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**Research Article**

# AMYLASE PRODUCTION FROM *GASTRIMARGUS MUSICUS* CELLS IN CULTURE AND IMMOBILIZED STATE

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## ABSTRACT

A low cost insect cell culture medium is developed without use of serum or costly chemicals. Cells were cultured in three variations of the basal medium i.e. with sucrose only, with creatine only and with both sucrose and creatine along with basal medium. The medium with sucrose only had the highest cell concentration followed by medium with creatine and with both sucrose and creatine. Basal medium had the lowest cell concentration. Amylase production was tested in cultured cells and it was found that amylase activity was highest in basal medium followed by medium containing only sucrose and basal medium and then by one containing only creatine and basal medium. The medium containing both sucrose and creatine had the lowest amylase activity. The cells from basal medium were used to immobilized cell in calcium aligenate. It was found that the immobilized cells also produced amylase.

***Key Words:*** *Gastrimargus musicus,* amylase, immobilization, insect cell culture



## INTRODUCTION

Insect cell culture is an established science. The report of maturation of testicular follicle cells to spermatocytes in Cercopia moth in an artificial medium provided a breakthrough for the work to be carried out in future (Lynn 2002; Sudeep *et. al.* 2005). Study of cell, production of recombinant proteins *etc.* have been different uses for which insect cell cultures have been tried (Maiorella, 1988; Lindsay *et al.*, 1991). Weiss *et. al.* (1993) developed a serum free medium for insect cell culture. Cell culture of cockroach has been tried for studying cuticle biosynthesis and production of chitin (Marks *et al.*, 2005). Many studies have highlighted the use of insect cell cultures for the production of recombinant proteins infected with baculovirus (Yamaji *et. al.* 2006). α-Amylases are ubiquitous in distribution, with plants, bacteria and fungi being the predominant sources. Amylases are industrial enzymes having approximately 25% of the enzyme market. The spectrum of amylase application has widened in many fields, such as the starch industry, clinical, medical, and analytical chemistries, as well as their wide spread application in starch sacccharification and in the textile, food, fermentation, paper, brewing and distilling industries (Reddy *et. al*. 2003). Adeleye (1990) studied extracellular α-amylase production induced in cultures of *Micrococcus varians*. Kathiresan *et al.* (2006) used *Penicillium fellutanum* for the production of amylase. The current study proposes the production of amylase from insect cells culture in artificial medium. Immobilization of cells and enzymes has been done for various purposes (Goosen 1993; Bugarski *et. al.* 2004; Shishido *et. al.* 2007). The advantage of immobilizing cells is that these immobilized cells can be used many times for the purpose they have been immobilized and downstream processing is easier. Various products can be easily purified after the cells producing them are immobilized and for this reason amylase production from insect cells was tested with immobilized cells.

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## MATERIALS AND METHODS

In the current we tried to develop cheap insect cell culture medium. Culture medium had the following constituents as given in Shukla et al. (2011): ammonium molybdate 0.035 mM/l, cobalt chloride 0.210 mM/l, cupric sulphate 0.117 mM/l, zinc sulphate 0.294 mM/l, ferrous sulphate 1.980 mM/l, aspartic acid 2.675 mM/l, Tween 80 25 mg, cholesterol 4.5 mg, ethanol 1 ml, inositol 2.220 mM/l, nicotinic acid 1.300 mM/l and thiamine 0.237 mM/l. All the constituents were mixed and autoclaved. Three different types of media were made from this basal medium. One medium was made by adding sucrose so that the final concentration was 1%. Second medium was made with final concentration of 1% creatine and the third had both sucrose and creatine. Addition was done aseptically. *Gastrimargus musicus* was selected for cell culture. For the cells to be cultured tissues from the legs were taken and were homogenized with phosphate buffered saline. 20 µl of the homogenized tissue sample was inoculated into separate flasks containing 100 ml of different media. Tissue sample was also inoculated into 100 ml basal medium. All the tests were performed in triplicates. The flasks were incubated at 27 ±ºC. After incubation of 7 days production of amylase was tested. For amylase test 1% starch was added with 1% agar autoclaved and poured into plates. 20µl supernatant from all the inoculated flasks were taken and poured on to the plates. Plates were kept for 15 minutes and then plates were flooded with iodine solution. Amylase activity in the supernatant was determined by the method of Yoo *et. al.* (1987). Extinction was read at 620 nm. Cells grown in basal medium were used for immobilization. A warm 1% sodium aligenate solution along with chilled calcium chloride solution was used for immobilization of cells. The immobilized cells in calcium

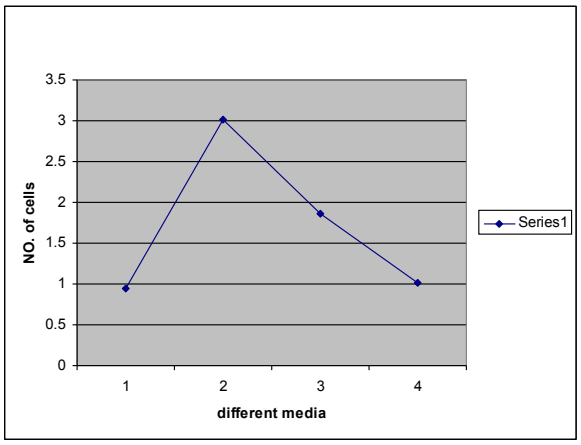
aligenate beads were kept submerged in starch solution. The hydrolysis of starch into glucose was confirmed by dinitro salicylic acid method (Miller, 1959).

