www.ThePharmaJournal.com

The Pharma Innovation

ISSN: 2277- 7695
TPI 2014; 3(7): 71-76

www.thepharmajournal.com
Received: 04-08-2014
Accepted: 28-08-2014

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Microbial production and optimization of media for organophosphate hydrolase

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ABSTRACT
Today, as use of neurotoxic Organophosphorus compounds are increasing, there is a great need that these compounds should be degraded through bioremediation. World Health organization recommended that Methyl parathion ($C_8H_{10}NO_5PS$) is an extremely hazardous organophosphorus pesticide which is successfully degraded by Organophosphorus hydrolase. In the present study, microbial production of organophosphorus hydrolase was carried out from an isolate from soil. Modification of culture conditions effect Organophosphorus hydrolase production. The yield of Organophosphorus hydrolase activity was improved to three fold after medium optimization in comparison to before optimized conditions. Maximum growth of the isolate was observed after 42 hours of incubation the basis of A_{600}. Sucrose (2.5%) and yeast extract (1.5%) were the optimal carbon and nitrogen sources, respectively.

Keywords: Organophosphate hydrolase (OPH); methyl parathion (MP); p-nitrophenol (PNP); organophosphorous (OP) compounds.

1. Introduction
Organophosphorus compounds are used as pesticides, air fuel ingredients and chemical warfare agents [1]. Organophosphorous insecticides contribute a share of ~ 36% in total world market of insecticides [2]. OP compounds are phosphotriesters with phosphorous linked by double bond to either oxygen or sulphur in case of oxon and thion, respectively [3]. Malathion, diazinon, chlorpyrifos, azamethiphos, dichlorvos, parathion and methyl parathion are characterized as organophosphates. OP compounds caused delayed neuropathy [4-5], Long QT syndrome [6], incident dementia [7] and diseases in other systems in humans [8-9]. Poisoning by OP compounds is a worldwide problem as it caused nearly 200,000 deaths annually [10]. Contamination of various ecosystems takes place because of excessive use of OP compounds [11]. OP compounds are neurotoxic as they inhibit the activity of acetylcholinesterase [12-16]. Acetylcholinesterase (EC 3.1.1.7) is responsible for breaking ester bond in acetylcholine and end of nerve stimulation [17]. AchE is a non-specific enzyme so working with it didn't produce any fruitful result to overcome the harmful effect of OP pesticides. To overcome this problem, Organophosphorus hydrolase is an enzyme of choice as it is specific in its nature and Organophosphorus compounds didn't affect its activity.
Organophosphorus hydrolase is composed of two units with molecular weight 72 kDa [18]. Organophosphorus hydrolase hydrolysed large no. of organophosphorus compounds having P-O, P-F, P-CN and P-S bonds [19-21]. Detoxification of substrate proceeds via attack of the bridging water molecule on phosphorous centre [18, 22-23]. Hydrolysis of methyl parathion leads to the formation of p-nitrophenol and thiophosphate [23-25]. In 1971 it was reported that efficacy of pesticide is affected by biodegradation [26]. Detoxification of methyl parathion reduced its toxicity by 120 fold [27]. In this present study, isolation of microorganism producing Organophosphorus hydrolase and optimization of culture conditions for the maximum production of Organophosphorus hydrolase has done.

2. Materials and methods
2.1 Materials
All the chemicals used were of analytical grade, commercially available from Himedia, Sigma, Merck etc. Culture used in the study was isolated from Bathinda field soil in the Biosensor lab.

2.2 Microorganism and culture medium

For the isolation of organophosphorus hydrolase producing bacteria, contaminated soil was collected from pesticide contaminated cotton field soil of Bathinda, India in steam sterilized okradges. 1 g of soil sample was mixed in 10ml of sterile water and was further serially diluted. Each dilution was used to inoculate modified Wakimoto media supplemented with 10 mg/l methyl parathion having the composition (in grams per litre): 15 g sucrose, 5 g peptone, 2 g $Na_2HPO_4.12H_2O$, 0.5 g $Ca(NO_3)_2.4H_2O$ and 0.5 g $FeSO_4.7H_2O$ [28] having pH 7.2. Culture was incubated at 30 ^{0}C for 42 hours on rotary shaker. The positive cultures were further streaked on agar plates having Wakimoto media for isolation and characterization.

