

Original Research Article

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Study the Optimum Parameters for Production of Cloned L-Asparaginase Type I by *Escherichia coli*

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ABSTRACT

Keywords

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In the present study, medium components and production conditions were different for carbon and nitrogen sources. The results have shown that the best carbon and nitrogen sources were lactose and yeast extract at concentration 1%. The different physiological factors include Temperature, pH, aeration and agitation. Besides, the results showed that the maximum production of L-Asparaginase was achieved after the incubation of the growth culture at 30°C and pH 7. Under these conditions, the activity of L-Asparaginase produced in culture medium was (10.3 IU/ml) and (10.5 IU/ml) respectively. The production of L-Asparaginase was modified during *E.coli* grown aerobically and when the shaker incubated at 220 rpm.

Introduction

L-asparaginase is an amidohydrolase enzyme (EC 3.5.1.1) that catalyzes the hydrolysis of L-Asparagine (L-Asn) to L-Aspartate (L-Asp) and releases ammonia (NH₃). The activity of L-Asparaginase is distributed in a number of microorganisms among plants, animals and others microorganisms, that include bacteria, yeast, fungi (Maria *et al.*, 2004) such as *E.coli* (Kamble *et al.*, 2012), *Pseudomonas sp.* (Ashraf *et al.*, 2004), *Erwinia sp.* (Jyoti *et al.*, 2011), *Cryptococcus nodaensis* (Sato *et al.*, 1999). *Penicillium* (Kavitha and vijayalakshmi, 2010). The activity of L-asparaginase plays an important role in

metabolizing the nitrogen for prokaryotic and eukaryotic cells (Michel, 2003). On the other hand, the activity of L-Asparaginase can be applied in different industrial and pharmaceutical purposes such as a processing aid in the food (David, 2005) and in the treatment of acute lymphoblastic leukemia (ALL) and some mast cell tumors (Bülbul and Karakuş, 2013). It also acts as a biosensor for monitoring the level of L-asparagine (Sabu *et al.*, 2000). The aim of this study is to optimize the condition of culture in order to increase the productivity level of Asparaginase in recombinant *E.coli* BL21 DE3. The success of L-Asparaginase

production mainly depends on the type of the microorganism and the media components that are used. Similarly, different physiological parameters have a greater effect on the production of enzymes. *E.coli* are reported to be very suitable for L-Asparaginase production (Siddalingeshwara and Lingappa, 2011).

This can be achieved by

1. Studying the effect of pH;
2. Studying the effect of incubation temperature;
3. Studying the effect of different carbon and Nitrogen source;
4. Studying the effect of agitation (rpm);
5. Studying the effect of aeration.

Materials and Methods

Growth Media and Preparation of Solutions (Ausuble *et al.*, 2007)

Luria-Bertani Broth (L.B): This media was prepared by dissolving 10 g of NaCl, 5 g of yeast extract and 10 g of trypticase peptone in one liter of distilled water (dH₂O) (Bertani, 1952).

L.B Agar: This medium was prepared by dissolving 7.5g of agar in one liter of LB broth media. M9 Media (Paglla *et al.*, 2013). It was prepared by mixing 0.6 g (Na₂HPO₄ · 2H₂O); 0.3 g (KH₂PO₄); 0.05 g (NaCl); 0.5 g (Asparagine); 0.2 g (MgSO₄ · 7H₂O); 0.01g (CaCl₂ · 2H₂O); 20% of glucose stock and 3% of phenol red solution (2.5%).

Tris Buffer (0.5M, pH 8) :This buffer was prepared by mixing 6.05 g of Tris/HCl, in 80ml of sterilized dH₂O. The pH was adjusted at 8, then the volume of buffer was completed to 100 ml.

L-asparagine (0.04M) and L-glutamine (0.04 M) solutions: Both solutions were prepared by dissolving (0.05) g and (0.06) g of L-asparagine and L-glutamine respectively in 10 ml of sterilized Tris base buffer pH 8.

Bacterial Strains and Culture Conditions (Ausuble *et al.*, 2007)

Recombinant BL21(DE3) pLysS-T1R *E. coli* were grown overnight at 37°C with 220 rpm shaker stored at -80°C for further analysis.

BL21 (DE3) *Escherichia coli* Strain was grown overnight in a shaking incubator at 37 °C and 220 rpm. The overnight culture was diluted 1:100 into fresh M9 media. The cultures were incubated at 37 °C with shaking at 220 rpm until the optical density OD₆₀₀ nm wave length approximately reached to 1.7. The growth culture was centrifuged at 10,000 rpm for 15 min, then the supernatant was used for the quantitative analysis of L-Asparaginase.

