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Hairy Root Culture of *Polygonum tinctorium* for Indirubin Production in a Split-flow Air-lift Bioreactor

Choong Sik Chung, Sung Hyun Hong, Chang Heon Kim, Youn Hyung Lee, Hyong Joo Lee[1], Young Am Chae[1] and In Sik Chung*

Department of Genetic Engineering, Kyung Hee University, Suwon 449-701
[1]Research Center for New Bio-Materials in Agriculture, Seoul National University, Suwon 441-744, Korea

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Indirubin and indigo-related compounds are of significant interest as natural colorants and for the treatment of chronic granulocytic leukemia.[1,2] The *in vitro* production of indirubin and indigo-related compounds in plant cell cultures is important because native plants such as *Indigofera* spp., *Isatis tinctoria*, *Polygonum tinctorium*, and *Lonchocarpus cyanescens* only produce these compounds in small amounts over the 1-2 years of growing period.[3] We have previously reported on the indirubin production using indole-supplemented suspension culture of *P. tinctorium*.[4-7]

Hairy root cultures provided a promising alternative to the biotechnological exploitation of plant cell cultures.[8] The attractiveness of hairy root cultures can be attributed to their genetic and biochemical stabilities, rapid growth in phytohormone-free media, and morphological differentiation,[8] suggesting that they can be used to enhance the biotransformation process. In this study, the feasibility of indole-supplemented hairy root cultures of *P. tinctorium* for indirubin production in a split-flow air-lift bioreactor was determined.

The hairy roots of a Korean cultivar of *P. tinctorium* were initiated using *Agrobacterium rhizogenes* 15834 and maintained as described previously.[9] For hairy root culture, an SH medium[10] supplemented with 30 g sucrose per liter was used. The initial pH of the medium was adjusted to 5.5 before autoclaving. Indole dissolved in ethanol was kept as a stock solution. In all experiments, 20 mM indole was added to the hairy root culture medium.

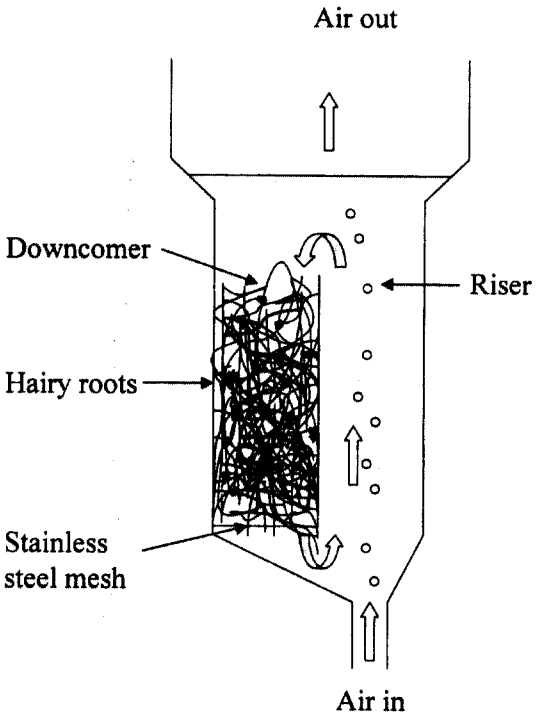

Fig. 1. Schematic diagram of a split-flow air-lift bioreactor for hairy root cultures of *P. tinctorium*.

The bioreactor set-up for the hairy root culture (Fig. 1) was identical to that described elsewhere.[11] The bioreactor was operated at an aeration rate of 100 ml/min and 27°C with 170 ml working volume. The indirubin concentration was analyzed either 30 or 60 days after the indole was added. For fresh weight determination, the hairy root cultures were gently pressed on filter papers to remove excess water and were weighed. The indirubin content was determined by measuring the absorbance at 534 nm as described elsewhere.[7] Figure 2 shows the effects of inoculum level on indirubin production after 30 and 60 days of incubation in a split-flow air-lift bioreactor. Indirubin production increased up to an inoculum of 120 g FW/l and then decreased. The decreased indirubin production at a higher than 120 g FW/l inoculum was probably due to mass transfer resistance in the liquid-solid boundary layers surrounding the hairy root tissues. The presence of optimum inoculum size was also observed for the hairy root cultures of *Atropa belladonna*[12] and *Catharanthus roseus*.[13]

Maximum indirubin production was about 120 μg/l in hairy root cultures. This value is lower than that (81-112 mg/l) reported for an air-lift bioreactor culture of suspension cells of *P. tinctorium*.[7] The reason for the lower indirubin production in hairy root culture is not yet fully understood, but it may be related to the differentiated property of hairy roots.

In conclusion, hairy root cultures of *P. tinctorium*

*Corresponding author
Phone: 82-331-201-2436; Fax: 82-331-202-9885
E-mail: ischung@nms.kyunghee.ac.kr

Abbreviations: FW, fresh weight.

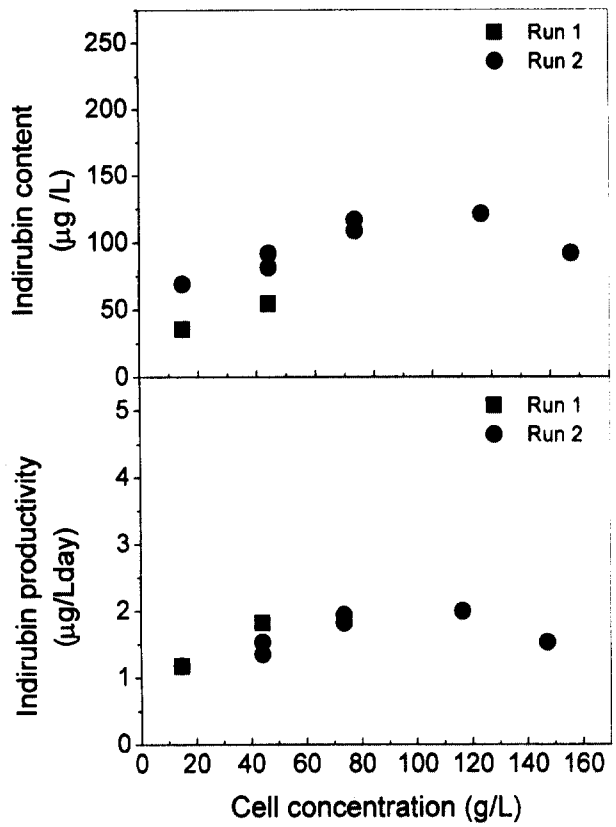

Fig. 2. Effect of inoculum level on indirubin production in hairy root cultures of *P. tinctorium*. The indirubin concentration was analyzed either 30 (Run 1) or 60 days (Run 2) after the indole was added.

displayed reasonable growth performance in the split-flow bioreactor. Indirubin production reached maximum at an inoculum of about 120 g FW/l. Decreased indirubin production at a higher than 120 g FW/l inoculum was probably due to poor mass transfer in dense hairy root cultures.

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