2.3 Growth of bacterial culture (before medium optimization)

Growth profile of bacterial culture was studied to know about the growth characteristics i.e., duration of lag, log and stationary phase. Absorbance at 600 nm for growth of the isolate was taken after every 6 hours up to 48 hours. Growth parameters were optimized for the better yield of organophosphorus hydrolase. For the optimization of medium components, firstly different concentration % inoculum was studied for inoculation.

2.4 Extraction of intracellular enzyme

100 ml medium was pelleted by centrifugation at 5000 rpm for 10 min having temperature 4 ^{0}C. Biomass was washed thrice and suspended in 1 ml of 50 mM Tris buffer. Cell pellet was disrupted by sonicating it for 10 min. at pulse on and off 15 sec and 10 sec respectively having 50% amplitude. Again centrifugation of sample was done to collect the supernatant containing desired intracellular organophosphorus hydrolase. Enzyme assay was performed to check enzyme activity by Mulbury and Karns method [29]. Protein content was determined using Bovine Serum Albumin as standard [30].

2.5 Optimization of media components for Organophosphorus Hydrolase production

Different carbon sources were used such as sucrose, dextrose and lactose having the same concentration (1.5%) for maximum growth of the isolate. Carbon source in which isolate having maximum growth was further optimized at different concentrations to get maximum enzyme activity at different time intervals. After centrifugation, followed by sonication enzyme was collected and enzyme activity was determined by above mentioned method. Then, Nitrogen source was optimized using Peptone, beef extract and yeast extract at same concentration of 0.5% and then Organophosphorus hydrolase was determined at different concentration of above optimized nitrogen source at different time intervals. pH of medium was set having range between 6.0-8.0 for its optimization. After pH optimization, Temperature was optimized for the maximal growth of bacterial culture. Bacterial culture was incubated between temperature range 25-40 ^{0}C at rotary shaker. After medium optimization growth profile of bacterial culture was studied again to determine the effect of optimization on growth of isolated microorganism.

3. Results and discussion

3.1 Growth characteristics

Isolated microorganism was identified as Gram positive, rod shaped as shown in Fig.1: (b). Maximum growth of isolate was observed at 10% (v/v) inoculum used for inoculation of culture media (Fig.2). It was depicted from Fig.3 that lag phase of bacterial culture lasts for 5 hours and growth was maximum at 42 hours. Similarly, Enzyme activity of organophosphorus hydrolase was observed maximum at 42 hrs (10.25 ± 1.120). Protein content of isolate before medium optimization was found to be 0.994 ± 0.009 mg/ml.

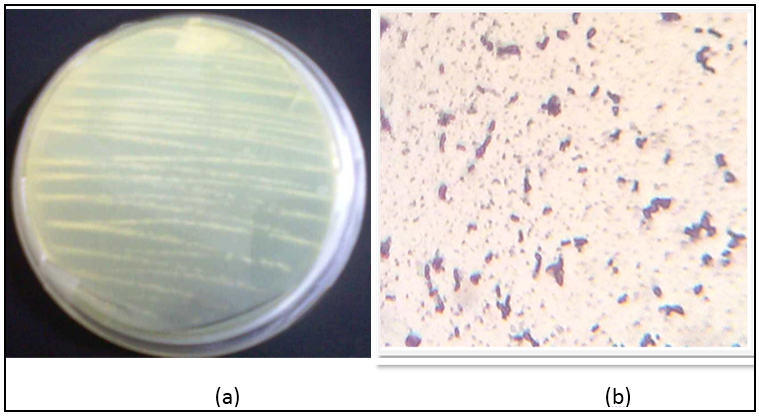

Fig 1: (a) Yellow color colonies of isolate on agar plate having Wakimoto media. **(b)** Gram staining of the isolate showing violet colored rod shaped cells.