## RESULTS AND DISCUSSIONS

Insect cell culture was obtained in the basal medium as well as all the three media developed. The culture developed as submerged colonies. The cells were visualized by microscope and counted by hemocytometer. The medium with only sucrose had the highest cell concentration i.e. 3.02 x $10^6$ cell per ml followed by the medium with only creatine 1.86 x $10^6$ cell per ml and then by the medium containing both sucrose and creatine i.e. 1.02 x $10^6$ cells per ml. Basal medium had the lowest cell concentration (0.95 x $10^6$ cells per ml) (Table 1, Graph -1).

**Table 1. Concentration of cells and amylase activity in different media**

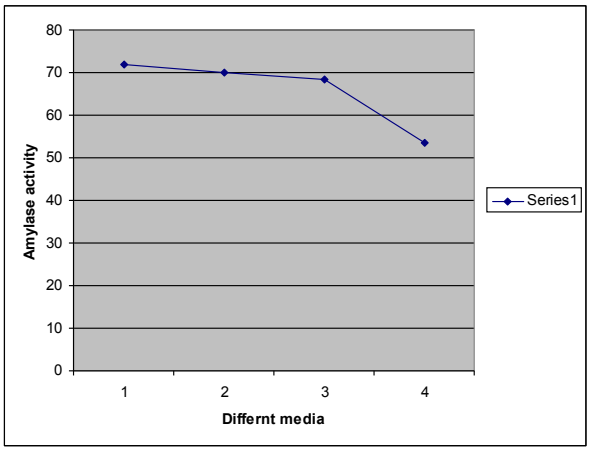
| S. No. | Medium | No. of cells/ml | Amylase activity (units/l) |
|---|---|---|---|
| 1 | Basal | 0.95 x $10^6$ | 72 |
| 2 | Basal with only sucrose | 3.02 x $10^6$ | 69.9 |
| 3 | Basal with only creatine | 1.86 x $10^6$ | 68.5 |
| 4 | Basal with both sucrose and creatine | 1.02 x $10^6$ | 53.4 |




**Graph 1- No. of cell in different medium.**

on X axis 1 = Basal medium, 2 = Basal with only sucrose, 3 = Basal with only creatine 4 = Basal with both sucrose and creatine on Y axis No. of cells in $10^6$

Amylase test was given by the supernatant of all the four media. Following flooding by iodine solution zone of clearance was observed for all the four media. Amylase activity was ascertained by the method of Yoo *et. al.* (1987) and found to be highest for the basal medium (72 units/l) followed by sucrose and basal medium (69.9 units/l) and then by the one containing creatine and basal medium (68.5 units/l). Medium containing both sucrose and creatine had the least amylase activity (53.4 units/l) (Table – 1, Graph - 2). This suggests that in the basal medium the cells secrete more amylase than in the medium with sucrose or creatine or with both as the concentration of cells in basal medium is the least. Even though the number of cells in basal medium is far less than those in other variations of the medium the amylase activity far exceeds the other medium. It suggests that sucrose and creatine alone hinders the production of amylase by the cells. In the medium containing both sucrose and creatine the amount of amylase activity is far less than that of other media suggesting that the hindrance caused by sucrose and creatine is added in combination. Calcium aligenate was formed when warm sodium aligenate solution was added into chilled calcium chloride solution along with cell culture in basal medium. The beads formed were small about 2-3 mm in diameter. The beads were kept submerged in starch solution and after incubation starch solution gave positive result for formation of glucose with DNS method.




**Graph 2- Amylase activity in different medium.**

on X axis 1 = Basal medium, 2 = Basal with only sucrose, 3 = Basal with only creatine 4 = Basal with both sucrose and creatine on Y axis amylase activity

Insect cell culture was obtained without addition of serum or expensive chemicals and amino-acids. The basal medium and other three additions are cheap and have less chances of getting contaminated as serum is not added. Amylase is an important industrial enzyme. It has been traditionally isolated from either bacteria or fungi both potentially harmful if not contained. There are chances of workers getting infections accidentally in both the cases. We here present a novel method of production of amylase by which chances of any accidental infection will be zero. Downstream processing for purification of amylase will also be not labour intensive with the present method as the cells grow in the culture at the bottom and amylase can be isolated from the supernatant liquid. This also minimizes the production costs as downstream processing involving separation of bacterial cells or fungal mat is not required. On immobilization also the cells produced amylase. The cells can be used for production of amylase either in culture or immobilized conditions which further minimizes the downstream processing.

### Conclusions

We conclude that the current work of developing a cheap insect cell culture medium was fruitful. Cells were easily cultured in all the different variations of medium tried. Amylase production was positive from the cultured cell. Amylase activity was found highest from cell cultured in the basal medium. The process can be scaled up and developed into a cost effective production method of amylase because the costs on downstream processing required for separation of cells or fungal mats is removed as cultured cells grow submerged at the bottom leaving a clear liquid at the top from which amylase can be isolated and purified. Also with immobilization of cultured cells the purification costs will be further minimized.

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