Enhancement of Shikonin Production in Suspension Cultures of *Lithospermum erythrorhizon* Cells by Gamma-irradiation

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1. Introduction

The shikonin and several derivatives produced by the roots of Boraginacae family plants are purple compounds that have been used in several parts of the World as antimicrobial and antitumor agents in human pharmaceuticals. Shikonin has been reported as the most successful specimen of the mass production of plant secondary metabolites by cell suspension culture. Numerous studies have elucidated the regulation of production of these compounds in cell suspension cultures. It has known that ultrasonic and gamma-irradiation can enhance the production of secondary metabolites. Thus, in present study, we investigate the effects of gamma-irradiation on the shikonin production and the key enzymes in the shikonin biosynthetic pathway of *L. erythrorhizon* cells.

2. Material and Methods

2.1 Plant Cell Culture & Gamma-irradiation

The *L. erythrorhizon* cell lines used in this study were induced by the root from *L. erythrorhizon* plant, which was routinely maintained on LS [1] solid medium containing 2 mg·L^{-1} benzyladenin (BA), 0.2 mg·L^{-1} indole-3-acetic acid (IAA) and 3% (v/v) sucrose at 25^{o}C under darkness.

The suspension culture was propagated in glass flask and bioreactor, each containing LS liquid medium (BA 2 mg·L^{-1} and IAA 0.2 mg·L^{-1}). The gamma-radiation was generated by a gamma irradiator (^{60}Co, *ca*. 150 TBq of capacity, AECL) in Korea Atomic Energy Research Institute.

2.2 Shikonin content & Enzyme analyses

The shikonin contents in callus and medium of cell lines were determined using a spectrophotometer according to Mizukami *et al.* [2]. The *p*-hydroxybenzoate (PHB)-geranyltransferase and PHB-glucosyltransferase were extracted from cells and quantified following the procedure of Heide *et al.* [3].

3. Results

3.1 Selection of Cell Line of L. erythrorhizon for Shikonin Mass Production

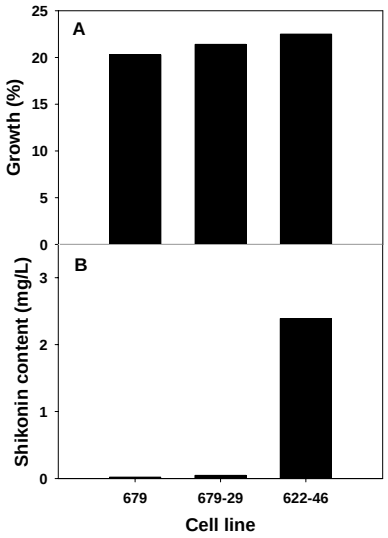

Figure 1. Increasing rate of growth (A) and shikonin contents (B) from callus culture of 3 cell lines of *L. erythrorhizon* for 14 days on the LS medium supplemented with BA 2 mg·L^{-1} and IAA 0.2 mg·L^{-1}.

The cell lines 679, 679-29 and 622-46 of *L. erythrorhizon* could be selected on LS agar medium containing BA 2 mg·L^{-1} and IAA 0.2 mg·L^{-1} for the production of shikonin in cell suspension culture. The accumulation of shikonin derivatives was significantly increased in suspension culture of cell line 622-46, compared to other two cell lines of *L. erythrorhizon* in LS liquid medium containing BA 2 mg·L^{-1} and IAA 0.2 mg·L^{-1} in the dark (Figure 1).

3.2 The Production of Shikonin by the Gamma-irradiation in Cell Suspension Culture

The effect of gamma-irradiation on the production of shikonin derivatives was investigated in suspension culture with callus exposed to gamma-irradiation. The shikonin content in the liquid medium was significantly increased by 2 Gy-irradiation in cell line 622-46 (Figure 2A). However, the shikonin content in callus was the

highest in the 16-Gy-irradiated group, compared to other groups (0, 2 and 30 Gy) (Figure 2B).