3.2 Optimization of media components for Organophosphorus Hydrolase production

Carbon source was used as a sole energy source. From various carbon source used, the maximum organophosphorus hydrolase activity from the isolate was obtained using sucrose (1.5%) as a carbon source (Fig. 4). While other carbon sources lactose and glucose showed comparatively less activity. At various concentrations of sucrose, maximum organophosphorus hydrolase activity (16.86 ± 1.162 U/ml) was assessed at 2.5% of sucrose at different time intervals (Fig. 5)

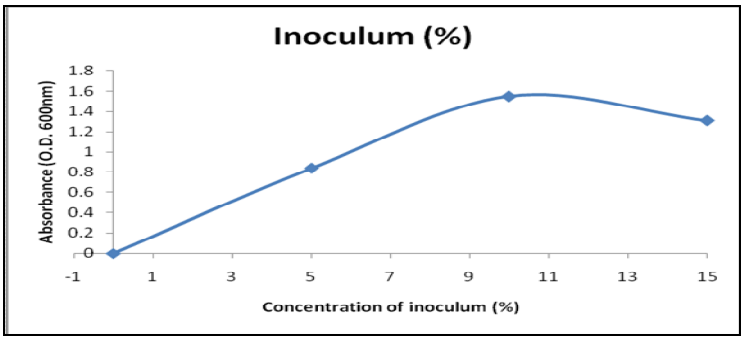

Fig 2: Growth of isolate using different concentration of inoculum (% v/v)

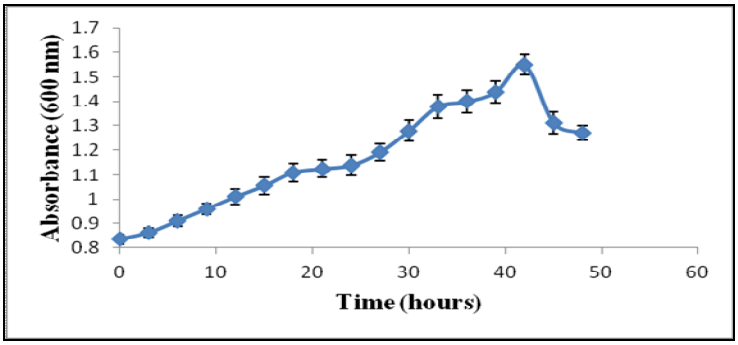

Fig 3: Growth Profile of bacterial culture on the basis of absorbance (600 nm)

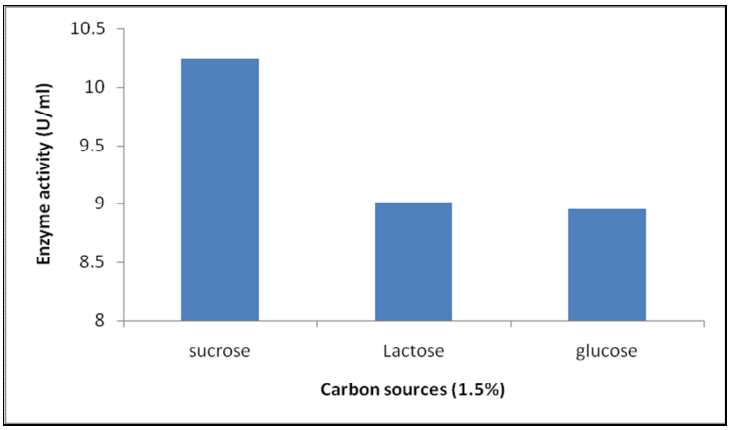

Fig 4: Dependence of Organophosphorus hydrolase activity on different carbon sources (1.5%)

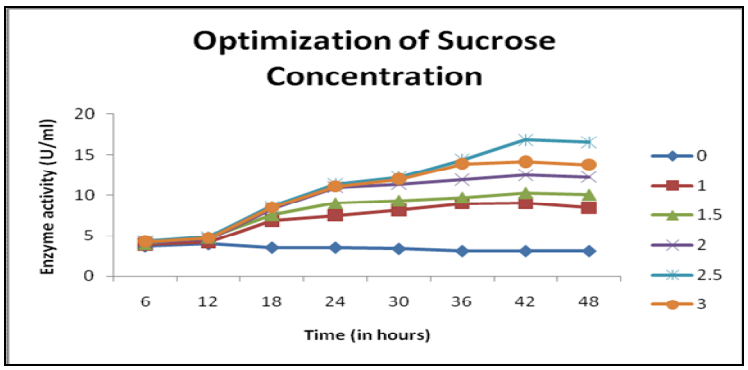

Fig 5: Enzyme activity of Organophosphorus hydrolase at different concentrations of sucrose at various time intervals.

