PRODUCTION OF BLUE PIGMENTS FROM THE CALLUS CULTURES OF *LAVANDULA AUGUSTIFOLIA* AND RED PIGMENTS (BETALAIN) FROM THE HAIRY ROOT CULTURE OF *BETA VULGARIS*

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ABSTRACT

Plants are used to produce many secondary metabolites that are too difficult, expensive or impossible to make by chemical synthesis. Conventional cultivation of plants is of course subject to vagaries of weather, pests and availability of land; hence, the interest in highly controlled culture of plant cells and hairy roots in bioreactors as methods of producing various products. This project focussed on production of blue and red colors of *Lavandula augustifolia* and *Beta vulgaris*, respectively. Callus and suspension cell culture were successfully produced from *L. augustifolia* after extensive trials, but hairy roots could not be generated from this species. In contrast, a successful protocol was developed for consistently producing hairy roots from *B. vulgaris*, but calli could not be generated from this species.

Effects of medium composition on growth of *L. augustifolia* calli and freely suspended cells and production of the blue pigment by the latter, were investigated. Optimal production of callus occurred in full-strength Murashige and Skoog (MS) medium supplemented with 2 mg/l of indole-3-acetic acid (IAA) and 1 mg/l of kinetin. Stable suspension cultures could be produced and maintained in full-strength MS medium supplemented with 1 mg/l each of IAA and kinetin. In suspension culture in full-strength MS medium, the following hormone combinations were tested: (1) 1 mg/l each of indole-3-acetic acid (IAA) and kinetin; (2) 2 mg/l of IAA and 1 mg/l of kinetin; (3) 2 mg/l of IAA and 1 mg/l of benzyl amino purine (BAP); and (4) 2 mg/l each of IAA and BAP. Combination (3) maximized cell growth, but the highest cell-specific production of the blue pigment was seen in combination (2), although pigment production occurred at all hormone combinations. The medium formulation that gave the best production of the pigment in shake flasks was scaled up to a 2 L aerated stirred tank bioreactor, but both the biomass and pigment productivities were reduced in the bioreactor apparently due to the high shear stress generated by the Rushton turbine impeller.

Compared to suspension cultures of *L. augustifolia*, the hairy root cultures of *B. vulgaris* grew extremely rapidly. Hairy roots also produced large amounts of the red pigments. Growth of hairy roots was influenced by the composition of the medium.

Although the full strength MS medium better promoted biomass growth compared to the half-strength MS medium, the final concentration of the biomass and the pigment were nearly the same in both media. Attempts were made to enhance production by using various hormones (i.e. naphthalene acetic acid, BAP, IAA added individually at a concentration of 0.5 mg/l), but none of the hormones proved useful. BAP adversely affected the growth of hairy roots.

In summary, production of pigments by suspension culture of *L. augustifolia* and hairy root culture of *B. vulgaris*, is technically possible, but requires substantial further optimization for enhancing productivity than has been possible in this project.

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