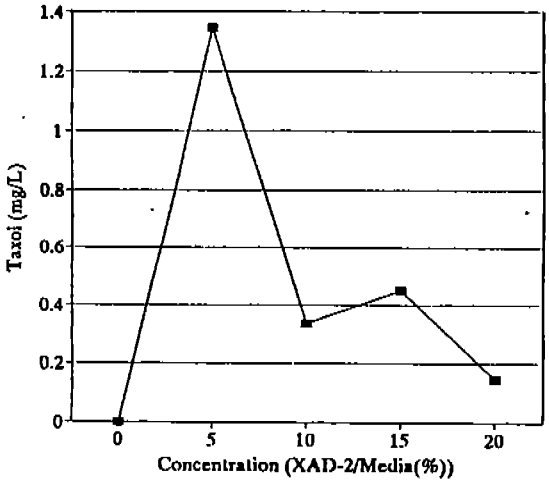
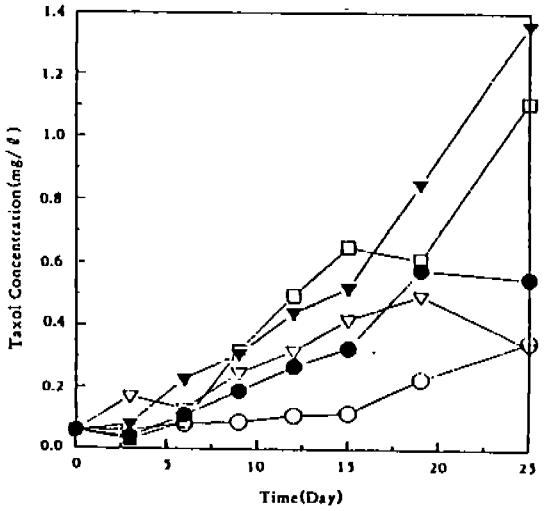


Fig. 6. Two-phase culture effects on taxol accumulation



Batch profiles of taxol production at various sucrose concentrations(○, 20 g/ℓ; ●, 40 g/ℓ; ▽, 60 g/ℓ; ▼, 80 g/ℓ; □, 100 g/ℓ sucrose)

Fig. 7. Sucrose effects on taxol production

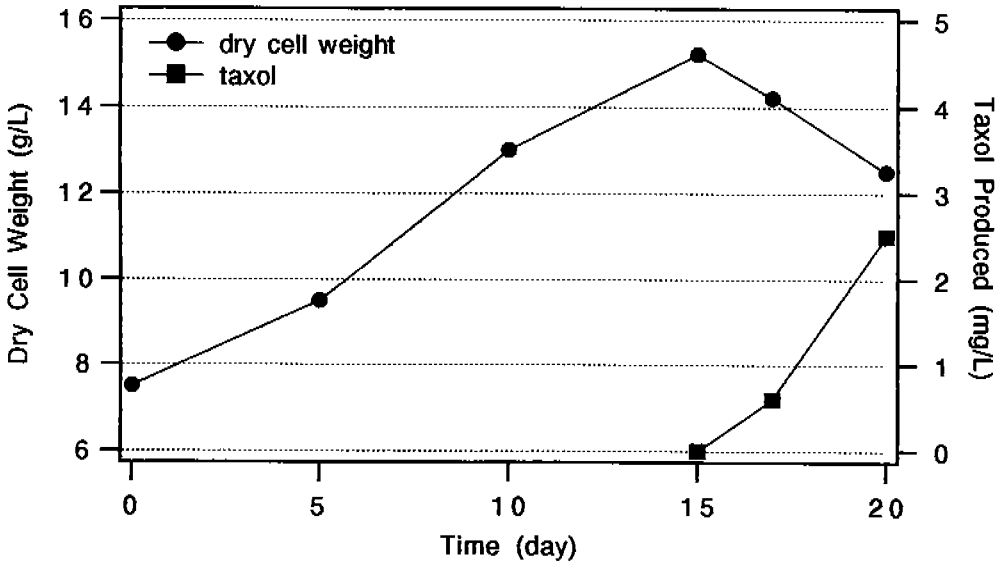


Fig. 8. Time course changes of cell growth and taxol production in 10 L STR bioreactor

Economics

Production of secondary metabolites by plant cell culture is known as an inherently expensive technique. Limited number of secondary metabolites of substantial price coupled with some other concerns can be justified as targets for commercial application. Prof. Shuler in Cornell University suggested the primary determinant of economic viability is the volumetric productivity of the reactor component of the process. He used volumetric productivity and wholesale price to calculate the production cost. According to his formular the wholesale price is 12 c/L-d ±60%.

Based on the concept of volumetric productivity and wholesale price, this analysis suggests an approximate lower bound on the volumetric productivity of taxol by plant cell culture. If the target production cost of taxol is $500/g ±60%, the volumetric productivity by cell culture should be higher than 0.24 mg/L-d. This value is much lower than those of other normal plant cell culture systems. This analysis, although very crude, suggests that taxol production by plant cell culture can be the alternative source because of far low allowable volumetric productivity. This is mainly due to high market price of taxol as an anticancer drug. In addition to volumetric productivity, separation and purification costs should be considered. And the market size of taxol can influence the capital costs. Considerable effort is required for optimization and economic analysis. Because of very high market price, taxol production by plant cell culture is believed to justify such a developmental effort.

References

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