STUDIES ON THE PRODUCTION OF COMPACTIN BY FUNGAL FERMENTATION

By

RITU CHAKRAVARTI

DEPARTMENT OF BIOCHEMICAL ENGINEERNG & BIOTECHNOLOGY

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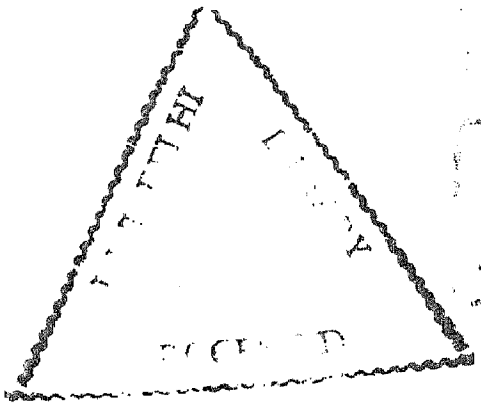

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CERTIFICATE

This is to certify that the thesis entitled "**Studies on the Production of Compactin by Fungal Fermentation**", submitted by **Ms. Ritu Chakravarti** to the Indian Institute of Technology-Delhi, for the award of the degree of **"Doctor of Philosophy"**, is a record of bonafide research work carried out by her under my supervision and guidance, is in conformity with the rules and regulations of 'Indian Institute of Technology-Delhi'. The research reports and results presented in the thesis have not been submitted to any other university or institute for the award of any other degree or diploma.

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Dr. Vikram Sahai, Associate Professor

Dept. of Biochemical Engineering & Biotechnology,

Indian Institute of Technology-Delhi,

Hauz Khas, New Delhi-110016

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Ritu Chakravarti

ABSTRACT

Compactin is an industrially important molecule with hypocholesterolemic activity. This is a specific, potent and competitive inhibitor of HMG-CoA reductase, a regulatory enzyme of cholesterol biosynthesis. It is also a precursor of another potent HMG-CoA reductase inhibitor, pravastatin. Compactin is generally obtained from fungal sources by submerged fermentation using complex medium. The compactin production has been carried out using many different fungi like *Penicillium citrinum*, *P. cyclopium*, *Aspergillus terreus*, *Trichoderma* sp. etc. The present work is aimed at selection of suitable fungi, medium formulation, study of the kinetics of compactin production, selection of suitable feeding strategy for enhanced compactin production and isolation of compactin.

Three different fungal strains were studied for compactin production and *P. citrinum*, being the most productive strain (37 mg/l), was selected for strain improvement. It was subjected to UV mutagenesis and 62 mutants were tested for their ability to produce compactin. One of the mutants, producing highest compactin concentration (3.7 fold of wild type strain), designated as *P. citrinum* VR-12 was selected for the present work. A suitable chemically defined production medium was developed and compactin concentration of about 145 mg/l was obtained after 12 d. This medium also facilitated the development of an easy spectrophotometric analysis of compactin. The use of chemically defined seed medium did not support the growth of *P. citrinum* VR-12, therefore, use of complex medium at seed stage was retained. Various complex nitrogen sources were studied at seed stage for their effect on compactin production, and water-soluble extract

of soyabean meal along with peptone and sodium nitrate was found best for compactin production and biomass formation. The cultural and environmental parameters were optimized using statistical tools for improved compactin production. Six parameters (urea, glucose, harvesting time, glycerol, inoculum age and $MgSO_4$), out of eleven parameters studied, were identified as important for compactin production by Plackett-Burman Design. Among these, five parameters were optimized by Response Surface Methodology using Central Composite Rotatable design. The batch study was conducted in shake flask and 7 l fermenter, using the optimized parameters (glucose, 4.65 g/l; glycerol, 15.8 g/l; urea 0.61 g/l; inoculum age 4.24 d; harvesting time, 8.9 d), resulting in compactin concentration of 456 mg/l and 420 mg/l respectively. Effect of various parameters like spore concentration, Tween 80, C/N ratio, preculturing, light, trace elements, agitation and catabolite repression was studied on the compactin production. It was found that light has profound effect on compactin production as its absence reduced the compactin production by 40%. Two different precursors, sodium acetate and tri-sodium citrate, were studied for their effect on compactin production. Use of 10 mM sodium acetate since the beginning of fermentation resulted in compactin production of 2700 mg/l, whereas, addition of 30 mM tri-sodium citrate during the cultivation increased the compactin production to 3000 mg/l.

To enhance the yield and overall average volumetric productivity of compactin, fed-batch fermentations were carried out in shake flask and fermenter. Use of whole medium of C/N ratio of 22 was used for feeding during cultivation at regular time intervals resulting in doubling of compactin production. To increase the productivity of compactin, pulse feeding of nutrients during cultivation was done in two ways, either to replenish the

nutrients just sufficient for growth and compactin production, or to increase the instantaneous nutrients concentration in the culture broth by more than 2X to the previous increase in concentration with every addition of feed. The use of one such strategy increased the compactin concentration to 3650 mg/l with overall average volumetric productivity of 26.9 mg/(l.h) as compared of 3.9 mg/(l.h) of batch fermentation in 7 l fermenter.

It was found that compactin in the culture broth remained in two phases; one, free in the broth and second, associated with the cell membrane. The partitioning of compactin between culture medium and cell membrane was a function of pH. Silica gel column chromatography was used to recover the compactin from the culture broth. Using simple sample preparation procedure and gradient elution with dichloromethane and methanol, crystals of compactin were obtained.

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