As after optimizing carbon source, nitrogen source was optimized. Fig. 6 shows the effect on organophosphorus hydrolase activity using different nitrogen sources having concentration 0.5% (peptone, beef extract, yeast extract). Maximum organophosphorus hydrolase activity was shown by yeast extract (28.63 ± 2.09 U/ml) used as nitrogen source (Fig. 7).

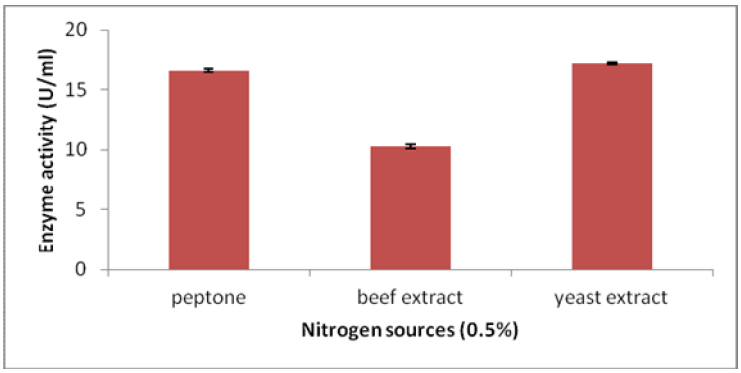

Fig 6: Comparison of Organophosphorus hydrolase activity at different nitrogen sources (0.5%) (Peptone, beef extract, yeast extract)

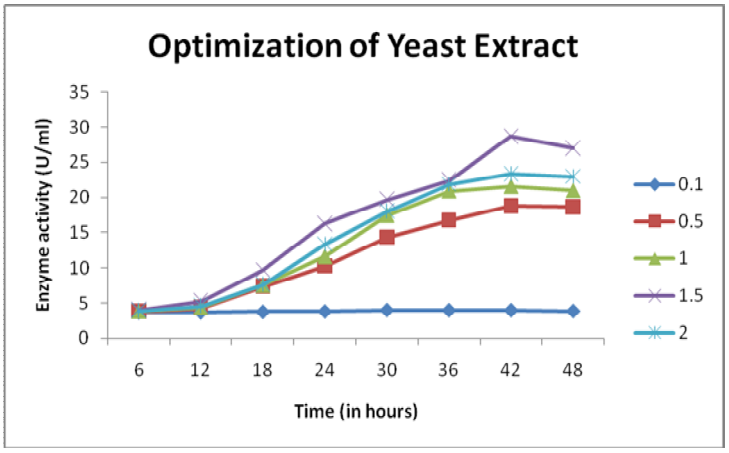

Fig 7: Organophosphorus hydrolase activity at different concentrations of yeast extract at various time intervals.

pH and temperature play an important role in the production of isolate producing organophosphorus hydrolase. From pH range 6.0-8.0, maximum enzyme activity (29.411 ± 1.123 U/ml) was observed at pH 7.2 having temperature 30 ^{0}C (Fig. 8 and Fig. 9) As temperature deviation disrupts the structure of Organophosphorus hydrolase.