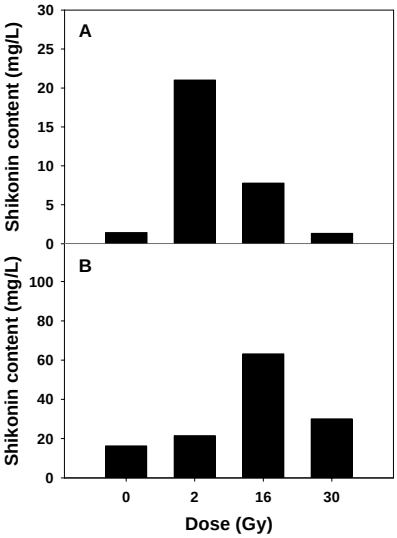

Figure 2. Shikonin contents in the suspension culture medium (A) and callus (B) of cell line 622-46 of *L. erythrorhizon* exposed to 0, 2, 16 and 30 Gy, and cultured in LS medium containing BA 2 mg·L^{-1} and IAA 0.2 mg·L^{-1} in the dark for 14 days.

3.3 The Effects of Gamma-irradiation on Enzyme Activities for Shikonin Biosynthesis

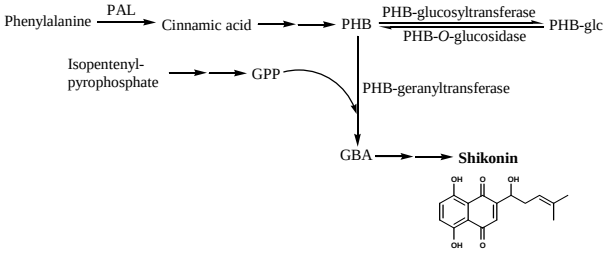

Figure 3. Biosynthetic pathway of shikonin [4]

Shikonins are synthesized from two key precursors, PHB from the shikimate-phenylalanine pathway and geranylpyrophosphate (GPP) from the isoprenoid pathway, respectively (Figure 3). The two precursors are linked in the PHB-geranyltransferase reaction to form *m*-geranyl-*p*-hydroxybenzoic acid (GBA), which provides the complete carbon skeleton of shikonins. It has been found previously that the increase of PHB-geranyltransferase activity led to shikonin production [3]. The PHB-geranyltransferase activity was increased in 2 and 16 Gy-irradiated groups, but the activity of PHB-glucosyltransferase was not significantly regulated (Figure 4).

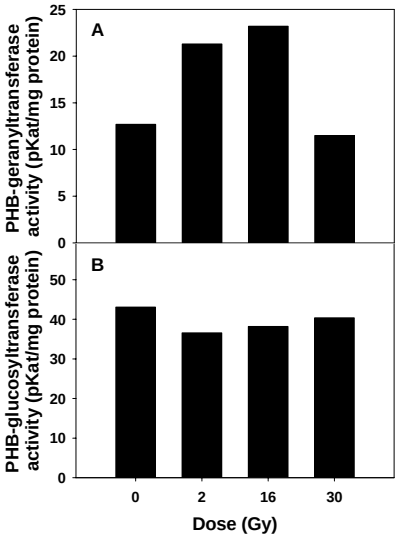

Figure 4. Activities of PHB-geranyltransferase (A) and PHB-glucosyltransferase (B) in cell suspension culture of cell line 622-46 of *L. erythrorhizon* exposed to 0, 2, 16 and 30 Gy, and cultured in LS medium containing BA 2 mg·L^{-1} and IAA 0.2 mg·L^{-1} in the dark for 14 days.

4. Conclusion

Base on the results obtained, we demonstrated the major positive effect of gamma-irradiation on secondary metabolite production, especially shikonin, in plant cell cultures, including the stimulation of the enzyme activities for secondary metabolite biosynthesis.

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