MASS PROPAGATION OF HAIRY ROOT CULTURE OF *CATHARANTHUS ROSEUS* IN A BIOREACTOR FOR AJMALICINE PRODUCTION

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by

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DEDICATED TO MY LOVED ONES

CERTIFICATE

This is to certify that the thesis entitled "**Mass propagation of Hairy root culture of *Catharanthus roseus* in a Bioreactor for Ajmalicine production"** being submitted by **Ms. Dhara Thakore** to the Indian Institute of Technology Delhi, for the award of "Doctor of Philosophy", is a record of bonafide research carried out by her, which has been prepared under my supervision and guidance in conformity with the rules and regulations of Indian Institute of Technology Delhi. The results contained in it have not been submitted in part or full to any other university or institute for award of any degree/ diploma.

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Date: **Dhara Thakore**

Place:

ABSTRACT

Plants have been a rich source of strategic compounds which are extensively used in healthcare, cosmetics as well as dietary supplements. Currently most of the plant metabolite production is based on traditional methods involving the use of whole plants which have some obvious decisive disadvantages of slow growth rate, low variable content and seasonal availability. Alternate production technologies like plant cell/organ cultures are desperately needed to supplement the plant secondary metabolite production. Hairy root culture obtained by *Agrobacterium rhizogenes* mediated transformation has been considered as a prospective alternative considering its vigorous growth in hormone free medium and better biochemical /genetic stability than specialized plant cell cultivation.

The hairy roots of *Catharanthus roseus* clone CP 32 var. Prabal used in the present investigation were induced by National Institute of Plant Genome Research (NIPGR), New Delhi. The kinetics of hairy root growth, substrate utilization and product formation was then established under these optimized medium and environmental conditions (medium composition: Potassium nitrate: 2.75 g/l, sodium phosphate monobasic: 37.5 mg/l, along with Gamborg B5/2 Major salts + B5/2 Minor salts + B5 vitamins + 16 g/l sucrose). The dry biomass accumulation at the end of 30 days cultivation period was 5.02 ± 0.46 g/l. The maximum content of ajmalicine in the roots was 24.9 ± 1.2 mg/l (4.97 ± 0.3 mg/g) on day 30 after which its concentration decreased. This translated to an overall volumetric productivity of 0.8 mg/l-d.

Several yield enhancement strategies were attempted in shake flask cultivation to further enhance the Ajmalicine yield (mg/g) in the growing hairy roots of *C.roseus*. The independent addition of Jasmonic acid (JA), Methyl jasmonate (MeJa) and Potassium

chloride (KCl) resulted in increased ajmalicine concentration than control. Attempt was made to elucidate the possible synergistic or antagonistic effect of combined addition of above elicitors on ajmalicine production. Statistical optimization protocol was therefore utilized for identification of appropriate concentrations of JA, MeJa and KCl which when added together should be able to enhance ajmalicine production. Time of exposure of hairy roots to elicitors was independently optimized as 48 h which featured the highest ajmalicine accumulation of 78.3 mg/l (15.9 mg/g) (as opposed to 24.9 mg/l in untreated batch control).

The hairy root growth and product formation kinetics was studied during shake flask cultivation to identify the key kinetic parameters and develop mathematical model for analysis of system behavior and its operation strategy(ies). It was observed that the developed model adequately described the batch cultivation kinetics and its extrapolation to fed batch cultivation (particularly constant/increasing nutrient feeding and pseudosteady state with respect to substrate) enhanced the ajmalicine accumulation in the hairy roots. Experimental implementation of model simulated substrate feeding lead to an ajmalicine accumulation of 52.9 ± 4.12 mg/l (10.62 ± 0.9 mg/g), 46.45 ± 3.9 mg/l (5.26 ± 0.25 mg/g) and 63.12 ± 3.4 mg/l (10.4 ± 0.86 mg/g) in constant, pseudosteady state of substrate and increasing feed rate fed batch cultivations respectively (as opposed to 24.9 ± 1.2 mg/l, 4.97 ± 0.3 mg/g in batch). A 60 % increase in overall ajmalicine productivity and 2.5 fold increase in volumetric yield was obtained by the increasing nutrient feed rate strategy compared to batch cultivation.

To further improve the alkaloid accumulation, addition of optimized elicitor mixture to growing *C. roseus* hairy roots along with fed batch cultivation (increasing feed rate) was attempted in shake flask cultures. This resulted in an ajmalicine accumulation of 123.2 ± 8.6 mg/l (16.2 ± 2.3 mg/g) (a 4 fold increase as compared to batch).

Mass cultivation of the hairy root culture was, thereafter, attempted in several bioreactor configurations (conventional as well as custom made) suiting the culture conditions and requirement (such as nutrient/oxygen transfer, root support etc). In the experiments conducted on different bioreactor configurations, following overall volumetric productivities of Ajmalicine (mg/l-d) were obtained, Conventional Bubble column Bioreactor: 0.52 mg/l-d, Rotating drum vessel with a perforated cylindrical root support: 0.15 mg/l-d and modified bubble column bioreactor (stirred tank reactor with impeller removed) with polypropylene mesh as support: 1 mg/l-d. The productivity, though, slightly higher than shake flask batch cultivation studies (0.8 mg/l-d), yet further improvement could be expected by application of different absorbent materials for providing appropriate support (anchorage) to the growing hairy roots. Modified bubble column with Polyurethane (PUF) foam support was, thereafter, investigated in the bioreactor cultivation which led to a volumetric productivity of 1.13 mg/l-d.

Finally, a novel absorbent material, super absorbent polymer (SAP), used in agriculture was examined as a support for hairy root during the bioreactor cultivation. An exceptionally high biomass (8.86 ± 0.32 g/l), ajmalicine accumulation (44.6 ±1.31 mg/l, 5 ± 0.04 mg/g) and productivity (1.48 mg/l-d) was obtained in this particular bioreactor configuration.

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