PHB BIOSYNTHESIS IN *STREPTOMYCES*: ENZYMOLOGY OF β-KETOTHIOLASE OF *STREPTOMYCES LIVIDANS*

by

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Submitted

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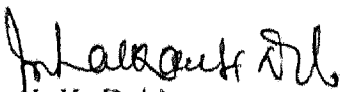
CERTIFICATE

This is to certify that the thesis entitled **"PHB biosynthesis in *Streptomyces*: enzymology of *β*-ketothiolase of *Streptomyces lividans*"** being submitted by **Ms. Shivani Verma** to the Indian Institute of Technology, Delhi, for the award of the degree of **' Doctor of Philosophy'**, is a record of the bonafide research work carried out by her under my supervision and guidance in conformity with the rules and regulations of the 'Indian Institute of Technology', Delhi. The research work and the 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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ABSTRACT

Polyhydroxyalkanoates (PHAs) have received a lot of attention in the recent years owing to the fact that this group represents natural polymers. These polymers came to light when their thermoplastic and biodegradable nature was discovered. Polyhydroxybutyrate (PHB) is the most well known and well-studied member of this family. PHB has been isolated from a number of microorganisms and two out of three of the enzymes leading to its production have been purified and characterized in some of these microbes. The genetic organization of the enzymes leading to PHB production and its degradation has also been reported for some of these organisms. Although a lot of attention has been focussed on some of them, such as *Ralstonia eutropha* from which PHB is commercially isolated, little attention has been focussed on some other PHB producers. *Streptomyces*, a group of high versatility from the family actinomycetes has received almost no attention in this regard.

The present study was, therefore, aimed at screening various *Streptomyces* sp. for their ability to produce PHB and purification and characterization of the first enzyme of the PHB biosynthetic pathway viz. β-ketothiolase from *Streptomyces lividans*. *Streptomyces* sp. were found to produce PHB and were able to grow well on cheap carbon sources and produce the polymer. *Streptomyces olivaceous* was found to produce higher polyhydroxyalkanoates besides PHB. β-ketothiolase was purified from *Streptomyces lividans* using ion-exchange chromatography, hydroxylapatite column chromatography and hydrophobic interaction chromatography. The pure enzyme obtained was found to have a molecular weight of 200 kDa. It was detected to be a tetramer of equal subunits of molecular mass 50 kDa. These data corresponds to the β-ketothiolases of the other organisms. The enzyme was found to have a K_m of 1.4×10^{-3} M

and a V_{max} of 7.3 x 10^{-11} moles/ml/sec for condensation reaction. A K_m value 1.3 x 10^{-5}M and V_{max} of 1.75 x 10^{-10} moles/ml/sec for thiolysis reaction keeping CoA constant and varying the concentration of Acetoacetyl CoA was obtained whereas K_m value of 1.6 x 10^{-5} and V_{max} of 1.83 x 10^{-10} moles/ml/sec was seen for thiolysis reaction when acetoacetyl CoA was kept constant and the concentration of CoA was fixed. The enzyme required sulfhydryl reagents like DTT for maintenance of its activity. An enzyme inhibitor that co-existed with the enzyme and had a non-proteinaceous nature was also studied.

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