Enzyme Assay

Amidohydrolase enzyme assay was assayed according to Mannan *et al.*, (1995): International Unit (IU): One international unit of Putative L-asparaginase or L-glutaminase is the amount of enzyme which liberates 1 μmol of ammonia per minute per ml [μmole/ml/min].

Optimal Conditions for L-Asparaginase Production

Optimal conditions for the production of L-asparaginase from recombinant *E.coli* BL21 DE3 were studied in order to obtain a higher enzyme production, as follows:

Optimum Carbon Source

The effect of different carbon sources on L-Asparaginase production (glucose, lactose, D-sorbitol and maltose) by recombinant *E.coli* BL21 DE3 was used to determine the optimum carbon source for enzyme production. Each carbon source was individually added to the production media of about 1% (w/v) as a final concentration

Effect of Nitrogen Source

Different nitrogen sources were tested to determine the optimum one for L-Asparaginase production. Nitrogen sources used in this study involved 1% w/v (Yeast extract, peptone, ammonium sulphate and mixture of L-asparagine and L-Glutamine).

Effect of Temperature on Production of L-asparaginase

The productivity level of L-Asparagine was determined after incubating the bacterial culture at different temperature values ranging from 25 to 50°C.

Effect of pH on Production of L-asparaginase

Activity of L-asparaginase in the production media was measured at various pH values to study the effect of pH on the production of both enzymes. The pH media was adjusted at different pH values (pH 5.0-9.0). Bacterial cultures were incubated at 37°C and the activity of the enzyme was measured.

Effect of Agitation (rpm)

The effect of agitation on the production of L-asparaginase was determined at vary agitation speeds of 50,100,220 and 250 rpm at 37°C for 18 h.

Effect of Aeration (volume of media to air-space of flask)

In order to study the effect of aeration on the production of L-asparaginase, different volumes (50, 100, 150 and 200 ml) of production medium were used in 250 ml flasks, and then tested for a maximum L-asparaginase production.

Results and Discussion

Effect of Carbon Sources on the Production of L-Asparaginase

L-Asparaginase production responded differently to various carbon sources at 1% (w/v). The results in figure (1) showed that the efficient L-asparaginase production occurred when lactose was used as a carbon source in a basal medium. The activity of L-asparaginase in a crude culture reached (10.89) U/ml while the low activity was when glucose, maltose and D-sorbitol were used as carbon sources.

Effect of Different Nitrogen Sources

Different nitrogen sources were used to determine their effect on the productivity of Asparaginase. As illustrated in figure (2), the results showed that the yeast extract at 0.5% (w/v) produced a maximum L-Asparaginase. Whereas other supplemented nitrogen sources showed a significant viable effect on both amidohydrolase activities.

Effect of Incubation Temperature

The maximum production of L-Asparaginase was observed when the culture media incubated at 30 °C (figure.3). Under this condition the L-Asparaginase activity reached 10.3IU/ml.