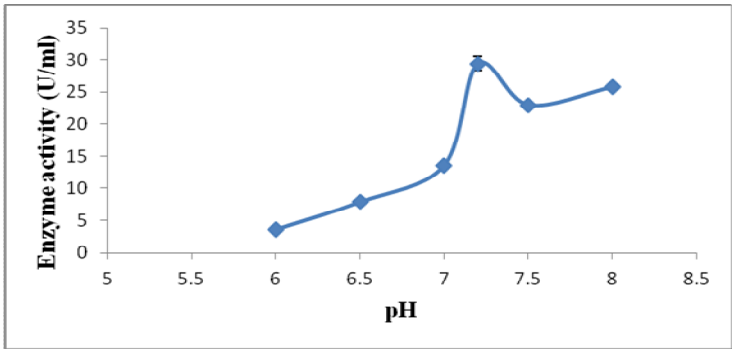

Fig 8: Dependence of Organophosphorus hydrolase activity on the pH of the media

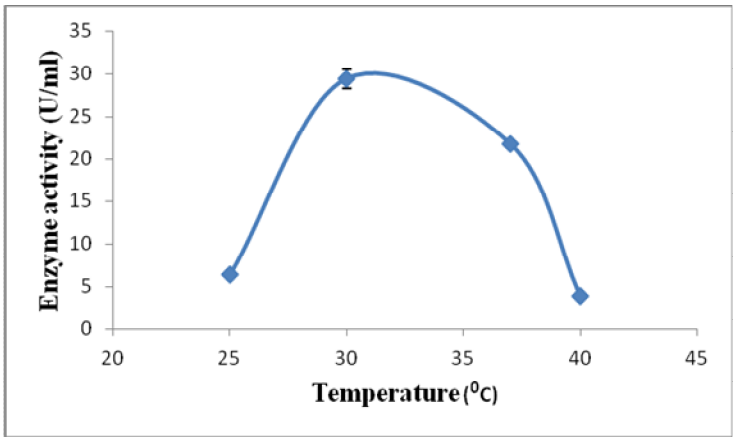

Fig 9: Effect of temperature on hydrolyzing ability of Organophosphorus hydrolase

3.3 Growth profile of bacterial culture after medium optimization

Maximum growth was obtained at 42 h after optimization of medium same as that was obtained before optimization of medium. Protein content of Organophosphorus hydrolase was determined to be 1.655 ± 0.005 mg/ml after medium optimization. It was increased to 0.7 fold after optimization of medium.

3.4 Prescribed medium for OPH production

Conditions for growth was optimized to get maximal production of biomass and Organophosphorus hydrolase (Fig. 10). From the above work it was concluded that increase in biomass production leads to increment in Organophosphorus hydrolase activity. Sucrose (2.5%) and yeast extract (1.5%) was optimized as carbon and nitrogen source, respectively.

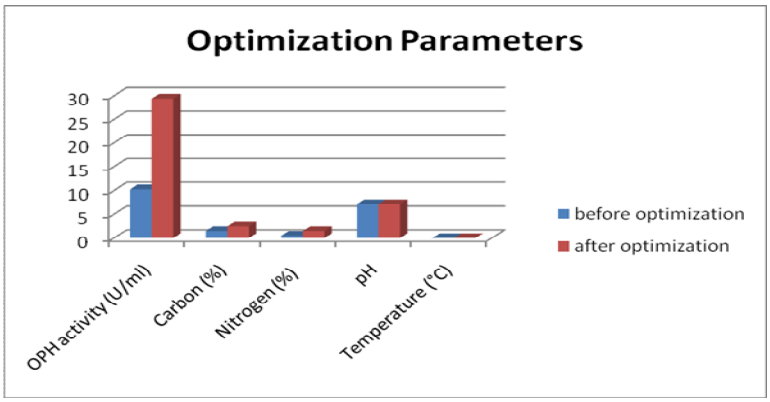

Fig 10: Comparison of conditions before and after optimization of medium

4. Conclusion

Organophosphorus hydrolase producing microorganism was isolated from methyl parathion sprayed field soil. Different medium components were optimized for the highest yield of Organophosphorus hydrolase. Recommended medium for OPH production should contain sucrose 2.5% as carbon source, yeast extract 1.5% (nitrogen source). pH and temperature should be maintained 7.2 and 30 ^{0}C respectively. Storage stability of Organophosphorus hydrolase producing isolate was 35 days at 4 ^{0}C.

5. Acknowledgement

The authors are grateful to Development of Science and Technology, Government of India for providing financial support for this project.

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