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A Study on Production and Evaluation of L-Asparaginase Obtained From *BACILLUS SUBTILIS*

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Abstract

L-asparaginase is commonly referred as one of the most acceptable clinical anti-tumor agents against lymphosarcoma and its associated leukemia. L-asparaginase (L-asparaginase amino hydrolase) mediates the catalysis through hydrolysis of L-asparagines into aspartic acid and ammonia. L-asparaginase was isolated from bacteria (Bacillus subtilis) by submerged fermentation process. The productions of this enzyme were subjected to physical parameters like pH, temperatures and different days. The outcome of this research indicated the pH 8, 300 C, and days 3 were best suited and optimal environmental condition for Bacillus subtilis. The enzyme was partially purified using the acetone method and stored to proceed further.

Key words:*L. Asparginase, Bacillus substilis, PH, Temperature, Days, Anti-cancerous activity, etc.*

I. INTRODUCTION

The organism was identified and named as Vibrio subtilis during the year 1835. It was further renamed as Bacillus subtilis in 1872. This bacterium is also known as Bacillus uniflagellatus, Bacillus globigii, and Bacillus natto. Bacillus subtilis was one among the first bacteria studied. Bacillus subtili are a gram-positive, rod shaped bacteria, usually found in soil and vegetative parts of plants. The optimal temperature for its growth stands around 25–350 C (Entrez genome project), which is in the mesophilictemperature range. Bacillus was isolated by soil sprinkle technique and it plays a significant role in antibiotics production too. The antibiotic, bacitracin is found most effective on gram-positive bacteria only (Jamil, 2007). Bacillus subtilis tends to secrete commercially important enzymes and its production bags 60% out of all commercially produced industrial important enzymes (Morikawa, 2006). The bacteria are known to be non-pathogenic and are responsible for contamination. They are commonly employed as fungicide for plants, vegetables, soybean seeds and it shall also colonize on root systems. It competes with fungal organisms and limits the cause of diseases.

The enzyme plays an effective antineoplastic agent, which is commonly used in acute lymphoblastic leukemia chemotherapy. L-asparaginase is an enzyme with an amidase group which shall catalyzes the substrate with end products such as L-aspartic acid and ammonia. L-asparaginase is commonly used in cancer treatment, as it interferes and limits the growth of cancerous cells. It also disrupts DNA, RNA and protein synthesis during G1 phase of cell

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cycle. L-asparaginase effectively acts against tumor cells and destroys the same; in turn it also induces apoptosis. Therefore, it is noteworthy to retrieve the new variant of L-asparaginase in order to optimize production conditions (Niharika and Supriya, 2014).

Microbes had proven as a user friendly source for the production of L-asparaginase enzyme. The precise mechanism on the mode of its action is still unknown; however to our understanding that hydrolysis occurs by two steps mediated reaction through a beta-acyl enzyme (Siddalingeshwara and Lingappa, 2010). Physical parameters, such as pH and temperature, play a large role in production of enzymes. The optimal PH is known as 8 and temperature of 35° C were commonly known as suitable conditions for the growth of Aspergillusterreus. Their studies have proved that L-asparagine induces the production of more enzymes from the culture available for the study. They had also increased the production of the enzyme (Moses, et al. 2011). Whereas, no pink zone was observed in control plates bearing Czapekdox agar plate in the absence of L-asparagine. The formation of a pink zone indicates the production of L-asparaginase. Actinomycetes are also a potential source for a increased yield of the L-asparaginase enzyme and high substrate specificity, with an optimum growth parameters as 2800 C and at pH 7 (Dhanam and Kannan, 2013). This enzyme was extensively studied in an L-asparagine biosensor which is commonly used for leukemia detection, and it is also applied in the food industry to minimize the level of acrylamide in fried and baked foods (Soniyamby et al. 2011).

MATERIALS AND METHODS

Specimen Collection

The bacterium, Bacillus subtilis, was obtained from Bioline Laboratories, Coimbatore. The inoculum was prepared by inoculating 5 mL of nutrient broth with 18 hours old culture, which was further incubated at 37oC for 16 to 24 hours.

Production of L-asparaginase

L-asparaginase production was carried out by submerged fermentation. 50 ml of the sterilized production medium was inoculated with 100 µl of Bacillus subtilis and was incubated at 370 C for 24 hours. L-asparagine, a substrate for production was procured from sigma chemicals and used in this study.

