Production And Purification Of Nitrophenol Oxygenase From Bacillus Subtilis

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Abstract- *Oxygenases are the key enzymes for aerobic biodegradation of aromatic compounds like p- Nitrophenol which catalyze the ring cleavage of the aromatic compounds. A bacterial culture of Bacillus subtilis, isolated from farm soil was used for the production of enzyme nitrophenol oxygenase using nutrient broth containing p- nitrophenol. The suspension was then used to extract nitrophenol oxygenase. It was purified by subjecting extract to centrifugation and ultrasonication at 150rpm for 15 min. further it was subjected to ammonium salt precipitation, dialysis and ion exchange chromatography.*

Keywords- Ammonium salt precipitation, Bacillus subtilis, Ion exchange chromatography, Nitrophenol Oxygenase, production.

I. INTRODUCTION

The excess use of pesticides, paints fertilizers deposits nitrophenol, a aromatic compound with ability to resist the degradation. This leads to accumulation and hazard to human life, if consumed. Because of this very reason the possible solution for *p*-Nitrophenol (PNP) degradation is its biodegradation. There are different enzymes involved in its biodegradation, of which oxygenases are the key enzymes. These enzymes catalyze the ring cleavage of the aromatic compounds under aerobic conditions which is the essential step for the complete mineralization of these compounds [1]. There are two types of oxygenases: Monooxygenases, transfer one oxygen atom to the substrate, and reduce the other oxygen atom to water and Dioxygenases, or oxygen transferases, incorporate both atoms of molecular oxygen (O_2) into the product(s) of the reaction [6]. Monooxygenases involved in denitrification of nitroaromatic compounds are 4-nitrophenol 4-monooxygenase, 4-nitrophenol 2-monooxygenase and 2-nitrophenol 2-monooxygenase (4-Nitrophenol 4-monooxygenase has been purified and characterized from *Pseudomonas* sp. strain WBC-3 [1]. Two pathways have been characterized among PNP degrading bacteria; one degradation process leads to the formation of 4-Nitrocatechol (4-NC) in *Bacillus* sp. and other leads to formation of hydroquinone (HQ) [7], [8].

The present study deals with production and purification of nitrophenol oxygenase from *Bacillus subtilis* isolated from farm soil.

II. MATERIALS AND METHODS

Bacillus subtilis isolated by inoculating filtered soil suspension derived from farm soil into enrichment media (Na_2HPO_4-5.8g, KH_2PO_4-3g, NaCl-0.5g, NH_4Cl-1 g, PNP-20mg, Distilled water- 1000 ml) with PNP (20mg/lit) was named C1 and used as inoculum [5], [8]. The cells from log phase were used as inoculum for 50ml nutrient broth supplemented with 1mM PNP. Inoculated broths were incubated at 37^0C for 24 hrs at 100rpm. The cells were harvested from each broth by centrifuging at 15,000 rpm for 5 min at 4^0C. The supernatants were discarded and pellet was washed twice with 0.2M sodium phosphate buffer of pH 7.4 and resuspended in same buffer. The pellet of cell was disrupted by sonication at 150 rpm for 15 sec at 4^0C. The cell debris was removed by centrifugation at 20,000 rpm for 20min at 4^0C. The resulting supernatant was referred to as crude extract and used for purification [2]. The isolated extract was purified by ammonium salt precipitation [3], dialysis method and ion exchange chromatography using DEAE Cellulose resin [4].

III. RESULT AND DISCUSSION

The nitrophenol oxygenase was produced by using *Bacillus subtilis* culture. Fig.1. shows the color change of nutrient broth containing PNP, indicates growth of bacteria. The Nitrophenol Oxygenase was extracted from bacterial samples using sonication.

The extracted enzyme sample was purified by ammonium sulfate precipitation and dialyzed. The dialyzed sample was purified by ion exchange chromatography using DEAE Cellulose as resin.

The detailed characterization of enzyme nitrophenol oxygenase with enzyme activity, molecular weight determination be the target of further studies, towards the aim of exploiting it for bioremediation of contaminated sites.

IV. FIGURES

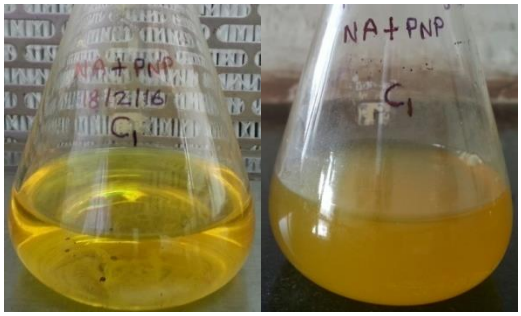

Figure 1. Color Change of Broth Indicating Bacterial Growth**.**

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