Effect of Methyl Jasmonate on Production of Paclitaxel and Related Taxanes in a Hydrophobic Ionic Liquid-Medium Two Phase Culture System

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We report here a hydrophobic ionic liquid (IL), 1-butyl-1-methylpyrrolidinium bis(trifluoromethanesulfonyl)imide (P14-TFSI), that contributed to increasing the amount of production of paclitaxel and the related taxanes of 10-deacetyl baccatin III, baccatin III and cepharomannine with in situ extraction from an aqueous medium in the IL-medium two phase culture system. Addition of an elicitor, methyl jasmonate (MJ) to the cell culture system enhanced the amount of production of the taxanes in the culture including 2.5 % IL. The total amount of the taxanes in the culture including 10 µM MJ and 2.5 vol% IL was more than 200 times greater than those in the control culture in the absence of IL and MJ, and more than two times greater than those in the culture including 2.5 vol% IL alone.

1. Introduction

Paclitaxel (PTX), a diterpenoid alkaloid, is an excellent anticancer drug due to its distinctive antitumor activities against a variety of cancers by stabilizing their depolymerization of microtubules. PTX is so expensive that its production cost needs to be decreased for cancer patients to keep their quality of life (QOL). Manufactured production of PTX is carried out by a semi-synthetic method which requires many reaction steps with hazardous organic solvents from precursors such as baccatin III (BIII) or 10-deacetyl baccatin III (10-DAB) extracted from needles [1-2]. A plant cell culture using callus induced from the needles is one of the promising methods for the cost-effective and safe production of PTX. However, there is a problem of feedback-inhibition of the PTX. Two phase culture systems using water-immiscible liquids such as organic solvents and ionic liquids (ILs) have been proposed for the in situ extraction of hydrophobic PTX from the culture medium via their hydrophobic interaction to decrease the PTX’s inhibition, [3-4]. We reported on the use of more hydrophobic organic solvents such as lauryl alcohol and ILs such as 1-hexyl-3-methylimidazolium hexafluorophosphate (HMIM-PF₆) for the in situ extraction of paclitaxel from the aqueous medium [5-6]. As harmless solvents in contrast to conventional organic solvents, ILs have attracted attention because of their special features such as high thermal stability, negligible volatility, and selective solubility. ILs have been applied for extraction and separation of bioactive compounds of hydrophobic 3-indole-butyrlic acid, ferulic acid and caffeine acid found in plants [7-13]. Magnetic ILs such as benzyltrioctylammonium bromotrichloroferrate (III) and 1,12-di(3-hexadecylbenzimidazolium) dodecane bis[(trifluoromethyl)-sulfonyl]imide bromotrichloroferrate (III) have higher extraction efficiencies for DNA molecules [14]. We reported the enhancement of PTX and
the related taxanes of 10-DAB, BIII and cepharomannine (CM) in the plant cell culture with the in situ extraction of these compounds by HMIM-PF₆ [6]. Figure 1 shows the metabolic pathway of PTX and the related taxanes from geranylgeranyl diphosphate [11]. Recently, we reported that a novel hydrophobic IL such as 1-butyl-1-methylpyrrolidinium bis(trifluoromethanesulfonyl)imide (P14-TFSI) increased the productivities of the taxanes [15].

Elicitors such as methyl jasmonate (MJ) and jasmonic acid have been used to increase the productivities of the secondary metabolites such as the taxanes [6, 16]. In the present research the effect of MJ addition on the enhancement of the production amount of the taxanes by the in situ extraction in plant cell culture including P14-TFSI was investigated.

2. Experimental

2.1 Cell, medium and reagents

Callus induced from the needles of *Taxus cuspidata* was used for the culture. A modified Gamborg's B5 medium including 20 g/L sucrose, 0.5 mg/L 1-naphthaleneacetic acid and 0.05 mg/L benzyl adenine was used as the medium [3]. A hydrophobic IL, 1-butyl-1-methylpyrrolidinium bis(trifluoromethanesulfonyl)imide (P14-TFSI), purchased from Tokyo Chemical Industry Co. (Tokyo, Japan) was used in the present research. The chemical formula and physicochemical properties of P14-TFSI are shown in Table 1. Methyl jasmonate (MJ, Figure 2), an elicitor stimulating production of secondary metabolites such as PTX and BIII plant cells [16], was used in the IL–medium two phase culture system.

The taxanes of 10-DAB, BIII, CM and PTX purchased from Wako Pure Chem. Co. (Osaka, Japan), were used as standard reagents for HPLC analysis.

| Chemical formula | Solubility in aqueous medium [mM] | Partition coefficient of paclitaxel in IL-medium two phase system [-] |
|------------------|----------------------------------|---------------------------------------------------------------|
| P14-TFSI         | 16.1                             | 45.4                                                          |

Table 1. Chemical formula and physicochemical properties of P14-TFSI used in this research [15]

Figure 1. Metabolic pathway of paclitaxel and the related taxanes from geranylgeranyl diphosphate [11].