Fig.1 Effect of Carbon Source on Production of L- Asparaginase

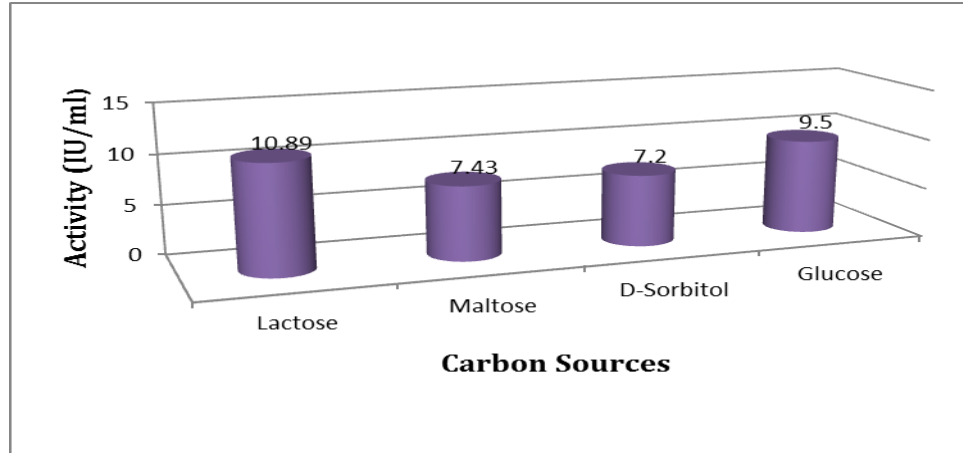


Fig.2 Effect of Different Nitrogen Sources on L-Asparaginase Production

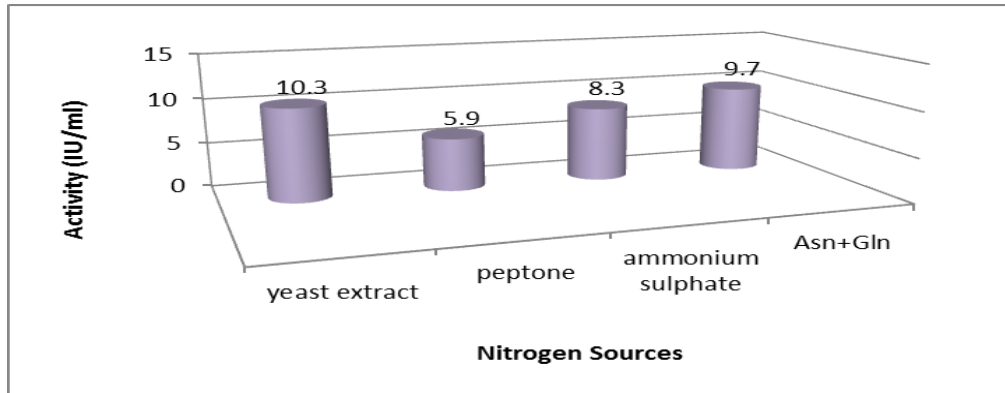


Fig.3 The Optimum Temperature Value for Maximum L-Asparaginase Production

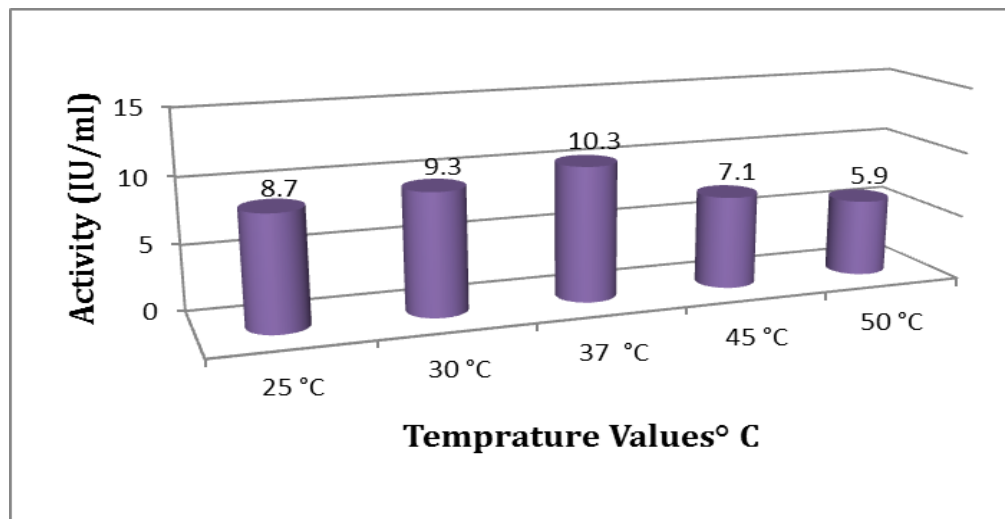


Fig.4 Effect of pH Values on Production of L-Asparaginase

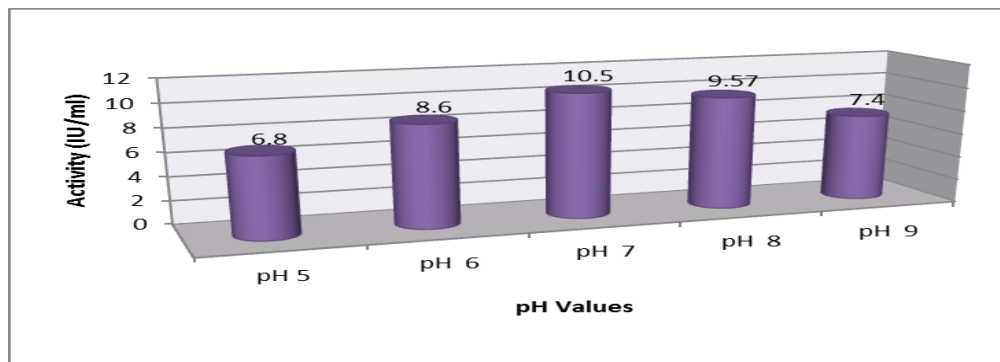


Fig.5 Effect of Agitation on the Production of L-Asparaginase

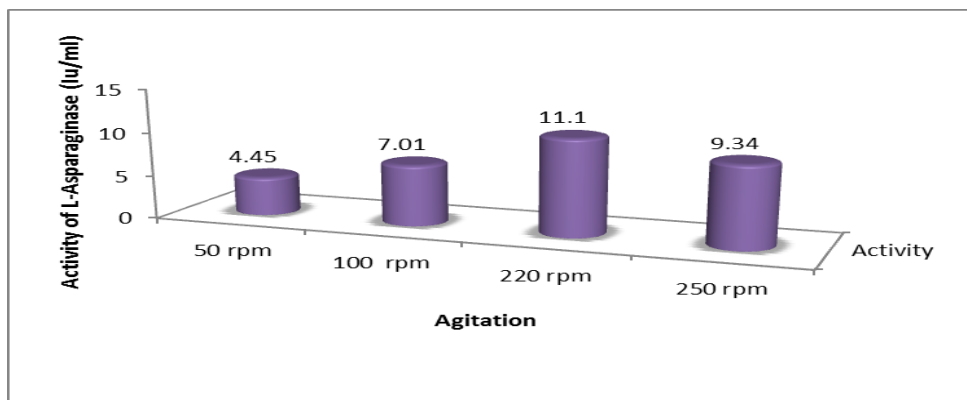
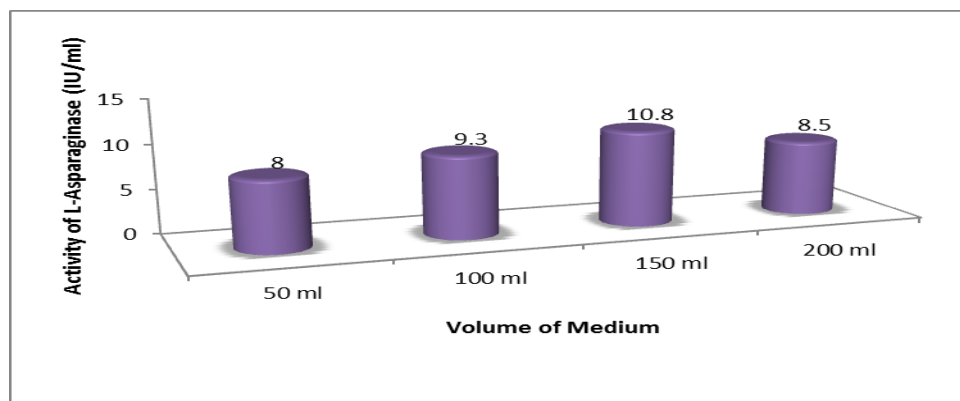


Fig.6 Effect of Aeration (Volume of Media to Space of Flask)



Effect of Initial pH Medium

The effect of initial pH media on amidohydrolase activity was tested at various pH values. As it is shown in figure (4), the maximum activity of L-

Asparaginase was obtained when the culture media of recombinant *E.coli* was adjusted at pH 7. At this pH value, the activity of L-Asparaginase was (10.5 U/ml)

E. coli has the ability to produce large amounts of L-asparaginase in alkaline and conditions more than acidic conditions. The change that occurred in pH value of culture media may be attributed to the metabolism of bacteria. This change may have an effect on the biological cells and solubility and on ionizing the nutrition substrates. All of these reasons negatively affect the enzyme activity and may lead to a distortion in the three dimension of enzyme (Tortora *et al.*, 2004; Hammami *et al.*, 2007).

Effect of Agitation Speed (rpm)

The effect of agitation speed on L-Asparaginase was reported in figure (5). The results shown that there was a gradual increase in the activity of the L-Asparaginase at 220 rpm. It was found that the activity of L-Asparaginase was (11.1 U/ml).

Effect of Aeration on L-Asparaginase Production

The results shown in figure 6, is howed that the optimum production of L-Asparaginase was found when the volume of media was 100 ml. The activity of L-Asparaginase was 10.8 (IU/ml).

In conclusion, there were many potential bacteria strains with a good L-asparaginase activity and they have been successfully isolated. Different strains showed the highest L-asparaginase activity (11.1 IU/ml). It is recommended to isolate the gene that is responsible for the production L-Asparaginase type I and to optimize the production of enzyme from new thermophilic bacterial strains that could be considered important sources to be used in the industrial L-asparaginase production. The latter is considered a potential source of useful microbes that can be used as a source

in different biological products such as enzymes.

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