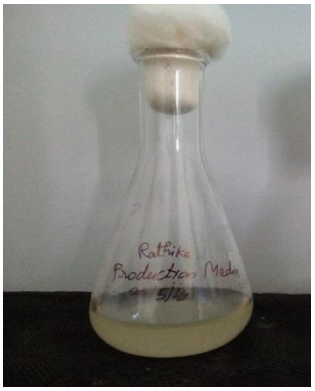

Fig.1 The production medium for creating L-asparaginase from Bacillus subtilis

Estimation of L-asparaginase activity:

Colorimetric method was used to estimate the activity of enzyme produced during the study (Imada et al., 1973). In assistance with UV visible spectrophotometer, Nessler reagent was also used to estimate the ammonia produced during the reaction. About 1 ml of fermented broth was centrifuged and supernatant was added with 500 µl of Nessler's reagent and incubated at 370 C for 30 min and optical density value was measured at 480 nm.

Optimization of L-asparaginase:

Effect of pH: The optimum pH and its stability were determined. The pH of the medium was adjusted with 5N- NaOH and 5N- HCl. The experimental pH was selected in the range 5, 6, 7, and 8. From the four pH values, the optimum pH was determined.

Effect of temperature: The optimum temperature was determined. The production medium employed during the study was incubated at diverse range of temperature in order to study its successive effect during the enzyme production. The ranges of

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temperatures used in this study were 300 C, 400 C, and 500 C. Out of which, optimum temperature was determined and recorded.

Optimum day: The optimal day was also determined for the enhanced production of enzyme. The production medium was centrifuged and the supernatant was preceded with Nesslerization, then the colorimetric value was determined. The process was repeated at regular intervals of 24 hours, 48 hours, and 72 hours.

Results:

Estimation of L-asparaginase activity:

The selected isolate synthesized L-asparaginase through submerged fermentation. The colorimetric value at 480 nm shows the presence of available protein in the media provided for its growth.

Optimization of L-asparaginase:

Effect of pH on enzyme production:

The best enzyme activity was obtained at pH 5 in the production medium with an duration of 24 hours. Enzyme production happened with the lesser time in other pH ranges. The optimum pH level for L-asparaginase enzyme was found to be 8. The results were tabulated below.

S. No	pH	Optical density value (480 nm)
1	5	1.5
2	6	1.5
3	7	1.5
4	8	1.648

Table 1. Optical density values for different pH levels

Effect of temperature on enzyme production:

The selected experimental temperatures were 30^0 C, 40^0 C, and 50^0 C. The optimum temperature was determined to be 30^0 C.

S. No	Temperature	Optical density value (480 nm)
1	30^0	0.683
2	40^0	0.618
3	50^0	0.220

Table 2. Optical density values for different temperatures

Optimum day:

Enzyme activity was measured for three days and the optimum day for the enzyme was determined as day 3 (72 hrs).

S. No	Days	Optical density (480 nm)
1	Day1	1.373
2	Day2	1.173
3	Day3	1.723

Table 3. Optical density values at different time intervals

DISCUSSION

Effect of pH and temperature

The enzyme was stable at an alkaline pH of 8.0 and retained 100% activity even after incubation for 30 and 60 min at 37^0 C. The results were indicative of the enzyme is more stable and comfortable in an alkaline pH rather than the acidic pH. Using pseudomonas stutzerimb–405, the enzyme obtained by Bacillus subtilis was stable at pH 8. According to Siddalingeshwara and Lingappa, 2011, were reported the better activity of the enzyme was observed between the ranges of 7.5 to 9.5 pH. This study is also in line with the earlier findings.

L-asparaginase activity was profound at 30^0 C. It is also active with a wide range of temperature as 30^0 C to 75^0 C. The increase in maximum temperature will ends up by making the enzyme unstable and will not used any more. The elimination of L-asparagines from the body shall achieve by increase in the body temperature up to certain extent. It can, therefore, be used to treat tumor patients. Siddalingeshwara and Lingappa (2011) found 37^0 C

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as an optimum temperature for the activity of enzymes. They also found no enzyme activity was lost during its pre-incubation at 700 C for 30 and 60 minutes respectively. At 80^0 C, the enzyme had shown 69% and 60% activity for 30 min and 60 min, respectively. Similarly, 100% of the enzyme activity was confirmed at 77^0 C. The results obtained from this study reflected other results (Siddalingeshwara and Lingappa, 2011).

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