*TASY*: taxadiene synthase, *DBBT*: taxane 2a-O-benzoyl transferase, *DBAT*: 10-deacetyl baccatin III-10-O-acetyltransferase, *BAPT*: baccatin III 13-o-(3-amino-3-phenylpropanoyl)transferase, *DBTNBT*: 3’-N-debenzoyl-2’-deoxytaxol-N-benzoyltransferase.

- 106 -
2.2 Plant cell culture

Suspension culture inoculated by the precultured cells was carried out in a 100 cm³ Erlenmeyer flask containing 20 cm³ of the modified B5 medium, 0.5 cm³ of the IL (2.5 vol%) and a defined amount of MJ on a rotary shaker (NR-150, Taitec, Saitama, Japan) at 110 rpm in the dark at 26 °C. During the culture the amounts of fresh cells, PTX and the related taxanes in the culture flask were measured.

2.3 Analysis

Cells were collected from cultures, washed with water, blotted on filter paper to remove excess liquid, and then weighed to determine the fresh cells weight. The amounts of the taxanes in the medium phase, the IL phase and the cells in all samples were analyzed by using a reversed-phase HPLC system according to the analytical procedures as described previously [4].

3. Results and Discussion

3.1 Effects of MJ addition on the productivities of paclitaxel and the related taxanes

Figure 3 shows the effect of MJ addition on the production amounts of PTX and the related taxanes (BIII, 10-DAB and CM). Significant enhancement in the amount of each taxane produced in the cultures including IL independent of the concentration of MJ compared to that in the control culture with no addition of both MJ and IL was observed. This enhancement was caused by an increase in the MJ concentration and partition of the produced taxanes into the IL by means of the strong hydrophobicity of P14-TFSI due to its lowered solubility in the aqueous medium (Table 1). The amount of PTX in the culture with 1 µM MJ was a maximum as shown in Table 2. The slight decrease in PTX in the culture with 10 µM MJ shown in Figure 3, might be due to the promotive production of CM prior to PTX with increasing MJ concentration. In contrast, the total sum of the taxanes in the culture with 10 µM MJ was optimal and more than 200 times greater than that in the control culture. This is caused by the increase in the amount of 10-DAB produced by increasing the MJ concentration. However, it was found the total sum of the taxanes

![Chemical structure of methyl jasmonate.](image)

![Amount of taxanes after a 14 d culture period in the culture including 2.5 vol% IL.](image)

![Table 2 Data on amount of PTX under the examined culture conditions](image)
in the culture with 100 µM MJ decreased, resulting from the inhibitory effect of MJ. A similar phenomenon, in which a higher concentration of MJ in the plant cell culture without IL decreased the productivities of PTX and BIII, was reported by Yukimune et al. [16]. In our present experiments, the production of 10-DAB was enhanced by IL and MJ, however, its subsequent conversion into BIII, PTX and CM only proceeded with difficulty. Activation of this conversion step catalyzed by the enzyme such as DBAT should be further examined.

Figure 4 shows the effect of MJ concentration on the cell growth. The cell growth in the culture with MJ, except at 100 µM, decreased compared with that in the control culture. The concentration of each taxane in the medium including IL independent of the MJ concentration was lower than that in the control culture (data not shown) because the produced taxanes partitioned to the IL phase rather than the medium phase. Though the decrease of cell growth in spite of the lowered concentrations of the taxanes in the medium phase is not clear, one of the most important reasons is considered to be that the nutrients preferred utilization of the cellular components to the production of the taxanes by MJ. Improvement of the cell growth in the culture with 100 µM MJ was observed. Though the meaning of this result is now unclear, the intracellular concentration of CM might be one of the reasons. The concentrations of taxanes except CM in the medium and the cells in the two phase culture system were smaller than those in the control culture (data not shown). The intracellular CM concentration in the culture including 100 µM was the smallest compared to that in other cultures as shown in Figure 5. This smallest concentration of CM might contribute to a decreasing inhibitory effect of CM, giving the improvement in the cell growth.

A method for back-extracting the target taxanes from the IL is crucial for the actual production process. It was reported that adjustment of the medium pH was useful for efficient back-extraction of hydrophobic ferulic acid and caffeine acid from HMIN-PF₆ [13]. The back-extraction of the taxanes from the P14-TFSI by adjusting pH in the culture medium was attempted, but produced no result. We have tried to back-extract the taxanes in the P14-TFSI with organic solvents which are volatile and immiscible with P14-TFSI. It was found that dimethyl ether was one of the valuable organic solvents for this purpose and
this is under examination.

From the experimental data described above, P14-TFSI could be an excellent extractant and stimulator for the enzymes related to the production of 10-DAB in the metabolic pathway of PTX. A strategy of conversion of 10-DAB to BIII and PTX should be further examined for an effective production process.

4. Conclusion

For more enhanced production of the taxanes an elicitor, MJ, was added to the plant cell culture including P14-TFSI which stimulated their production. It is shown that MJ stimulated the amount of production of the taxanes in the culture including P14-TFSI and 10 µM MJ was optimal for the amount of production of taxanes, suggesting that P14-TFSI could be an excellent extractant and stimulator for the enzymes related to the conversion of 10-DAB. A strategy for promotive conversion of 10-DAB to BIII and PTX should be further examined.

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